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Effects of Enriching Nitrogen and Phosphorus on the Growth of *Sargassum Podacanthum* Cultured in Potassium-Fortified Inland Saline Water

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Abstract: Potassium-fortified inland saline water (K^+ISW) has shown potential for growing marine species, including seaweed species. The response of a brown seaweed species, *Sargassum podacanthum,* to nitrogen and phosphorus enrichments were evaluated by culturing the species for 84 days in K^+ISW and comparing it with Ocean Water (OW). The culture media were enriched weekly with ammonium chloride and sodium dihydrogen phosphate, with ammonium and phosphate ratios of 10:1 at five different concentrations 80:8, 120:12, 160:16, 200:20 and 240:24 µM. The culture medium with no enrichment was used as a control. The water quality and biomass of *S. podacanthum* were measured fortnightly. The *S. podacanthum* biomass increase significantly with different concentrations of the nutrient supplementations. The standing biomass and Specific Growth Rate (SGR) of *S. podacanthum* were similar in OW and K^+ISW in the absence of any nutrient supplementation and at the supplement concentration of ammonium and phosphate 160:16 µM. However, from day 42 onwards, at the ratios of 80:8, 120:12, 200:20 and 240:24, *S. podacanthum* cultured in OW grew significantly faster than in K^+ISW. In K^+ISW, optimal growth of *S. podacanthum* was observed at the 160:16 and the increase in biomass was significantly higher than the initial biomass until day 70, whereas at the other four nutrient supplement concentrations, the *S. podacanthum* biomass remained unchanged during the entire culture period. The nitrite, total Kjeldahl nitrogen and phosphate concentrations in water were found to be significantly ($p<0.05$) and negatively correlated ($p<0.05$) with *S. podacanthum* biomass. Therefore, the results showed that the enrichment of 160 µM ammonium and 16 µM phosphate is required in the K^+ISW for *S. podacanthum* to achieve optimal growth.

Keywords: Inland Saline Water, *Sargassum podacanthum,* Nutrient Enrichment, Potassium Fortification, Ammonium, Phosphate

Introduction

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

At the same salinity, the level of potassium (K^+) concentration in ISW is lower than in Ocean Water (OW) in Australia (Allan and Fielder, 1997; Dinh, 2016) and USA (Boyd and Thunjai, 2003; Forsberg *et al.*, 1996) although other ionic profiles can be similar (Fotedar *et al.*, 2011; Prangnell and Fotedar, 2006a). Potassium is vital

for aquaculture and K^+ deficiency in ISW can negatively affect the growth of the aquatic animals (Mourad *et al.*, 2012). For example, the survival of juvenile mulloway (*Argyrosomus hololeptidotus*) (Doroudi *et al.*, 2006), juvenile snapper (*Pagrus auratus*) (Fielder *et al.*, 2001) and red drum (*Sciaenops ocellatus*) (Forsberg *et al*., 1996) is adversely affected when cultured in low K^+ environment. Therefore, fotifying ISW with K^+ to achieve similar concentration in OW is essential to sustain the growth of shrimp (Prangnell and Fotedar, 2006b; Tantulo and Fotedar, 2006), fish (Fielder and Allan, 2003), red seaweed *Lomentaria* sp. (Bui *et al.*, 2017a) and brown seaweed *Sargassum linearifolium* (Bui *et al.*, 2017b).

Sargassum spp. commonly used as a source of fertilizers and soil conditioners (Huisman, 2000), are the dominant taxa in near shore reef areas along Perth beaches (Womersley, 1996). *Sargassum* spp. are prevalent sources of compounds used in pharmaceutical (Hur *et al.*, 2008) and agriculture industries (Ara *et al.*, 1997). The extracts from *Sargassum* can be used as in the treatment of neurological disorders (Natarajan *et al.*, 2009), dementia (Pangestuti and Kim, 2010) and HIV (Thuy *et al.*, 2015). The fact that *Sargassum* is active in antioxidant activity, cholinesterase inhibition activity, neuroprotective activity, anti-cancer and cytotoxic activity has made it a popular ingredient in health enhancement products (Yende *et al.*, 2014). *S. fusiforme* has been cultivated in Korea and Japan as a food source (Bast, 2014). *Sargassum* has many branches growing from a short stipe (Huisman, 2000). The length of *Sargassum*'s thallus is about 0.1–2 m, while its stipes are 1–20 cm long from a discoid-conical holdfast (Womersley, 1987). Of the *Sargassum* species, *S. podacanthum* is distributed from Point Peron (Western Australia (WA) to Port Noarlunga (South Australia) (Womersley, 1987) and thus can be an important candidate for growing in local ISW. The branches of *S. podacanthum* are typically terete, but more angular at the top, usually with short, scatter spines, which branch out radically (Womersley, 1987). *S. podacanthum* is thalli monoecious with bisexual receptacles, simple or branched and its conceptacles are unisexual (Womersley, 1987). Though nutrient uptake and nutrient enrichment of various species of seaweeds, including some *Sargassum* spp. have been extensively studied (Coutinho and Zingmark, 1993; Pérez-Mayorga *et al.*, 2011; Perini and Bracken, 2014, Reef *et al.*, 2012, Schaffelke and Klumpp, 1998), there is no information available on the impacts of ammonium (NH_4-N) and phosphate (PO_4^{3-}-P) supplementation on *S. podacanthum* productivity, particularly when cultured in K^+-fortified ISW (K^+ISW).

Nutrients, such as nitrogen (N) and phosphorus (P), are limiting factors for photosynthesis of seaweeds (Larned, 1998). N is a limiting nutrient in the growth of *Sargassum* spp. cultured in Hawaii (Larned, 1998) and Taiwan (Hwang *et al.*, 2004), P is also considered a limiting factor for *S. natans* and *S. fluitans* growth in the western North Atlantic (Lapointe, 1986). As nutrient limitation on the growth of seaweed is species dependent (Larned, 1998), the majority of seaweed species grow faster in ammonium-enriched than in phosphate-enriched media (Larned, 1998). Supplying (NH_4-N) is more efficient than nitrate (NO_3^--N) for seaweed growth (Atkinson and Smith, 1983). Thus, the combined NH_4-N and phosphate (PO_4^{3-}-P) has a stronger effect on the growth of *S. baccularia* than a single nutrient (Schaffelke and Klumpp, 1998). However, the information on the impacts of nutrient supplementation during *S. podacanthum* culture, in K^+ISW, is lacking. The present study aims to examine the effects of different N and P concentrations, through the supplementation of NH_4-N and PO_4^{3-}-P, on the growth of *S. podacanthum* in K^+ISW of WA under the laboratory conditions.

Materials and Methods

Sargassum podacanthum Collection

The entire thallus length of *S. podacanthum,* identified by WA Herbarium, were hand-picked from Point Peron, WA (latitude 32° 16.3S, longitude 115° 41.2E) and immediately transported in OW containers to Curtin Aquatic Research Laboratory (CARL). The *S. podacanthum* was acclimated in aerated OW at 22°C in three 114 L aquaria, under a down-welling photo-lux density of 120 µmol photon m^{-2} s^{-1} and 14:10 h light: dark cycle (Hanisak and Samuel, 1987). The thalli were washed to remove all epiphytes and then rested one more day in aerated OW. The middle of the thalli at the same developmental stage were cut to pieces of approximately 3,500 mg each and then immediately transferred into the cultured media.

Experimental Setup

The 45 ppt ISW was procured from a lake in Wannamal, WA (31°15S, 116°05E) and brought to CARL and kept in 10,000L reservoir. The ISW and fresh water were filtered through a 0.5 µm glass fibre membrane and then mixed to achieve 35 ppt water. At the salinity of 35 ppt, the [K^+] in OW and ISW was, respectively, 351.1 and 84.4 mg L^{-1}; therefore 508.5 mg L^{-1} of anhydrous potassium chloride was added to ISW to achieve the [K^+] in ISW equivalent to the [K^+] in OW. The OW at 35 ppt was sourced from Hillary Habour (31°83S, 115°74E) and stored at CARL and was also filtered through a 0.5 µm glass fibre membrane before using in the trial.

A total of fifty-two (52) 1.5 L-glass beakers filled with 1 L of water were used for the 84 day-trial. The

growth of the *S. podacanthum* was determined at five levels of nutrient concentrations in OW and K^+ISW. The nutrients were provided as molar NH_4-N:PO_4^{3-}-P equal to 10:1 by the weekly addition ammonium chloride (NH_4Cl) and sodium dihydrogen phosphate (NaH_2PO_4) mixtures (Campbell, 2001). Five different concentrations of NH_4-N:PO_4^{3-}-P were 80:8, 120:12, 160:16, 200:20 and 240:24 µM (Liu *et al.*, 2004). The needed amount of NH_4Cl and NaH_2PO_4 for NH_4-N:PO_4^{3-}-P 80:8, 120:12, 160:16, 200:20 and 240:24 µM were, respectively, 4.28 and 0.96, 6.42 and 1.44, 8.56 and 1.92, 10.70 and 2.40, 12.84 and 2.88 mg, weighed and stirred to dilute in a part of the cultured media which were taken out from the cultured beakers. The waters were then returned back to the beakers and diluted in the 1 L cultured medium by a small glass stick. Thirteen treatments were set up in four replicates (including five nutrient concentrations in OW (OW_80, OW_120, OW_160, OW_200, OW_240), five nutrient concentrations in K^+ISW (ISW_80, ISW_120, ISW_160, ISW_200, ISW_240) and the controls of ambient OW (OW_0), ambient K^+ISW (ISW_0) and a raw ISW at 35 ppt.

The salinity was maintained at 34-35 ppt by adding freshwater to compensate for any losses due to evaporation throughout the trial. All beakers were directly exposed under the white fluorescent lights of 90 µmol photon m^{-2} s^{-1} on a 14:10 h light:dark cycle and ambient room temperature of 20–26°C (Hanisak and Samuel, 1987).

Data Collection

Water quality parameters of the culture media and the fresh biomass of *S. podacanthum* were measured every 14 days and at the commencement and termination of the trial.

Nitrate (NO_3^--N) was measured by the Cadmium Reduction Method (Method 8171 for 0–5 mg L^{-1} and Method 8039 for higher concentrations). Nitrite (NO_2^--N) was measured by the Diazotization Method (Method 8507 for concentration less than 0.350 mg L^{-1}) and the Ferrous Sulfate Method (Method 8153 for concentration higher than 0.350 mg L^{-1}). Ammonium (NH_4-N) was measured by the Salicylate Method (Method 8155 for concentration less than 0.05 mg L^{-1}; Method 10023 for concentration higher than 0.05 mg L^{-1}). Phosphate (PO_4^{3-}-P) was measured by the Amino Acid Method (Method 8178 for 0–30 mg L^{-1}). These parameters were determined by using a Hach DR/890 handheld meter (Hach, Loveland, Colorado, USA). Total Kjeldahl Nitrogen (TKN) was measured according to the Official Method of the AOAC (Helrich, 1990) (method 937.48) by analysing nitrogen using Kjeltec Auto 1030 analyser (Foss Tecator, Höganäs, Sweden).

All *S. podacanthum* thalli were removed from the beakers by a small net and then dried by soft hand towels (Ahmad *et al*., 2011). The thalli were quickly transferred to a mechanical weighing scale (Model GX-4000, A&D Company Limited, Tokyo, Japan). After weighing, the thalli were immediately returned back to the cultured media. At the commencement, three pieces of *S. podacanthum* which were the same developmental stage and similar parts of the *S. podacanthum* thalli were used for culturing in the trial were weighed and placed on aluminium foil trays to dry in an air oven at 60°C for 72 h to get dried biomass (McDermid and Stuercke, 2003). The ratio of dried/fresh biomass were applied to calculate the dried biomass of the *S. podacanthum* throughout the trial. All the fresh *S. podacanthum* by the termination was weighed and dried similarly.

The cumulative specific growth rates (SGR) was calculated as: $\mu_a = (\ln A_t - \ln A_o) \times 100/t$. Where: μ_a was the SGR of *S. podacanthum* (% d^{-1}); A_t and A_o were the dried weights (mg) of the total *S. podacanthum* biomass at a current time (t, d) and the commencement of the trial (0, d); t was the current time of the trial (days).

Every day at 9–11 AM, the salinity was recorded by a portable refractometer (RHS-10ATC, Xiamen Ming Xin Instrument, Xiamen, Fujian, China) and the pH was recorded by a pH metre (CyberScan pH 300, Eutech Instrument, Singapore).

Data Analysis

All data were analysed using SPSS for Windows version 24.0. The Levene's test was used to test the homoscedasticity of variances of the samples. When the homoscedasticity of the variances of the samples was violated, the translog or square root transformation was undertaken before proceeding with the variance test. The significant differences at $p<0.05$ among the means of variables were compared by using Analysis of Variance (ANOVA), pair samples t-test and Least Significant Difference (LSD) post hoc tests. Correlation analysis was used to determine relationship among variables.

Results

Biomass of Sargassum podacanthum

Although the standing biomass of the *S. podacanthum* varied as the time progressed, different nutrient enrichments resulted in significant ($p<0.05$) differences in the growth of *S. podacanthum*. All *S. podacanthum* in ambient ISW (control) died after the first 14 days. The standing biomass of *S. podacanthum* increased with increased nutrient supplementation concentrations. The ratio 160:16 resulted in a significantly ($p<0.05$) higher *S. podacanthum* standing biomass than the *S. podacanthum* exposed to all other nutrient concentrations throughout the trial.

Table 1: Dried biomass (mg) of *S. podacanthum* at control and five supplementation concentrations of NH_4-N:PO_4^{3-}-P in Ocean Water (OW) and K^+-fortifed Inland Saline Water (K^+ISW)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	441.39±3.49	442.63±10.36	451.33±4.32	445.73±3.10	446.36±2.89	449.46±7.16
	K^+ISW	445.11±9.84	439.83±16.75	439.83±13.97	443.25±10.35	443.25±11.26	445.11±3.56
Day 14	OW	472.76±3.88	493.26±15.84	457.23±26.55	515.00±21.33	448.84±34.71	506.93±29.87
	K^+ISW	477.11±13.86^{bc}	423.37±22.78^{bc}	486.11±37.54^{b}	566.88±43.09^{a}	403.18±32.26^{c}	490.15±27.59^{ab}
Day 28	OW	492.95±37.11	491.719±44.55	539.54±51.40	568.437±33.48	479.28±63.04	$_1$569.67±31.71
	K^+ISW	464.37±8.66^{b}	422.44±20.02^{b}	514.69±36.14^{ab}	601.66±43.48^{a}	403.80±37.64^{b}	$_2$492.64±28.49^{ab}
Day 42	OW	481.77±44.91	$_1$492.64±44.84	$_1$600.42±57.46	604.77±42.64	$_1$506.62±82.57	$_1$593.59±42.32
	K^+ISW	444.80±27.35^{b}	$_2$387.03±39.91^{b}	$_2$468.10±38.01^{b}	635.21±59.00^{a}	$_2$409.08±33.39^{b}	$_2$422.75±22.27^{b}
Day 56	OW	432.69±58.20^{b}	$_1$545.13±57.77^{ab}	$_1$683.36±66.35^{a}	617.82±42.82^{a}	$_1$576.82±53.20^{ab}	$_1$620.30±39.67^{a}
	K^+ISW	437.35±46.05^{b}	$_2$355.97±44.22^{b}	$_2$468.11±45.24^{ab}	619.68±58.70^{a}	$_2$371.19±52.48^{b}	$_2$444.49±47.70^{b}
Day 70	OW	403.18±54.33^{b}	465.30±20.39^{b}	$_1$689.57±111.90^{a}	647.95±64.84^{a}	$_1$610.05±43.83^{a}	575.57±62.18^{ab}
	K^+ISW	381.13±13.18^{ab}	331.74±42.97^{b}	$_2$392.00±79.77^{ab}	535.50±27.06^{a}	$_2$295.40±105.67^{b}	403.18±20.84^{ab}
Day 84	OW	366.84±57.45^{b}	393.55±12.25$_{bc}$	$_1$585.82±63.37^{a}	563.77±49.53^{a}	$_1$531.15±49.49^{ac}	$_1$525.56±76.90^{ac}
	K^+ISW	354.10±15.63^{ab}	286.70±31.34^{b}	$_2$332.36±64.39$_{ab}$	448.22±16.55^{a}	$_2$265.27±99.90^{b}	$_2$335.47±20.93^{ab}

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

In OW, *S. podacanthum* showed a significantly ($p<0.05$) higher biomass in OW_120, OW_160, OW_200 than the other nutrient concentrations in the second half of the trial period and reached the maximum biomass at day 70 and then declined (Table 1). In K^+ISW, the *S. podacanthum* biomass was significantly higher in ISW_160 throughout the trial and the biomass at the end was similar to that at the beginning. However, at all other nutrient concentrations, the biomass did not change during the first 70 days and then significantly declined (Table 1).

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

The biomass of *S. podacanthum* was significantly correlated ($R^2>0.7$, $p<0.05$) with time (in fortnights) (Table 2), water temperature and the concentrations of NO_2^--N, NH_4-N, TKN and PO_4^{3-}-P in waters (Table 3). The SGR of the *S. podacanthum* decreased towards the end of the trial and varied significantly ($p<0.5$) with the nutrient levels in two water types (Table 4). The SGR was significantly correlated with the water pH, temperature and water quality parameters, with the exception of NO_3^--N (Table 3). The SGR ranged from negative values to 1.70% d^{-1}, recorded in the first 14 days in ISW_160. The *S. podacanthum* SGR was significantly ($p<0.01$) affected by the water types from day 42 onwards, except at the waters without nutrient supplementation and the ratio of 160:16.

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

A negative SGR of *S. podacanthum* was recorded in ISW_80, ISW_200 and ISW_240, as the trial progressed. In other nutrient levels in K^+ISW the SGR gradually decreased from the beginning to the end of the trial. An exception SGR data were recorded at ISW_160, where highest ($p<0.05$) SGR among five nutrient concentrations over time were observed and showed the only positive SGR of the *S. podacanthum* in K^+ISW during the whole trial (Table 4).

Water Quality

The pH of the OW was similarly in five different nutrient concentrations and control during the trial. However, from the day 42 onwards, the pH in the ISW_240 was significantly ($p<0.05$) higher than that in all other supplementation levels in K^+ISW (Fig. 1a). The temperature of the culture media was similar in all the nutrient concentrations, ranging from 20 to 26°C (Fig. 1b).

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

The [N] remained unchanged in the early stages of the trial and significantly ($p<0.05$) increased by the end of the trial in both water types. During the trial, [NO_2^--N] was similar at all the nutrient levels. Both NO_2^--N and NO_3^--N varied widely in K^+ISW, but in ISW_160, NO_2^--N was stable as the time progressed (Table 5), while NO_3^--N decreased significantly by the end of the trial (Table 6).

Table 2: The regression correlation of the *S. podacanthum* biomass in mg (y) with the time in fortnight (x) in Ocean Water (OW) and K^+ fortified Inland Saline Water (K^+ISW) at control and five supplementation concentrations of NH_4-N:PO_4^{3-}-P (µM)

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

Table 3: Pearson correlation of *S. podacanthum* biomass and water quality parameters (N=48)

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

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

Table 4: Specific growth rate (% d^{-1}) of *S. podacanthum* at control and five supplementation concentrations of NH_4-N:PO_4^{3-}-P in Ocean Water (OW) and K^+-fortifed Inland Saline Water (K^+ISW)

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

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

Table 5: The concentrations of NO_2^--N in water cultured *S. podacanthum* at control and five supplementation concentrations of NH_4-N:PO_4^{3-}-P in Ocean Water (OW) and K^+-fortifed Inland Saline Water (K^+ISW)

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

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

Table 6: The concentrations of NO_3^--N in water cultured *S. podacanthum* at control and five supplementation concentrations of NH_4-N:PO_4^{3-}-P in Ocean Water (OW) and K^+-fortifed Inland Saline Water (K^+ISW)

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

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

Table 7: The concentrations of NH_4-N in water cultured *S. podacanthum* at control and five supplementation concentrations of NH_4-N:PO_4^{3-}-P in Ocean Water (OW) and K^+-fortifed Inland Saline Water (K^+ISW)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	Negligible^{f}	$_1$0.977±0.009^{e}	$_1$1.717±0.014^{d}	$_1$2.087±0.005^{c}	$_1$2.643±0.005^{b}	2.750±0.000^{a}
	K^+ISW	Negligible^{f}	$_2$1.003±0.005^{e}	$_2$1.617±0.009^{d}	$_2$1.987±0.010^{c}	$_2$2.607±0.006^{b}	2.750±0.000^{a}
Day 14	OW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	K^+ISW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Day 28	OW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	K^+ISW	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Day 42	OW	0.017±0.002	0.007±0.002	0.010±0.007	$_1$0.010±0.007	$_1$0.030±0.021	0.033±0.017
	K^+ISW	0.010±0.004^{c}	0.040±0.028^{c}	0.080±0.018^{cb}	$_2$0.173±0.062^{ab}	$_2$0.193±0.110^{a}	0.033±0.009^{c}
Day 56	OW	Negligible^{b}	$_1$0.040±0.028^{a}	0.010±0.007^{b}	Negligible^{b}	Negligible^{b}	0.027±0.015^{ab}
	K^+ISW	Negligible^{b}	$_2$Negligible^{b}	Negligible^{b}	Negligible^{b}	0.020±0.014^{ab}	0.037±0.012^{a}
Day 70	OW	$_1$Negligible^{c}	0.063±0.023^{b}	$_1$0.133±0.046^{a}	$_1$0.093±0.023^{ab}	0.043±0.010^{b}	0.127±0.002^{a}
	K^+ISW	$_2$0.137±0.029^{b}	0.080±0.015^{bc}	$_2$0.047±0.006^{bc}	$_2$0.207±0.010^{a}	0.035±0.007^{c}	0.103±0.017^{b}
Day 84	OW	0.053±1.650	$_1$0.030±0.004	0.133±0.028	$_1$0.033±0.013	0.207±0.104	$_1$0.073±0.025
	K^+ISW	0.179±0.036	$_2$0.248±0.021	0.249±0.085	$_2$0.191±0.067	0.137±0.015	$_2$0.217±0.017

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

Table 8: The concentrations of TKN in water cultured *S. podacanthum* at control and five supplementation concentrations of NH_4-N:PO_4^{3-}-P in Ocean Water (OW) and K^+-fortifed Inland Saline Water (K^+ISW)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	0.42±0.03^{f}	$_1$1.07±0.02^{e}	$_1$1.84±0.04^{d}	$_1$2.33±0.02^{c}	$_1$2.71±0.02^{b}	$_1$3.01±0.06^{a}
	K^+ISW	0.54±0.07^{f}	$_2$1.80±0.02^{e}	$_2$1.98±0.04^{d}	$_2$2.17±0.03^{c}	$_2$3.39±0.04^{b}	$_2$3.60±0.10^{a}
Day 14	OW	2.03±0.06^{c}	$_1$2.45±0.14^{b}	2.10±0.06^{bc}	$_1$2.36±0.05^{bc}	$_1$2.45±0.09^{b}	$_1$3.15±0.19^{a}
	K^+ISW	2.36±0.23^{d}	$_2$3.55±0.07^{b}	2.47±0.23^{d}	$_2$3.13±0.06^{c}	$_2$4.27±0.03^{a}	$_2$3.74±0.09^{b}
Day 28	OW	$_1$0.23±0.06^{c}	$_1$1.12±0.10^{b}	$_1$0.96±0.07^{b}	$_1$1.17±0.16^{b}	$_1$1.31±0.03^{ab}	$_1$1.59±0.04^{a}
	K^+ISW	$_2$1.70±0.15^{e}	$_2$1.70±0.17^{e}	$_2$2.75±0.17^{c}	$_2$2.40±0.03^{d}	$_2$3.10±0.12^{b}	$_2$3.31±0.15^{a}
Day 42	OW	$_1$1.82±0.21^{b}	$_1$2.57±0.42^{b}	$_1$2.15±0.29^{b}	$_1$2.15±0.09^{b}	$_1$4.02±0.23^{a}	$_1$2.19±0.34^{b}
	K^+ISW	$_2$3.74±0.09^{c}	$_2$4.53±0.29^{c}	$_2$5.09±0.66^{b}	$_2$4.30±0.14^{c}	$_2$7.52±0.34^{a}	$_2$5.14±0.37^{b}
Day 56	OW	$_1$0.37±0.03^{d}	$_1$2.29±0.44^{cd}	$_1$1.59±0.09^{c}	$_1$1.96±0.23^{bc}	$_1$2.80±0.41^{b}	$_1$4.02±0.23^{a}
	K^+ISW	$_2$3.27±0.26^{c}	$_2$3.31±0.23^{c}	$_2$3.74±0.18^{c}	$_2$4.76±0.06^{b}	$_2$5.18±0.66^{b}	$_2$6.54±0.43^{a}
Day 70	OW	$_1$1.59±0.09^{bc}	4.11±0.34^{ab}	5.51±0.69^{a}	$_1$1.54±0.21^{c}	$_1$2.94±0.21^{b}	$_1$0.61±0.17^{c}
	K^+ISW	$_2$3.27±0.09^{b}	4.25±0.14^{ab}	5.56±0.52^{a}	$_2$4.81±0.18^{a}	$_2$4.11±0.41^{ab}	$_2$4.76±1.45^{ab}
Day 84	OW	$_1$1.88±0.46^{c}	$_1$3.41±0.36^{ab}	$_1$3.41±0.60^{ab}	$_1$3.02±0.37^{bc}	3.36±0.55^{ab}	4.76±0.66^{a}
	K^+ISW	$_2$4.61±0.49^{b}	$_2$6.76±0.30^{a}	$_2$5.26±0.73b	$_2$4.82±0.73^{b}	4.25±0.26^{b}	5.18±0.21^{b}

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

Table 9: The concentrations of PO_4^{3-}-P in water cultured *S. podacanthum* at control and five supplementation concentrations of NH_4-N:PO_4^{3-}-P in Ocean Water (OW) and K^+-fortifed Inland Saline Water (K^+ISW)

Time	Water	Control	80:8	120:12	160:16	200:20	240:24
Day 1	OW	1.00 ± 0.00^{c}	$_1 2.57\pm0.05^{b}$	$_1 2.50\pm0.12^{b}$	2.70 ± 0.07^{ab}	$_1 2.47\pm0.02^{b}$	$_1 2.83\pm0.02^{a}$
	K^+ISW	1.07 ± 0.02^{c}	$_2 2.80\pm0.04^{b}$	$_2 2.67\pm0.02^{b}$	2.60 ± 0.00^{b}	$_2 3.17\pm0.02^{a}$	$_2 3.27\pm0.05^{a}$
Day 14	OW	$_1 1.03\pm0.02^{b}$	$_1 1.17\pm0.02^{a}$	$_1 1.10\pm0.04^{b}$	$_1 0.63\pm0.02^{c}$	$_1 0.83\pm0.02^{c}$	$_1 0.83\pm0.02^{c}$
	K^+ISW	$_2 1.20\pm0.04^{b}$	$_2 1.30\pm0.04^{a}$	$_2 1.00\pm0.04^{d}$	$_2 1.30\pm0.04^{a}$	$_2 1.10\pm0.04^{c}$	$_2 1.23\pm0.02^{b}$
Day 28	OW	$_1 0.93\pm0.08^{ab}$	1.10 ± 0.07^{a}	0.97 ± 0.13^{ab}	$_1 0.67\pm0.02^{bc}$	$_1 0.60\pm0.11^{c}$	$_1 0.83\pm0.02^{b}$
	K^+ISW	$_2 1.17\pm0.02^{ab}$	1.30 ± 0.04^{a}	0.87 ± 0.13^{c}	$_2 1.30\pm0.04^{a}$	$_2 0.97\pm0.08^{bc}$	$_2 1.23\pm0.02^{a}$
Day 42	OW	0.30 ± 0.11^{b}	0.33 ± 0.02^{b}	$_1 0.37\pm0.13^{b}$	$_1 0.33\pm0.08^{b}$	$_1 0.57\pm0.02^{b}$	$_1 1.23\pm0.06^{a}$
	K^+ISW	0.50 ± 0.22^{cd}	0.27 ± 0.06^{d}	$_2 0.77\pm0.10^{bc}$	$_2 0.83\pm0.02^{b}$	$_2 1.03\pm0.02^{b}$	$_2 1.53\pm0.05^{a}$
Day 56	OW	0.60 ± 0.11	0.90 ± 0.12	0.97 ± 0.08	0.53 ± 0.06	$_1 0.77\pm0.18$	0.57 ± 0.10
	K^+ISW	1.03 ± 0.17^{ab}	1.13 ± 0.20^{ab}	1.07 ± 0.18^{ab}	1.27 ± 0.16^{a}	$_2 1.10\pm0.45^{ab}$	0.77 ± 0.02^{b}
Day 70	OW	0.47 ± 0.02	0.73 ± 0.08	$_1 0.43\pm0.05$	0.60 ± 0.11	$_1 0.50\pm0.04$	0.43 ± 0.08
	K^+ISW	1.13 ± 0.02^{bc}	0.63 ± 0.02^{c}	$_2 1.37\pm0.17^{b}$	0.87 ± 0.33^{c}	$_2 2.53\pm0.63^{a}$	0.57 ± 0.02^{c}
Day 84	OW	1.17 ± 0.33^{ab}	1.27 ± 0.02^{ab}	1.43 ± 0.34^{ab}	1.30 ± 0.32^{ab}	$_1 1.87\pm0.46^{a}$	1.07 ± 0.17^{b}
	K^+ISW	1.43 ± 0.05^{bc}	1.90 ± 0.15^{b}	1.23 ± 0.25^{bc}	1.43 ± 0.06^{cb}	$_2 3.50\pm0.36^{a}$	0.93 ± 0.08^{c}

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

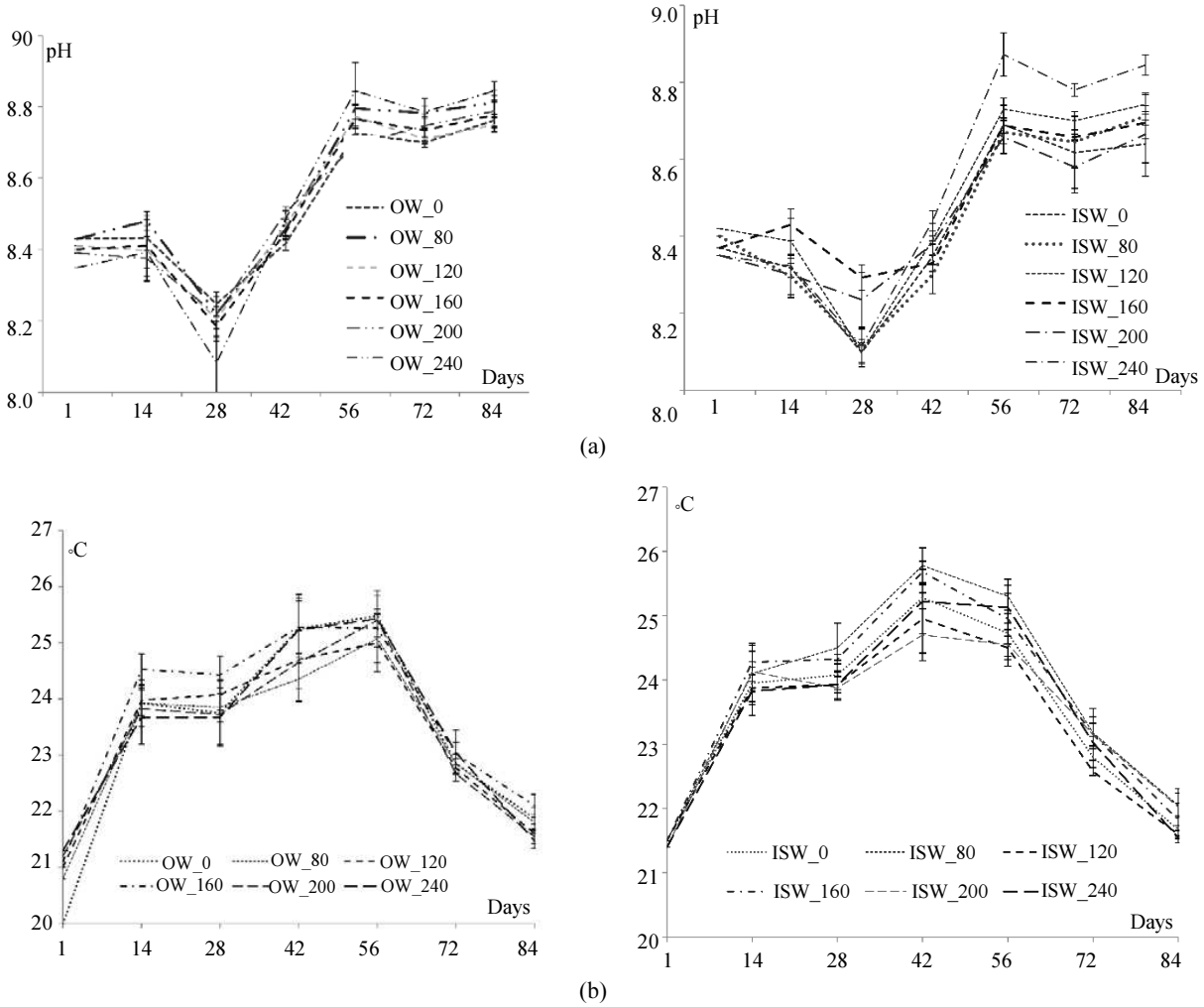

Fig.1: The pH (a) and temperature (b) of the water cultured *S. podacanthum* at controls and five supplement concentrations of NH_4-N:PO_4^{3-}-P 80:8, 120:12, 160:16, 200:20 and 240:24 μM were respectively supplied to ocean water (OW_0, OW_80, OW_120, OW_160, OW_200, OW_240) and K^+-fortified inland saline water (ISW_0, ISW_80, ISW_120, ISW_160, ISW_200, ISW_240)

The NH_4-N and TKN concentrations significantly rose with increasing nutrient enrichment levels and were higher in K^+ISW than in OW. However, after releasing *S. podacanthum* into the water, [NH_4-N] was approximately negligible over the first 56 days, then increased to a maximum of 0.25 mg L^{-1}, which was lower than at the commencement of the trial (Table 7). Conversely, TKN decreased to a minimal value at day 28 and significantly increased by the end of the trial (Table 8).

The [PO_4^{3-}-P] in both water types decreased significantly ($p<0.05$) during the trial compared than at the beginning of the trial. It was higher in K^+ISW than OW at all nutrient supplementation concentrations greater than 80:8 (Table 9).

Discussion

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

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

The *S. podacanthum* died in raw ISW after one fortnight reconfirmed the need of K^+ fortification in ISW for acceptable growth of *Sargassum.* The [K^+] in ISW significantly affected the growth of *S. linearifolium,* which reached an optimal growth in K^+ISW at a similar concentration of K^+ in OW (Bui *et al*., 2017b). Therefore, the nutrients were enriched into the K^+ISW at the similar concentration of K^+ in the OW. K^+ is essential for the growth of plants (Blumwald *et al.*, 2000; Talling, 2010), particularly for seaweed, as it is recognised as an important internal cation (Kirst, 1977) playing a role in protein and starch synthesis and metabolic processes in living cells (Evans and Sorger, 1966). Moreover, K^+ balances the osmotic gradient in aquatic plant cells (Malhotra and Glass, 1995) and maintains a standard cellular sodium to potassium ratio (Blumwald *et al*., 2000). At 35 ppt, the [K^+] in ISW is less than one-third of the [K^+] in OW and this low level caused the total mortality of the *S. podacanthum* after two weeks of cultivation even though the N and P in raw ISW were similar to ISW_0. When the *S. podacanthum* died due to lack of K^+, nutrients were not consumed, so that the NO_3^--N and NH_4-N concentrations in raw ISW did not change during the first two weeks of culture and the data from this treatment was not continued collected.

The *Sargassum*'s growth and development is seasonal (Schaffelke and Klumpp, 1998; Vuki and Price, 1994) and varies according to the species (McCourt, 1984). The fertile receptacles of the temperate *Sargassum* are shed in summer and the tropical *Sargassum* is abundant in winter (McCourt, 1984). The Australian *Sargassum*, *S. tenerrimum, S. fissifolium, S. olygocystum* achieve higher biomass and maximum length in summer (December–February), followed by a late summer peak in reproduction (March–May). *S. linearifolium*, on the other hand, achieves its peak in size in June–September and reproduction in September–January (Martin-Smith, 1993). The growth of *S. podacanthum* in OW and K^+ISW, without any nutrient supplementation resulted in similar life cycles as any other sub-tropical and/or temperate *Sargassum* spp. Our trial, lasted from September to December and the growths of *S. podacanthum* significantly increased in the first month and then decreased from late October. Wherein the *S. linearifolium* maximum growth occurrs during the late winter and early spring (August–October) and then starts to decline in November (Martin-Smith, 1994), which is also similar to *S. linearifolium* grown under the laboratory conditions (Bui *et al*., 2017b). In the nutrient enriched condition, the growing stage of *S. podacanthum* lasted up to 70 days, similar to *S. linearifolium* in the natural environment. This result demonstrated that the seasonal growth cycle of *S. podacanthum* in the laboratory condition was similar to the natural out-door conditions.

Temperature and pH play a crucial role in the growth of *Sargassum* (Chen and Zou, 2014; Choi *et al.*, 2009). The water temperature, with no significant differences among water types, was 20–26°C, without any controlled mechanism in place. This temperature range reflected similar OW temperatures during this season (https://www.seatemperature.org/australia-pacific/australia/western- australia/, downloaded 23 Dec 2016). According to Hanisak and Samuel (1987), this is a suitable temperature for the maximum growth of *Sargassum* spp. The correlation of the *S.*

podacanthum biomass (in the form of a standing crop) and temperature in the present study is similar to *S. polysystum, S. binderi* and *S. siliquosum* in the natural environment (May-Lin and Ching-lee, 2013). Although the pH of the culture media was in a suitable range for seaweed growth (Lignell and Pedersén, 1989), pH was lowest in the second fortnight in controls and the nutrient enrichment concentrations of 80:8, 120:12 and 200:20 in both water types, that coincided with the occurrence of *Sargassum* mortality (Tucker and D'Abramo, 2008). In contrast, *S. podacanthum* grew well in ISW_160, where the pH was relatively stable over time.

The maximal SGR of *S. podacanthum* in the present study was 1.7% d^{-1} in the first fortnight in the ISW_160, lower than the SGR (4.7% d^{-1}) of *S. horneri* (Turner) C. Agardh in the natural OW environment under similar temperatures (Gao and Hua, 1997, Yamauchi, 1984). The growth of *Sargassum* spp. are species-specific (Hanisak and Samuel, 1987) as *S. baccularia* reaches twice its growth in a NH_4-N:PO_4^{3-}-P ratio of 10:1, at 3–5 µM NH_4-N supplied continuously in OW and the growth rate is reduced when NH_4-N and PO_4^{3-}-P are supplemented beyond these ranges (Schaffelke and Klumpp, 1998). The SGR 8% d^{-1} of *S. baccularia* in nutrient enrichment OW (Schaffelke and Klumpp, 1998) is higher than that of *S. podacanthum* from this study in both OW and K^+ISW, again highlighting the different nutrient requirements among various species of *Sargassum*. At a similar concentration of N (including NO_3^--N and NH_4-N), the SGR of *S. podacanthum* in OW in the first month and in ISW_160 in the first five fortnights was around 0.3% d^{-1}, which is similar to the SGR of the adult stage of *S. muticum* under natural OW on the floating raft from August to May at Higashiura, where the temperature changed from 25 to 10°C and the NO_3^--N is from 1.0–4.1 µM, NH_4-N is from 5.8–11.0 µM and PO_4^{3-}-P is from 0.12–0.35 µM for five months (Yamauchi, 1984).

The standing biomass of *S. podacanthum* was significantly correlated to the nutrient levels in the culture media. In addition to the weekly-supplied nutrients, the N and P in water were also generated by the decomposition process of dying *S. podacanthum.* The soluble N and P concentrations in water are difficult to stabilise and measure as they are quickly cycled by living microbes (Downing, 1997). N and P have been consumed at different rates (Smith *et al.*, 1986), for example, at the same concentrations, NH_4-N uptake is faster than PO_4^{3-}-P (Wallentinus, 1984). *S. podacanthum* and bacteria in water quickly consumed the provided PO_4^{3-}-P and NH_4-N, resulting in a similar concentration of PO_4^{3-}-P in cultured media to that found in natural OW throughout the trial, particularly in ISW_160, and NH_4-N quickly reduced to negligible levels after enrichment. The [PO_4^{3-}-P] in the culture media was lowest from day 42 to day 70 of cultivation, that was found to be correlated to a high biomass of *S. podacanthum* in both water types. Towards the end of the trial, when a reduction of the *S. podacanthum* biomass was recorded, the PO_4^{3-}-P and NH_4-N supplements were not totally consumed and in turn, resulted in the increase of PO_4^{3-}-P and NH_4-N. A sharp increase in PO_4^{3-}-P and NO_3^--N in ISW_200 towards the end of the trial was associated with the highest reduction in the *S. podacanthum* biomass. The additional source of organic nitrogen was came from the decomposing dead *Sargassum* (Robards *et al.*, 1994), therefore, the higher TKN concentration in K^+ISW than in OW resulted from the higher mortality of *S. podacanthum* in K^+ISW. The present study confirmed that the main factor for the highest standing biomass of *S. podacanthum* in real-time was limited by N, represented by NO_3^--N and NO_2^--N rather than NH_4-N, as seaweed can consume NO_3^--N instead of NH_4-N when NH_4-N is insufficient (Jie *et al.*, 2008) or is lower than 0.135 mg L^{-1} (Balode *et al.*, 1998). During the present trial, NH_4-N was usually negligible and [NO_3^--N] was always available at 2–4 mg L^{-1}, which met the requirements of *S. podacanthum*. The [NO_3^--N] in water was found to be significantly correlated with the *S. podacanthum* biomass, given that [NO_3^--N] and the biomass of *S. podacanthum* were both unchanged at all nutrient levels in the first half of the trial. The increase of [NO_3^--N] towards the end of the trial resulted in a reduction of *S. podacanthum* biomass in both water types. In ISW_160, [NO_3^--N] was stable over the first four fortnights and then increased, indicating that the *S. podacanthum* biomass decreased after reaching its maximum biomass in the fourth fortnight.

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

Conclusion

The nutrient enrichment of 160:16 µM of NH_4-N:PO_4^{3-}-P, using NH_4Cl and NaH_2PO_4, in ISW which

was fortified with K^+ at similar K^+ concentration in OW at the same salinity, results in a similar biomass and SGR of *S. podacanthum* cultured in OW. This nutrient level is the most suitable water for growing *S. podacanthum* in K^+ISW. The *S. podacanthum* growth cycle in the laboratory conditions is similar to the natural conditions, wherein, the maximal growth season is from the late winter to early spring.

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Author's Contributions

Ha Thi Thu Bui: As the 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. All authors have read and approved the manuscript. No ethical issue that may arise after the publication of this manuscript.

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