

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 $\mu\text{mol L}^{-1}$ $NH_4:PO_4$ 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 $\mu\text{mol L}^{-1}$ 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. monochlamyptea* [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 Harbour (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 NH₄ enriched water, (3) the effects of temperature and NH₄ on the growth of *Lomentaria* sp., and (4) the effects of NH₄ and PO₄ 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	8,9.2 ± 0.26	8,8.72 ± 0.02	8,8.79 ± 0.08	8,8.67 ± 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_NH₄, ISW_NH₄, ISW66_NH₄ (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_NH₄. 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_NH₄ and ISW66_NH₄ (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 $\text{NH}_4\text{:PO}_4$ 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 $\text{NH}_4\text{:PO}_4$; (3) T3 - 150:15 μmol L⁻¹ $\text{NH}_4\text{:PO}_4$; and (4) T4 - 300:30 μmol L⁻¹ $\text{NH}_4\text{:PO}_4$.

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: $\mu_a = (\ln A_t - \ln A_0) \times 100/t$. 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 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

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. NH₄ was negligible as the experiment progressed, whereas NO₂ decreased and NO₃ increased in all waters toward the end of the experiment. There was no significant difference of [NO₃] 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 [NO₃] than other waters (Table 7). However, NO₃ showed no significant correlation with the biomass of *Lomentaria*, but NO₂ did.

PO₄ 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.

NH₄ 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_NH₄ and ISW66_NH₄ resulted in significantly higher biomass and SGR_w of *Lomentaria* sp. than ISW and ISW66, respectively (Table 8). NH₄ presented the highest effectiveness when used in ISW66_NH₄; this resulted in higher biomass and SGR_w of *Lomentaria* sp. by the end of the experiment than OW_NH₄ and ISW_NH₄. In the waters not enriched with NH₄, 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	₄ 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	₂ 0.8 ± 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 NH₄ 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_NH₄ 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_NH₄ 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_NH₄. 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 [NO₂] and [PO₄] 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 NH₄:PO₄ levels. In OW, T2 resulted in the highest biomass and SGR, and the dried content of *Lomentaria sp.* cultured in T4 was lowest.

Although NH₄ was provided weekly, NH₄ in water was negligible during the experiment. By the beginning of the experiment, [NO₃] in ISW was higher than in OW, and in both waters, [NO₃] and [PO₄] were significantly increased in higher nutrient enrichment levels. However, by day 25, [NO₃] in ISW was only higher than OW at T3, and lower at T4. [NO₃] and [PO₄] were similar in OW. [NO₂] 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 sea 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	₃ 3.20 ± 0.13	₃ 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.

Criteria	OW				ISW66			
	T1	T2	T3	T4	T1	T2	T3	T4
Biomass								
Day 1	3.37 ± 0.01	₃ 3.38 ± 0.02	3.40 ± 0.01	₃ 3.40 ± 0.02	₃ 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 $80 \mu\text{mol L}^{-1} \text{NH}_4$ than at $200 \mu\text{mol L}^{-1}$ [44]. However, in this study, the *Lomentaria* sp. showed no response in $100 \mu\text{mol L}^{-1} \text{NH}_4$ 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 $18\text{-}19^\circ\text{C}$ 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 $21\text{-}22^\circ\text{C}$ and $25\text{-}26^\circ\text{C}$ 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 $21\text{-}26^\circ\text{C}$. 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 $21\text{-}26^\circ\text{C}$ in the temperature-effect experiment and K^+ -fortification effect experiment at $18.5\text{-}21^\circ\text{C}$ 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 $21\text{-}22^\circ\text{C}$, 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 $[\text{PO}_4]$ was lower at the termination of the experiment than at the beginning in the last experiment, showing *Lomentaria* sp. growth.

In OW, the $\text{NH}_4\text{:PO}_4$ ratio at $75\text{:}7.5 \mu\text{mol L}^{-1}$ 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, $\text{NH}_4\text{:PO}_4$ 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 $21\text{-}26^\circ\text{C}$.

Conclusions

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

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