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

Gracilaria cornea grow well in the Florida Keys at 15-25°C [31,32]. At 15°C, *Chondrus crispus* and *Furcellaria lumbricalis* reach their maximum growths [29]. However, at temperatures exceeding 30°C, an inferior growth of *Hypnea cervicornis* and *H. musciformis* was recorded [30,32].

Studies on seaweed culture in ISW in Australian is limited to *Gracilaria cliftonii* Withell, Miller and Kraft, and *Sargassum linearifolium* [19] even though there are abundant studies about seaweed growth, chemical and nutrient uptakes worldwide [33-38] . This study is the first attempt to grow *Lomentaria* sp. in the laboratory, testing the growth feasibility of *Lomentaria* sp. in OW and ISW, targeting on consuming the available ISW source to reduce adverse impacts of ISW on environment and agriculture [24].

Material and Methods

Seaweed collection

Lomentaria sp., was identified by WA Herbarium, was collected at Matilda Bay, Swan River, WA (latitude 31°97.9S, longitude 115°82.2E). This species currently is identifying by WA Herbarium and it maybe a new species. The *Lomentaria* sp. was transported in tanks holding ambient river salty water to Curtin Aquatic Research Laboratory (CARL) immediately after collection. The *Lomentaria* sp. were thoroughly cleaned in OW to remove all epibiotics.

Before using in experiments, the *Lomentaria* sp. was then acclimated for one day in aerated OW at 30‰ at 22°C 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 [33].

Experimental setup

ISW had a salinity 45% was procured from a lake at Wannamal, WA (31°15″S, 116°05″E). OW had a salinity of 35‰ was procured at Hillary Habour (31°.83″ S, 115°.74″E). 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 using in the experiments. OW and ISW were then diluted with filtered fresh water to achieve needed salinity waters at 30‰.

A series of four experiments were conducted in order to determine (1) suitable [K^+] levels for growing *Lomentaria* sp. in ISW, (2) the growth feasibility of *Lomentaria* sp. in NH_4 enriched water, (3) the effects of temperature and NH_4 on the growth of *Lomentaria* sp., and (4) the effects of NH_4 and PO_4 enrichment on the growth of *Lomentaria* sp.

Water salinity was maintained at 30-31‰, similar to the salinity of Swan River where the *Lomentaria* sp. was collected, by adding 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 at 25-26°C or 21-22°C.

Lomentaria sp. growth in K^+-fortified ISW(K^+ISW)

A total of 20 glass beakers, with a capacity of 1.5 L, holding 1 L culture medium were used for five fortnights from 19/6-27/8/2013. The experiment determined the growth rate of *Lomentaria* sp. in four replicates at three levels of [K^+] in ISW with two controls of OW and ISW at ambient room temperature. KCl was used to fortify ISW to approximately 100%, 66%, and 33% (ISW100, ISW66, and ISW33 respectively) of [K^+] in OW at 30‰ salinity. [K^+] at 30‰ 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‰ to achieve ISW100, ISW66, ISW33, respectively.

The pH of cultured media was similar over the experimental period except at day 14, when ISW66 resulted in the highest pH among the five waters ($P<0.05$). The experiment was conducted in ambient room temperature, reflecting seasonal temperature changes during 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 1).

Effect of ammonium enrichment in OW and ISW on the growth of Lomentaria sp.

Lomentaria sp. were cultured in 24 glass tanks 25/8-24/9/2013, receiving the results from previous experiment, when the ISW66 resulted in highest SGR of *Lomentaria* sp. Approximately 180 g of *Lomentaria* sp. was grown in each tank holding 45 L water in three replicates with aeration provided, at room temperature 17-19°C. The water included OW, ISW and ISW66 as control

Table 1. *The water pH and temperature in K^+ISW for culturing Lomentaria sp.*

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

and the weekly enriched with NH_4 100 µmol by NH_4Cl to give OW_NH_4, ISW_NH_4, ISW66_NH_4 (Table 2).

Effects of temperature on Lomentaria sp. cultured in OW and K⁺ISW

The effects of temperature on the growth of *Lomentaria* sp. were determined in two experiments.

The first experiment was conducted over four weeks, 25/8-24/9/2013. Approximately 180 g $tank^{-1}$ of 45 L cultured medium in four replicates, aeration provided were tested in three temperature conditions at 25-26°C, 21-22°C and 18-19°C. The water included OW, and OW enriched with 100 µmol NH_4 by NH_4Cl, OW_NH_4. An automatic heater (Sonpar, HA-200, Zhongshan, Guangdong, China) was used in each tank to maintain the temperature. The pH and temperature of waters at the same temperature levels were similar over the experimental period (Table 3).

The second experiment was conducted 26/9-28/10/2013, at two temperature levels (25-26°C and 21-22°C) with three waters OW, OW_NH_4 and ISW66_NH_4 (the last two waters were enriched with 100 µmol L^{-1} NH_4 by NH_4Cl), achieving the outcomes of NH_4 enrich for ISW66 and the two temperature levels in the first experiment of temperature effect. The *Lomentaria* sp. was selected by whole fond weight of approximately 3.5 g L^{-1}, grown 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. The pH and temperature of the water were similar at the same temperature levels (Table 4).

Effects of ammonium and phosphate enrichment on the growth of Lomentaria sp. in OW and K⁺ISW

A total of 24 1.5 L beakers were used for eight treatments in three replicates for the experiment 28/10-23/11/2013. *Lomentaria* sp. was cultured at a density of 3.5 g L^{-1}. The beakers were placed randomly into tanks filled with water. One automatic heater (Sonkar, HA-200, Zhongshan, Guangdong, China) and a pump (Grant Model GD 120, England) were used in each tank to maintain water temperature at 25-26°C, the optimal temperature for *Lomentaria* sp. growth (achieved from the temperature effect experiments). The water salinity was kept constant at 30-31‰ by adding filtered fresh water to compensate for evaporation.

Four levels of NH_4:PO_4 were provided weekly for OW and ISW66, by NH_4Cl and Na_2HPO_4: (1) T1 - no nutrients provided; (2) T2 - 75:7.5 µmol L^{-1} NH_4:PO_4; (3) T3 - 150:15 µmol L^{-1} NH_4:PO_4; and (4) T4 - 300:30 µmol L^{-1} NH_4:PO_4.

Table 2. *pH and temperature in NH4 enriched water.*

Waters	NH_4^+ (µmol L^{-1})	pH	Temp (°C)
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

Table 3. *pH and temperature of the temperature-effect experiment in OW.*

Waters	NH_4^+ (µmol L^{-1})	pH	Temp (°C)
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

Data collection

Water quality: The NH_4, NO_3, NO_2 and PO_4 concentrations in water were determined fortnightly applying the methods described by Bui et al. (in press).

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).

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 [33]. The thalli were immediately transferred to a weighing scale (AW220, d=0.1mg, Shimazu, Japan).

The cumulative 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).

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.

Table 4. *pH and temperature in the temperature-effect (second experiment).*

Factors	21-22°C			25-26°C		
	OW	OW_NH_4	ISW66_NH_4	OW	OW_NH_4	ISW66_NH_4
Temperature (°C)	21.64 ± 0.13	21.64 ± 0.06	21.69 ± 0.16	25.78 ± 0.22	25.67 ± 0.03	25.50 ± 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

Results

Lomentaria sp. growth in K⁺ISW

Lomentaria sp. 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 increase of the biomass during the culture period (P<0.05), 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 5). On average, ISW66 resulted in higher biomass growth than other waters in the first 56 days.

Note: (for all Tables throughout the article) 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).

Over time, the biomass and SGR were significantly (P<0.05) different among treatments. In the first two fortnights, the SGR of *Lomentaria* sp. 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 *Lomentaria* sp. presented a similar fortnightly SGR over the last three fortnights of the experiment (Table 6). In the first 42 days of the culture period for growing *Lomentaria* sp., either ISW66 or ISW33 gave higher biomass gains than other water sources.

In the first two fortnights, the *Lomentaria* sp. showed promising signs of growth when new axial filament growth from different parts of the thallus, and the red colour of *Lomentaria* sp. remained. However, although the fresh biomass of the *Lomentaria* sp. increased until day 42, a sign of discolouration appeared, and defragmentation of the thallus began. By the end of the experiment, most of the red colour of the *Lomentaria* sp. disappeared and few tissues remained, providing small amounts of fresh biomass of the *Lomentaria* sp.

The [N] in water varied differently at different points of the culture period. NH_4 was negligible as the experiment progressed, whereas NO_2 decreased and NO_3 increased in all waters toward the end of the experiment. There was no significant difference of [NO_3] 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] than other waters (Table 7). However, NO_3 showed no significant correlation with the biomass of *Lomentaria*, but NO_2 did.

PO_4 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 *Lomentaria* sp.

Effect of ammonium enrichment in OW and ISW on the growth of Lomentaria sp.

NH_4 did not affect the growth of *Lomentaria* sp. in OW, but it did show a significant effect on *Lomentaria* sp. growth in ISW. Both ISW_NH_4 and ISW66_NH_4 resulted in significantly higher biomass and SGR_w of *Lomentaria* sp. than ISW and ISW66, respectively (Table 8). NH_4 presented the highest effectiveness when used in ISW66_NH_4; this resulted in higher biomass and SGR_w of *Lomentaria* sp. by the end of the experiment than OW_NH_4 and ISW_NH_4. In the waters not enriched with NH_4, the three water types gave similar biomass and SGR_w of *Lomentaria* sp. However, a significant reduction was found in the biomass of *Lomentaria* sp. over the experimental period in all waters.

Effects of temperature on Lomentaria sp. cultured in OW and K⁺ISW

Temperature significantly (P<0.05) affected the biomass and growth rate of *Lomentaria* sp. during the four weeks growing

Table 5. *The biomass (g) of Lomentaria sp. 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}

Table 6. *The SGR (% d^{-1}) of Lomentaria sp. 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

Table 7. *The water quality parameters of Lomentaria sp. cultured in K^+ISW.*

Time	OW	ISW	ISW33	ISW66	ISW100
NO_2					
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					
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					
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					
Day 1	1.55 ± 0.12^{a}	$_{1}$1.68 ± 0.05^{a}	$_{1}$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}

Table 8. *Biomass (g) and SGR_w (% d^{-1}) of Lomentaria sp. in NH_4 enriched water.*

Parameters	OW		ISW		ISW66	
	OW	OW_NH_4	ISW	ISW_NH_4	ISW66	ISW66_NH_4
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}$161.6 ± 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}

in the tanks. The ambient temperature of 18-19°C resulted in the lowest *Lomentaria* sp. biomass and SGR_w. However in the OW_NH_4 water, 25-26°C gave a higher biomass and SGR than 21-22°C (Table 9).

In the second experiment, when only two levels of temperature and three water types were used, mortality of *Lomentaria* sp. started occurring on day 25. By day 45, there was no sign of living *Lomentaria* sp. in the beakers; therefore, the data of biomass and SGR_w were collected by day 25 of the experimental. At the 25-26°C, 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 *Lomentaria* sp. than ISW66_NH_4. On the other hand, the temperature showed no effect on the growth of *Lomentaria* sp. in all waters, while at the same temperature levels, the three water sources resulted in a similar SGW_w. The length of the *Lomentaria* sp. showed no significant change over the culture period in all waters and temperatures (Table 10).

Effects of ammonium and phosphate enrichment on the growth of Lomentaria sp. in OW and K^+ISW

Following the results from the previous experiment, this experiment lasted for only 25 days, to collect the dried biomass of the *Lomentaria* sp. By the end of the experiment, with no nutrient enrichment, ISW66 resulted in a significantly higher biomass and SGR_w of *Lomentaria* sp., and higher [NO_2] and [PO_4] content in water than in OW; however, these were similar at other nutrient levels (Tables 11 and 12).

Nutrient enrichment did not significantly affect the growth of *Lomentaria* sp. in ISW66. The biomass, SGR_w and dried content of *Lomentaria* sp. were similar after 25 days of culture in four NH_4:PO_4 levels. In OW, T2 resulted in the highest biomass and SGR, and the dried content of *Lomentaria* sp. cultured in T4 was lowest.

Although NH_4 was provided weekly, NH_4 in water was negligible during the experiment. By the beginning of the experiment, [NO_3] in ISW was higher than in OW, and in both waters, [NO_3] and [PO_4] were significantly increased in higher nutrient enrichment levels. However, by day 25, [NO_3] in ISW was only higher than OW at T3, and lower at T4. [NO_3] and [PO_4] were similar in OW. [NO_2] 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] during the experiment, and [PO_4] was significantly correlated with the biomass of the *Lomentaria* sp (Table 12).

Discussion

This is the first study on growing *Lomentaria* sp. in artificial conditions, particularly in ISW. *Lomentaria* sp. showed an ability to grow in ISW under special conditions of K^+ISW and seasonal temperatures.

Potassium fortification was needed for ISW to sustain the growth of *Lomentaria* sp., when at the day 28, ISW66 resulted in higher *Lomentaria* sp biomass than in OW, and from the 28th day onward, the biomass of *Lomentaria* sp was similar in 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 [9,11]. The [K^+] in the seaweed cells should be between 100-200 mM for proper protein synthesis [39]. Intracellular [K^+] is regulated by internal and external [K^+] exchange mechanisms, which are determined by external [K^+] [39,40]. The osmotic gradient of aquatic plant cells is maintained by [K^+], and is facilitated by a suitable ratio between Na^+ and K^+ internally [39,41]. 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 [14,17,18,19,42]. Similarly, *Lomentaria* sp. also needs higher [K^+] than in ambient ISW for growing. 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 *Lomentaria* sp. than higher or lower [K^+], and it is similar to the preferred Na:K for *Ulva* growth at 47:1 [43]. This [K^+] range is lower than required by other rea seaweeds *Caloglossa leprieurii* and *Bostrychia radicans* [12]. If the culture period was less than one month, ISW66 would be a better choice than ISW33. However, *Lomentaria* sp. should not be cultured longer than 42 days for a higher biomass gain.

Table 9. *Biomass and SGR_w (% d^{-1}) of Lomentaria sp. in three temperature levels.*

Parameters	25-26°C		21-22°C		18-19°C	
	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	-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

Table 10. *Biomass (g), length (mm) and SGR (% d^{-1}) of Lomentaria sp. in two temperatures.*

Criteria	21-22°C			25-26°C		
	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

Table 11. *Biomass (g), SGR_w (% d^{-1}) and dried content (%) of Lomentaria sp. cultured in four nutrient levels.*

Crite-ria	OW				ISW66			
	T1	T2	T3	T4	T1	T2	T3	T4
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

Table 12. *The water quality OW and ISW66 in which Lomentaria sp. was cultured at different nutrient enrichment levels.*

Criteria	OW				ISW66			
	T1	T2	T3	T4	T1	T2	T3	T4
NO_3								
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								
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								
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

Ammonium is preferred source of N for seaweed growth over NO_3 [44], which is why NH_4 in water was negligible over the culture period, even in the waters supplied weekly with NH_4. In previous work, the red seaweed *Gelidium amansii* grew faster at 80 µmol L^{-1} NH_4 than at 200 µmol L^{-1} [44]. However, in this study, the *Lomentaria* sp. showed no response in 100 µmol L^{-1} NH_4 in both OW and ISW 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 19°C. This result was demonstrated in the temperature-effect experiment, where the reduction rate of *Lomentaria* sp. cultured in 18-19°C was higher than other two higher temperature levels. As the *Lomentaria* sp. cultured in tanks holding OW and OW_NH_4 showed different responses to the 21-22°C and 25-26°C 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-effect experiment, was also tested. A similar SGR_w was found for *Lomentaria* sp. 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 *Lomentaria* sp. cultured in captivity was 21-26°C. This prefer temperature range was similar to the green seaweeds *Ulva curvata* [45], *Ulva lactuca* [46], and *Ulva pertusa* [47], and the red seaweed *Hypnea cervicornis* J Agardh [30], but was higher than the need of the red seaweeds *Phycodrys rubens and Membranoptera alata* [48] .

Contrary to the negative SGR found in *Lomentaria* sp. cultured in all temperature conditions in tanks, the *Lomentaria* sp. cultured in beakers at 21-26°C in the temperature-effect experiment and K^+-fortification effect experiment at 18.5-21°C resulted in a positive SGR_w, revealing the scale of growing *Lomentaria* sp. This can only be explained by the different seasons of sampling. The *Lomentaria* sp. were collected from the field 2-3 days before the beginning of each experiment, reflecting the seasonal growth of *Lomentaria* sp. 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 *Lomentaria* sp. grew quickly and the canopy was largest. Furthermore, the *Lomentaria* sp. standing crop decreased gradually by the end of summer, and reappeared in the spring.

At 21-22°C, the length of *Lomentaria* sp. cultured in OW_NH_4 were reduced, resulting from apical cell breakage; however, the biomass gain was positive, indicating growth of the *Lomentaria* sp. The similarity of the SGR_w and SGR_L of the *Lomentaria* sp. cultured in ISW66_NH_4 and the sources of OW showed the ability of *Lomentaria* sp. to grow in ISW66_NH_4.

Although NH_4 was necessary for *Lomentaria* sp. growth in ISW66, the combination of NH_4 and PO_4 did not show the good effect than single NH_4. In addition to the weekly supplied NH_4/PO_4, N and P in water were also produced by the decomposition of *Lomentaria* sp. NH_4 combines with PO_4 result in a higher growth rate of *Sargassum baccularia* than single nutrient sources [24]. Soluble N and P in water are quickly cycled by living microbes, so their concentrations are not stable, difficult to measure [49]. They are also consumed at different rates [50]. At the same concentrations, NH_4 is uptaken faster than PO_4 by seaweeds [51]. Consequently, NH_4 was negligible in waters as the experiment progressed, NO_3 was reduced over the culture period, and [PO_4] was lower at the termination of the experiment than at the beginning in the last experiment, showing *Lomentaria* sp. growth.

In OW, the NH_4:PO_4 ratio at 75:7.5 µmol L^{-1} 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* [44]. However, in ISW, NH_4:PO_4 enrichment showed no effect on the growth of *Lomentaria* sp., 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 *Lomentaria* sp. to OW and OW_NH_4 at 21-26°C.

Conclusions

This study identified the suitable environmental parameters to grow *Lomentaria* sp. under laboratory conditions as a temperature of 21-26°C, a salinity of 30-31% and a supplied NH_4 concentration of no greater than 100 µmol L^{-1}. In ISW, K^+ fortification is needed at 33-66% of [K^+] in OW at 30‰ for higher biomass gain in the culture period of no longer than 42 days.

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References

1. Huisman JM. Marine Plants of Australia. Western Australia: University of Western Australia Press. 2000.
2. Womersley HBS. The marine benthic flora of southern Australia: Rhodophyta. Part IIIB. Gracilarialse, Rhodymeniales, Corallinales and Bonnemaisoniales (Vol. 5). Canberra & Adelaide: Australian Biological Resources Study & the State Herbarium of South Australia. 1996.
3. Nulsen B. Inland Saline Water in Australia. Paper presented at the Inland Aquaculture Workshop, Perth, ACIAR Proceedings. 1997;83:6-11.
4. Partridge GJ. Inland Saline Aquaculture: Overcoming Biological and Technical Constraints Towards the Development of an Industry. (PhD), Murdoch, Perth. 2008.
5. Timms BV. Salt lakes in Australia: Present problems and prognosis for the future. Hydrobiologia. 2005;552(1):1-15.
6. Allan GL, Heasman H, Bennison, S. Development of industrial-scale inland saline aquaculture: Coordination and communication of R&D in Australia. Fisheries final report series No. 100. Retrieved from New South Wales, NSW Department of Primary Industries and the Fisheries Research & Development Corporation. 2008.

7. Talling JF. Potassium- A Non-Limiting Nutrient in Fresh Waters? Freshwater Reviews. 2010;3(2):97-104.
8. Evans HJ, Sorger GJ. Role of mineral elements with emphasis on the univalent cations. Annual Review of Plant Physiology. 1966;17(1):47-76.
9. Kirst GO. Ion composition of unicellular marine and fresh-water algae, with special reference to Platymonas subcordiformis cultivated in media with different osmotic strengths. Oecologia. 1977;28(2):177-89.
10. Rupérez P. Mineral content of edible marine seaweeds. Food Chemistry. 2002;79(1):23-26.
11. Checchetto V, Teardo E, Carraretto L, et al. Regulation of photosynthesis by ion channels in cyanobacteria and higher plants. Biophysical Chemistry. 2013;182(0):51-57.
12. Yarish C, Edwards P, Casey S. The effects of salinity, and calcium and potassium variations on the growth of two estuarine red algae. Journal of Experimental Marine Biology and Ecology. 1980;47(3):235-49.
13. Dinh HQ, Fotedar R. Early development of the blue mussel Mytilus edulis (Linnaeus, 1758) cultured in potassium-fortified inland saline water. Aquaculture. 2016;452:373-79.
14. Fielder DS, Allan GL. Improving fingerling production and evaluating inland Saline Water Culture of Snapper, Pagrus auratus. NSW Fisheries Final Report Series No. 43. CRC Project No. C4.2. Retrieved from Nelson Bay, NSW Fisheries Final Report Series No. 43: 2003.
15. Mourad N, Kreydiyyeh S, Ghanawi J, et al. Aquaculture of marine fish in inland low salinity well water: potassium is not the only limiting element. Fisheries and Aquaculture Journal. 2012:42:12.
16. Tantulo U, Fotedar R. Comparison of growth, osmoregulatory capacity, ionic regulation and organosomatic indices of black tiger prawn (Penaeus monodon Fabricius, 1798) juveniles reared in potassium fortified inland saline water and ocean water at different salinities. Aquaculture. 2006;258(1-4):594-605.
17. Dinh HQ. Cultural biology of the blue mussel, Mytilus edulis (Linnaeus, 1758) in inland saline water in Western Australia. (PhD), Curtin University Perth. 2016.
18. Prangnell DI, Fotedar R. The effect of potassium concentration in inland saline water on the growth and survival of the western king shrimp, Penaeus latisulcatus Kishinouye, 1896. Journal of Applied Aquaculture. 2005;17(2):19-34.
19. Tantulo U, Fotedar R. Osmo and ionic regulation of black tiger prawn (Penaeus monodon Fabricius 1798) juveniles exposed to K+ deficient inland saline water at different salinities. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 2007;146(2):208-14.
20. Burgess RM, Pelletier MC, Ho KT, et al. Removal of ammonia toxicity in marine sediment TIEs: a comparison of Ulva lactuca, zeolite and aeration methods. Marine Pollution Bulletin. 2003;46(5):607-18.
21. Campbell S. Ammonium requirements of fast-growing ephemeral macroalgae in a nutrient-enriched marine embayment (Port Phillip Bay, Australia). Marine Ecology Progress Series. 2001;209:99-107.
22. Kim JK, Kraemer GP, Neefus CD, et al. Effects of temperature and ammonium on growth, pigment production and nitrogen uptake by four species of Porphyra (Bangiales, Rhodophyta) native to the New England coast. Journal of Applied Phycology. 2007;19(5):431.
23. Ramus J, Venable M. Temporal ammonium patchiness and growth in Codium and Ulva (Ulvophaceae). Journal of Phycology. 1987;23(4):518-23.
24. Schaffelke B, Klumpp DW. Nutrient-limited growth of the coral reef macroalga Sargassum baccularia and experimental growth enhancement by nutrient addition in continuous flow culture. Marine Ecology Progress Series. 1998;164:199-211.
25. Robards K, McKelvie ID, Benson RL. Determination of carbon, phosphorus, nitrogen and silicon species in waters. Analytica Chimica Acta. 1994;287(3):147-190.
26. Atkinson MJ, Smith SV. C:N:P Ratios of Benthic Marine Plants. Limnology and Oceanography. 1983;28(3):568-74.
27. Uchida T. The life cylcle of Sargassum horneri (Phaeophyta) in laboratory culture Journal of Phycology. 1993;29(2):231-35.
28. Taukulis FE, John J. Development of a diatom-based transfer function for lakes and streams severely impacted by secondary salinity in the south-west region of Western Australia. Hydrobiologia. 2009;626(1):129-43.
29. Bird N, Chen L, McLachlan J. (1978). Effects of temperature, light and salinity on growth in culture of Chondrus crispus, Furcellaria lumbricalis, Gracilaria tikvahiae (Gigartinales, Rhodophyta), and Fucus serratus (Fucales, Phaeophyta). Botanica Marina. 1978;22(8):521-27.
30. Ding L, Ma Y, Huang B, et al. Effects of seawater salinity and temperature on growth and pigment contents in Hypnea cervicornis J. Agardh (Gigartinales, Rhodophyta). BioMed Research International. 2013;10.
31. Dawes CJ, Orduña-rojas J, Robledo D. Response of the tropical red seaweed Gracilaria cornea to temperature, salinity and irradiance. Journal of Applied Phycology. 1998;10(5):419.
32. De Faveri C, Schmidt EC, Simioni C, et al. Effects of eutrophic seawater and temperature on the physiology and morphology of Hypnea musciformis J. V. Lamouroux (Gigartinales, Rhodophyta). Ecotoxicology. 2015;24(5):1040-52.
33. Ahmad SH, Surif M, Omar WMW, et al. Nutrient uptake, growth and chlorophyll content of green seaweed, Ulva reticulata: Response to different source of inorganic nutrients. Empowering Science, Technology and Innovation Towards a Better Tomorrow, UMTAS. 2011;542-48.

34. Coutinho R, Zingmark R. Interactions of light and nitrogen on photosynthesis and growth of the marine macroalga Ulva curvata (Kützing) De Toni. Journal of Experimental Marine Biology and Ecology. 1993;167(1):11-19.

35. Cruz-Suárez LE, León A, Peña-Rodríguez A, et al. Shrimp/Ulva co-culture: A sustainable alternative to diminish the need for artificial feed and improve shrimp quality. Aquaculture. 2010;301(1-4):64-68.

36. Pérez-Mayorga DM, Ladah LB, Zertuche-González JA, et al. Nitrogen uptake and growth by the opportunistic macroalga Ulva lactuca (Linnaeus) during the internal tide. Journal of Experimental Marine Biology and Ecology. 2011;406(1-2):108-15.

37. Perini V, Bracken MES. Nitrogen availability limits phosphorus uptake in an intertidal macroalga. Oecologia. 2014;175(2):667-76.

38. Reef R, Pandolfi JM, Lovelock CE. The effect of nutrient enrichment on the growth, nucleic acid concentrations, and elemental stoichiometry of coral reef macroalgae. Ecology and Evolution. 2012;2(8):1985-95.

39. Blumwald E, Aharon GS, Apse MP. Sodium transport in plant cells. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2000;1465(1-2):140-51.

40. Tromballa HW. Influence of permeant acids and bases on net potassium uptake by Chlorella. Planta. 1978;138(3):243-48.

41. Malhotra B, Glass ADM. Potassium Fluxes in Chlamydomonas reinhardtii: (I. Kinetics and Electrical Potentials). Plant Physiology. 1995;108(4):1527-36.

42. Prangnell DI, Fotedar R. The growth and survival of western king prawns, Penaeus latisulcatus Kishinouye, in potassium-fortified inland saline water. Aquaculture. 2006;259(1-4):234-42.

43. Yamashita M, Tomita-Yokotani K, Hashimoto H, et al. Sodium and potassium uptake of Ulva – Application of marine macro-algae for space agriculture. Advances in Space Research. 2009;43(8):1220-23.

44. Liu D, Amy P, Sun J. Preliminary study on the responses of three marine algae, Ulva pertusa (Chlorophyta), Gelidium amansii (Rhodophyta) and Sargassum enerve (Phaeophyta), to nitrogen source and its availability. Journal of Ocean University of China. 2004;3(1):75-79.

45. Malta E-J, Draisma S, Kamermans P. Free-floating Ulva in the southwest Netherlands: species or morphotypes? a morphological, molecular and ecological comparison. European Journal of Phycology. 1999;34(5):443-54.

46. Van Khoi L, Fotedar R. Integration of western king prawn (Penaeus latisulcatus Kishinouye, 1896) and green seaweed (Ulva lactuca Linnaeus, 1753) in a closed recirculating aquaculture system. Aquaculture. 2011;322-323:201-09.

47. Liu J, Dong S. Comparative studies on utilizing nitrogen capacity between two macroalgae Gracilaria tenuistipitata var. liui (Rhodophyta) and Ulva pertusa (Chlorophyta). I. Nitrogen storage under nitrogen enrichment and starvation. Journal of Environment Science (China). 2001;13:318-22.

48. Lüning K. Temperature tolerance and biogeography of seaweeds: The marine algal flora of Helgoland (North Sea) as an example. Helgoländer Meeresuntersuchungen. 1984;38(2):305-17.

49. Downing J. Marine nitrogen: Phosphorus stoichiometry and the global N:P cycle. Biogeochemistry. 1997;37(3):237-52.

50. Smith SV, Kimmerer WJ, Walsh TW. Vertical flux and biogeochemical turnover regulate nutrient limitation of net organic production in the North Pacific Gyre. Limnology and Oceanography. 1986;31(1), 161-67.

51. Wallentinus I. Comparisons of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. Marine Biology. 1984;80(2):215-25.

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