American Journal of Applied Sciences

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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Article history
Received: 08-12-2018
Revised: 02-02-2018
Accepted: 05-04-2018

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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

(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 growing-seasonal requirements under 28°C of the *S. linearifolium* (Martin-Smith, 1993).

At the same salinity, the ISW ionic profile in Australia can be similar to the Ocean Water (OW) (Fielder *et al.*, 2001; Prangnell and Fotedar, 2006a), but the potassium concentration ($[K^+]$) is much lower (Ingram *et al.*, 2002; Boyd *et al.*, 2007) and varies (Nurmi *et al.*, 1988; Nulsen, 1997). It is not feasible for marine shrimp, fish and mollucs to survive and grow without K^+ fortification, similar to K^+ levels to OW (Fielder and Allan, 2003; Doroudi *et al.*, 2006; Prangnell and Fotedar, 2006b; Dinh, 2016). The *S. linearifolium* also needs K^+-fortified at similar K^+ concentration in OW to sustain its growth in ISW (Bui *et al.*, 2017b). 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°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 (Nurmi *et al*., 1988; Nulsen, 1997; Taukulis and John, 2009). The pH varies from 3.9 to 9.7 in the wheat belt of WA (Nulsen, 1997; Taukulis and John, 2006), or 7.4 at 35 ppt in Broome (Lee, 1997; Taukulis and 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 5–40 ppt (Mazor and George, 1992), which is 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°C with an average of 17.7°C (Taukulis and 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 suitable pH for *S. honeri* zygote germination is 5–10 (Ogawa, 1984). Similarly, the temperature is a vital factor affecting *Sargassum* growth (Uchida, 1993). The optimal growth temperature for *S. muticum* is at 25°C (Hales and Fletcher, 1989), while *S. patents* prefers 20–30°C (Endo *et al.*, 2013).

ISW in WA is characterized by high changes in pH and temperature by location and seasons. In a contribution to the use of ISW for aquaculture to reduce the adverse impact of salinization (Kolkovski, 2010), an attempt to grow the *Sargassum* in K^+-fortified ISW (K^+ISW) has been investigated by investigating what temperature and pH are conducive to grow these two *Sargassum* species in ISW. Therefore, 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.

Materials and Methods

Preparation of Sargassum Species

Sargassum linearifolium and *S. podacanthum* were hand-picked from Point Peron, WA (latitude 32° 16.3'S, longitude 115° 41.2'E) and then transported for 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 h 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) wiping 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 145 g $fond^{-1}$, dried by paper towel, weighed (Model GX-4000, A&D Company Limited, Tokyo, Japan) and then placed into tanks to get stocking densities of and then placed into tanks to get stocking densities of 0.8 kg 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.

Preparation of Inland Saline Water

The ISW at a salinity of 45 ppt was procured from a lake in Wannamal, WA (31°15S, 116°05E) 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 0.5 µm 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 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 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.

Table 1: Eight treatments of the experiment testing the effect of two pH and two temperature levels on the growth of *Sargassum linearifolium* and *S. podacanthum* in K^+-fortified inland saline water

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)

Experimental Setup

The experiment was 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 *et al*., 2008), two water temperatures (ambient room temperature 21-22°C and higher at 26–27°C, which is the upper temperature level of ISW in WA (Taukulis and John, 2006) and two species of *Sargassum* (*S. linearifolium* and *S. podacanthum*) (Table 1). These eight treatments were randomly triplicated. The tanks were aerated by two air stones 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 h light:dark cycle (Hanisak and 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°C.

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 analyse N was applied to measure Total Kjeldahl Nitrogen (TKN) using a Kjeltec Auto 1030 analyzer (Foss Tecator, Hoganas, 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* 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_o 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 weighed and dried at 60°C for 72 h to get stable dried weights. They were then ground 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°C for 30 min.

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.

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. 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 Analysis of Covariance (ANCOVA) 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.

Results

Biomass of the Sargassum Species

At the commencement of the experiment, the fresh biomass (approximately 145 g tank^{-1}) of the *Sargassum* 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 interaction in the three-way interaction among species, pH and temperature on *Sargassum* SGR $F_{(2,24)} = 0.43$ at the first 14 days ($p>0.05$). 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 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.

Compositions of the Sargassum

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 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 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*.

Table 2: SGR, dried weight, ash and protein content of the *Sargassum* spp. cultured in K^+-fortified inland saline water at two levels of pH and two levels of temperature

	S. linearifolium				*S. podacanthum*			
	21-22°C		26-27°C		21-22°C		26-27°C	
Criteria	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^{b}	0.37±0.44^{a}	-3.52±0.000^{b}	-1.51±0.49^{ab}
Day 14-28	-0.39±0.59	$_{1,}$2-0.90±1.08	$_{2}$-4.94±0.38	-4.08±0.37	-9.23±6.39^{b}	0.38±1.35^{a}		-3.21±2.55^{ab}
Day 28-42	-0.22±0.23	$_{2}$-5.27±3.44			-4.64±0.86	-2.04±1.16		
Dried weight (%)								
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	12.98±0.19	12.98±0.19	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	11.41±0.72	11.98±0.00	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	11.20±1.36		12.89±2.03
Day 42	14.42±0.47	$_{1,2}$12.75±0.80			$_{2}$13.27±0.96	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^{b}	$_{2}$38.11±0.83^{a}		
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,}$29.98±0.16	$_{2}$10.45±0.28^{a}	$_{2}$9.76±0.16a	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 3: The chemical compositions of the *Sargassum* spp. cultured in K^+-fortified inland saline water at two levels of pH and two levels of temperature by day 1 and day 28 of the experiment

		S. linearifolium					*S. podacanthum*			
			Day 28					Day 28		
			21-22°C		26-27°C			21-22°C		26-27°C
Parameters	Unit	Day 1	pH 8	pH 5.5–6.5	pH 8	pH 5.5–6.5	Day 1	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^+-fortified inland saline water at 26-27°C, water pH of 8 at day 28 providing no samples for analysis

The chemical composition of the *Sargassum* is presented in Table 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 seaweed at day 28 than the commencement of the trial.

Water Quality Parameters

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 4).

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.

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 seaweeds (Table 5). 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 6). The temperature presented no effect on these water quality parameters.

The ionic composition of water is provided in Table 7, of which, after a month of cultivating *Sargassum*, the sodium ions were different from the commencement, while the $[K^+]$ remained unchanged over the cultured period 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.

At high temperatures, the DO gradually increased from early afternoon to noon of the next day; whereas, at low temperature, the DO reduced by 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.

Table 4: Quality parameters (mg L^{-1}) of the K^+-fortified inland saline water cultured *Sargassum* spp. at two levels of pH and two levels of temperature as the experiment progressed

	S. linearifolium				*S. podacanthum*			
	21–22°C		26–27°C		21-22°C		26-27°C	
Para-meters	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^b	$_1$0.150±0.021^a	$_1$0.020±0.002^b	$_{12}$0.150±0.021^a	$_1$0.020±0.002^b	$_1$0.150±0.021^a	$_1$0.020±0.002^b	$_1$0.150±0.021^a
Day 14	$_2$0.005±0.002^b	$_1$0.018±0.005^{ab}	$_1$0.026±0.007^{ab}	$_1$0.055±0.015^a	$_1$0.016±0.003^b	$_1$0.013±0.004^b	$_2$0.081±0.026^a	$_1$0.115±0.025^a
Day 28	$_2$0.002±0.000^c	$_1$0.317±0.030^a	$_2$0.102±0.033^{bc}	$_2$0.166±0.073^b	$_1$0.015±0.003^c	$_1$0.184±0.098^b	$_3$0.375±0.000^a	$_2$0.375±0.000^a
Day 42	$_3$0.013±0.003^b	$_2$5.333±0.667^a			$_2$0.008±0.002^b	$_2$5.333±0.667^a		
NO_3^--N								
Day 1	$_1$2.53±0.12^b	27.67±0.44^a	$_1$2.53±0.12^b	27.67±0.44^a	$_1$2.53±0.12^b	$_1$27.67±0.44^a	$_1$2.53±0.12^b	27.67±0.44^a
Day 14	$_2$1.03±0.24^b	27.87±0.37^a	$_2$1.07±0.09^b	33.13±0.95^a	$_2$1.43±0.12^b	$_1$225.37±1.23^a	$_1$2.40±0.38^b	19.47±8.00^a
Day 28	$_3$0.27±0.18^c	28.53±2.47^a	$_1$2.40±0.15^b	25.17±1.13^a	$_2$1.07±0.12^c	$_2$23.47±1.05^a	$_2$3.47±0.55^b	25.67±0.35^a
Day 42	$_1$2.30±0.25^b	25.40±0.42^a			$_1$2.53±0.18^b	$_2$22.27±1.22^a		
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	$_1$20.02±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^b	$_2$0.11±0.03^a	$_2$0.08±0.00^{ac}	$_2$0.04±0.02^{bc}
Day 42	$_{12}$0.01±0.01^a	0.00±0.00^b			0.00±0.00^b	$_3$0.05±0.03^a		
TKN								
Day 1	1.14±0.16^b	$_1$10.48±0.06^a	$_1$1.14±0.16^b	$_1$10.48±0.06^a	1.14±0.16^b	$_1$10.48±0.06^a	$_1$1.14±0.16b	$_1$10.48±0.06^a
Day 14	1.40±0.41^b	$_1$9.85±0.12^a	$_{12}$1.42±0.21^b	$_1$8.68±1.28^a	1.66±0.17^b	$_1$9.97±0.71$_a$	$_{12}$1.73±0.18b	$_1$9.34±1.03^a
Day 28	1.61±0.35^b	$_2$2.17±0.11^{ab}	$_2$1.77±0.20^b	$_2$3.67±1.22^a	1.56±0.45	$_2$1.70±0.17	$_2$2.45	$_2$2.10±0.18
Day 42	1.77±0.19	$_2$2.19±0.06			1.91±0.05	$_2$1.77±0.25		
PO_4^{3-}-P								
Day 1	10.93±0.09	$_1$1.13±0.03	0.93±0.09	1.13±0.03	$_1$0.93±0.09	1.13±0.03	$_1$0.93±0.09	1.13±0.03
Day 14	$_2$0.30±0.06^b	$_1$0.67±0.12^b	0.60±0.26^{ab}	3.17±2.45^a	$_2$0.37±0.03	0.83±0.30	$_2$1.57±0.29	0.87±0.18
Day 28	$_1$1.00±0.06^{bc}	$_2$2.50±0.52^a	0.93±0.20^c	1.60±0.29^b	$_3$1.30±0.06	1.00±0.00	$_{12}$1.13±0.07	1.57±0.03
Day 42	$_1$0.93±0.19	$_1$0.73±0.03			$_1$0.83±0.07	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 5: Pearson correlation of SGR (% d^{-1}) of the *Sargassum* spp. cultured in K^+-fortified inland saline water at two levels of pH and two levels of 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	54.000	54.000	54.000	53.000	55.000

Table 6: The effect of nitrogen on the SGR of the *Sargassum* spp. cultured in K^+-fortified inland saline water between the two pH levels and among the eight treatments

Group	Source	Type III sum of squares	df	Mean square	F	Significant	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

Table 7: Ionic profile (mg L^{-1}) of the K^+-fortified inland saline water cultured *Sargassum* spp. at two levels of pH and two levels of temperature by day 1 and day 28 of the experiment

		Day 28							
		S. lineariforlium				*S. podacanthum*			
		21–22°C		26–27°C		21–22°C		26–27°C	
Parameters	Day 1	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	0.66	0.76	0.72	0.86	0.86	0.77	0.67	0.81	0.84
Ca	583.00	554.00	520.00	576.00	606.00	570.00	474.00	536.00	618.00
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	1525.00	1384.00	1304.00	1526.00	1540.00	1492.00	1229.00	1464.00	1680.00
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	351.50	351.00	361.00	369.00	366.00	359.00	347.00	364.00	359.00
Na	8719.00	7886.00	7282.00	8591.00	8838.00	7251.00	7141.00	8308.00	9452.00
S	602.4.0	763.00	716.00	824.00	818.00	765.00	791.00	780.00	816.00
Zn	<0.05	<0.05	0.12	<0.05	<0.05	<0.05	<0.05	<0.05	0.24

Discussion

Temperature and pH strongly influence the growth of *Sargassum* (Choi *et al.*, 2007; Chen and Zou, 2014; Hwang *et al*., 2015). The *Sargassum* growth rate is strongly affected by the variation of temperature (Uchida, 1993; Endo *et al*., 2013) and the effect of temperature within the tested range was stronger than the pH, shown by the significant 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) and respiration rate (Davison *et al.*, 1991), ammonium and nitrogen uptake rate (Duke *et al.*, 1989; Hwang *et al.*, 2004). The range of studied temperature was within a preferred range of 20–30°C 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 22–24°C and reaches maximum wet weight and length in August to November when the temperature ranges from 24–28°C and ceases in summer when temperature reaches over 29°C (Martin-Smith, 1993). The temperature window in this experiment at 20–22°C, given the higher growth rate of *Sargassum* than the higher temperature of 26–27°C, is similar to the natural maximal growth rate condition. Both *Sargassum* species could not be sustained after a month at a high temperature of 26–27°C in K^+ISW. Similarly, the growth of young seedlings *S. henslowianum* reduced when temperature increased to 30°C (Chen and Zou, 2014). The SGR of the *Sargassum* in this trial, were at adult stages, at 20–22°C is higher than the adult stage of *S. muticum* (Yamauchi, 1984) but is much lower than the juvenile *S. horneri* (Choi *et al*., 2007) and juvenile *S. muticum* (Hales and Fletcher, 1989) at 15°C, presented the lower SGR of adults thalli and juvenile, which is similar to *S. horneri* (Lee *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 20°C (Taukulis and 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 20–22°C was 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 was lower than *S. naozhouense* (11.2%) (Peng *et al.*, 2013). Although the pH and temperature did 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* increased significantly as the trial progressed. This indicates the protein of *Sargassum* under the laboratory conditions was 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; Boyd and Thunjai, 2003) although the ionic profile of ISW can be similar to OW at the same salinity (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 *Lomentaria* sp., respectively (Bui *et al.*, 2017a; 2017b). 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 could 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 was 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 and Miyachi, 1986; Drechsler and 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 *et al*., 2017b), 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 *et al*., 2017b).

This study reveals that the suitable pH for long-term growth of *Sargassum* in ISW was the ambient pH of 7.0–8.0. This pH range is similar to the red seaweed *Gracilaria tikvahiae*, *G. secundata* and *G. manilaensis* needs for high production and maximum growth rate (Skirrow, 1975; Lignell and Pedesén, 1989; Hidayat *et al*., 2015). 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 did 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 and Murthy, 2007) which peaks at pH 4.5 (Figueira *et al.*, 1997; Davis *et al*., 2000). The *Sargassum* accumulated Fe and Zn, particularly at low pH, but released the Cu to the environment when Cu in water is lower than 0.05 mg L^{-1}, which is a possible explanation for lower Cu 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 was too weak. Therefore, instead of HCl and acetic acid, 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 had neither influenced SGR of *Sargassum* spp. nor it influenced the significant level pH and temperature on the SGR of *Sargassum* spp. Both the Pearson correltation 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 is 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 and 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 and Hurd, 2001) and the surplus P was stored in *Sargassum* tissue.

Conclusion

The *Sargassum linearifolium* and *S. podacanthum* grow faster in K^+ISW 35 ppt at pH 7–8.2 and temperature 20–22°C 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°C in K^+ISW up to 28 days. A further study about the higher than the ambient pH 8 and temperature of 15°C of K^+ISW effects on the growth feasibility of *Sargassum* spp. is recommended.

Acknowledgement

We thank Dr. Thomas Kalb, Mr. Chau Do, Dr. Van Thang Nguyen, Proof-reading-service.com Ltd for proofreading this paper.

Funding Information

This work was funded by the Vietnamese Ministry of Education and Curtin International Postgraduate Research Scholarship and conducted at Curtin University, Australia.

Author's Contributions

Ha Thi Thu Bui: Correspondent author, as a part of the PhD thesis, who was responsible for setting up and running the experiment, collecting and analysing data, writing the manuscript.

Trong Quoc Luu: Helped with seaweeds collection, experimental setup and data collection.

Ravi Fotedar: Supervised the research, edit and approved the manuscript.

Ethics

This article is original material. The corresponding author confirms that all authors have read and approved the manuscript. No ethical issue that may arise after the publication of this manuscript.

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