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Bioremediation efficiency of indigenous seaweeds of Chennai coast in brackishwater system

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Abstract
The accelerated development of high-density brackishwater shrimp farming necessitates the importance of bioremediation. Seaweeds have the potential to reduce nutrients from aquaculture systems and provide extra income when species of economic importance are used. Identification of suitable seaweed species which is locally available in abundance with bioremediation capacity in brackishwater system is paramount, and the present study addresses this issue. An exploratory monthly survey was undertaken in three brackishwater systems in Chennai coast viz. Muttukadu lagoon, Vennangupattu Lake and Pulicat Lake from March 2018 to February 2019 which led to a focus on species of the family Gracilariaceae. Identification of the species through taxonomical and molecular observations confirmed that seaweed from Muttukadu lagoon and Vennangupattu Lake is *Agarophyton tenuistipitatum* and that from Pulicat Lake is *Hydropuntia edulis*. Evaluation of the bioremediation potential of these two species indicated that they were similar with respect to ammonia and phosphate reduction efficiency whereas the specific growth rate of *A. tenuistipitatum* was significantly higher than *H. edulis*. Furthermore, the nutrient reduction efficiency and specific growth rate was significantly higher at biomass density of 3.5 and 4.5 g L^{-1} compared to 1.5 and 2.5 g L^{-1}. It could therefore be concluded that *A. tenuistipitatum* could be utilised for bioremediation as well as culture in brackishwater system at a biomass density ranging from 3.5 to 4.5 g L^{-1}.

Keywords Bioremediation · *Cox*2–3 spacer · Initial biomass · RuBisCo spacer · Seaweed

Introduction

Aquaculture in India has evolved as a viable commercial farming practice from the level of traditional backyard activity over the past few decades with considerable diversification in

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terms of species and systems and has been showing an impressive annual growth rate of 6–7%. Indian crustacean farming is brackishwater aquaculture wherein shrimps account for more than 90% of Indian crustacean production. Shrimp cultivation has been on a surge especially since 2009, after the introduction of *Penaeus vannamei* with production levels of 10–12 t ha crop^{-1} within a 135-day duration (Laxmappa 2016). The production of this species has reached a level of 622,327 tonnes during 2017–2018 (MPEDA 2018). At present, the major brackishwater-cultivated species is *Penaeus vannamei* which contributed about 52.9% among crustaceans in world aquaculture production (FAO 2020).

The aquaculture of marine animals is not an environmentally friendly activity. One of the most serious problems of aquaculture of invertebrates and fish is the loading of excessive nutrients into the local waters. In general, 52–92% of the nitrogen and 85% of the phosphorus enters the aquatic environment through feed wastage, excretion and faeces, which may easily induce eutrophication, induces algal bloom leading to anoxia, but both are part of the process (Zhou et al. 2006).

The use of brackishwater macroalgae capable of bioremediation would help to sequestrate the nutrient nitrogen and phosphorus to a great extent. Earlier studies have shown that in their normal growth and development, macroalgae absorb and metabolise large amounts of nutrients into their tissues (Lobban and Harrison 1994). Gracilariaceaean algae have been identified as valid and effective agents for nutrient bioremediation from different IMTA systems (Neori et al. 2004). In seawater temperature of 20–23 °C at irradiance of 250 μmol quanta m^{-2} s^{-1}, *Hydropuntia edulis* was able to remove around 95% of ammonium from shrimp farming system (Jones et al. 2001). At marine salinity regime and 9.5–19 °C temperature, *Gracilaria chilensis* was capable of removing 95% ammonium and 32% orthophosphate from a salmon culture system under natural daylight condition (Buschmann et al. 1996). In a mollusc-fish-seaweed–based IMTA studies under natural daylight, it was found that in seawater and 16–26.9 °C temperature condition, *Gracilaria conferta* removed 34% of ammonium and about 25% of orthophosphate (Neori et al. 1998). Martinez-Aragon et al. (2002) reported that *Gracilaria gracilis* with the condition of 18 °C and 240 μmol photon m^{-2} s^{-1} illumination in a flow-through system could remove around 93% and 62.2% of ammonium and orthophosphate respectively from sea bass (*Dicentrarchus labrax*) cultured effluent water within marine salinity regime. Apart from the bioremediation potential, Gracilariaceaean algae have economic importance as agarophyte and as food for humans and marine animals (Yang et al., 2015). Several Gracilariaceaean species are important in the multi-million dollar agar and agarose phycocolloid industry (Johnson et al. 2014).

Family Gracilariaceae has a worldwide distribution; Gracilariacean algae (or its representatives) grows in tropical and subtropical waters, and also found in temperate zone along the European coast up to Sweden and along the Asian coast up to Sea of Okhotsk. Among family Gracilariaceae, the genus *Hydropuntia* and *Agarophyton* include around 8 and 3 species, respectively (Gurgel et al. 2018; World Register of Marine Species (WoRMS) 2020). In India, the main species cultivated is *Hydropuntia edulis* (= *Gracilaria edulis*) (Krishnamurthy 1991); among thirty-two species of Gracilariacean algae have been reported for the country.

The main aim of this investigation was to identify the locally available brackishwater seaweed species (through morphological as well as molecular methods) which is abundant and has a commercial value along with its bioremediation potential, i.e. to estimate the minimum biomass density required for maximum sequestration of nutrients (N and P) with higher growth rate. From this point of view, the members of the Gracilariaceae are the most promising

objects among brackishwater seaweeds. Therefore, three naturally available Gracilariacean algae collected from Pulicat Lake, Muttukadu lagoon and Vennangupattu Lake were studied.

Materials and methods

Identification of seaweeds

Studies on seaweed species of brackishwater system are meagre. Monthly exploratory survey on three brackishwater systems in Chennai coast viz. Muttukadu lagoon, Vennangupattu Lake and Pulicat Lake from March 2018 to February 2019 aided in selecting seaweeds with the help of local fishermen. In all locations, Gracilariacean algae were found abundant almost throughout the year. Seaweeds were collected from the shallow (0.4–1 m) water bodies in the Pulicat Lake (13° 28′ 59.4″ N and 80° 15′ 46.1″ E) of salinity ranged between 12.5 and 61 g L^{-1}, and also a very shallow (< 0.5 m) zone of the Muttukadu lagoon (12° 48′ 42.1″ N and 80° 14′ 39″ E) and Vennangupattu Lake (12° 14′ 41″ N and 79° 58′ 58″ E) of salinity ranged from 10 to 27.4 g L^{-1} during the year at the low-tide period (Fig. 1). After collection, algae were transferred to the laboratory where epiphytes and encrusting organisms were removed. Thereafter, they were stored in a tank with seawater for further studies.

Morphological and anatomical methods

Fresh seaweeds were collected from the field and the genus were identified in the laboratory by studying morphological and anatomical features which were photographed using a Nikon DS-U3 DS-Fi2-U3 camera on a bright field microscope. For anatomical identification, the cross section of fresh thallus and cystocarp were mounted with 40% corn syrup.

Molecular protocol

Three samples (one per site) were collected and cleaned of surface epiphytes, debris and portions of each sample were kept separately for DNA extraction for which the Chelex protocol (Goff and Moon, 1993) as outlined in Zuccarello et al. (1999a) was followed. A fresh thallus tip of approximately 3–15 mm or 10–25 mg was used for each sample. Based on the earlier studies by Zuccarello et al. (1999a, b), two DNA markers from different cell organelles were used to identify the species. The first one was intergenic region between ribulose bisphosphate carboxylase/oxygenase large and small subunit viz. RuBisCo spacer region located in plastid and the second one was intergenic region between cytochrome oxidase subunit 2 and cytochrome oxidase subunit 3 viz. *Cox*2–3 spacer region located in mitochondria. RuBisCo spacer region was amplified using forward primer 5′-tatacttctacagacacagctga-3′ (rbcF1) and reverse primer 5′-atttcacacaggaaacagctatgacatgtcaaataatggtagtcccca-3′ (rbcR2-M2) and amplification of *Cox*2–3 spacer region was performed using forward primer (cox2-for) 5′-gtaccwtctttdrgrrkdaaatgtgatgc-3′ and a reverse primer (cox3-rev) 5′-ggatctacwagatgraawggatgtc-3′ (Zuccarello et al. 1999b; Byrne et al. 2002). Polymerase chain reaction (PCR) products were checked by electrophoresing in a 1.5% agarose gel, stained in ethidium bromide, visualised under UV light and photographed. Crude PCR products were prepared and sent to Eurofins Genomics India Pvt. Ltd. for sequencing. Sequenced data obtained from the two DNA markers for three specimens were analysed for similarity using nucleotide BLASTN programme online.

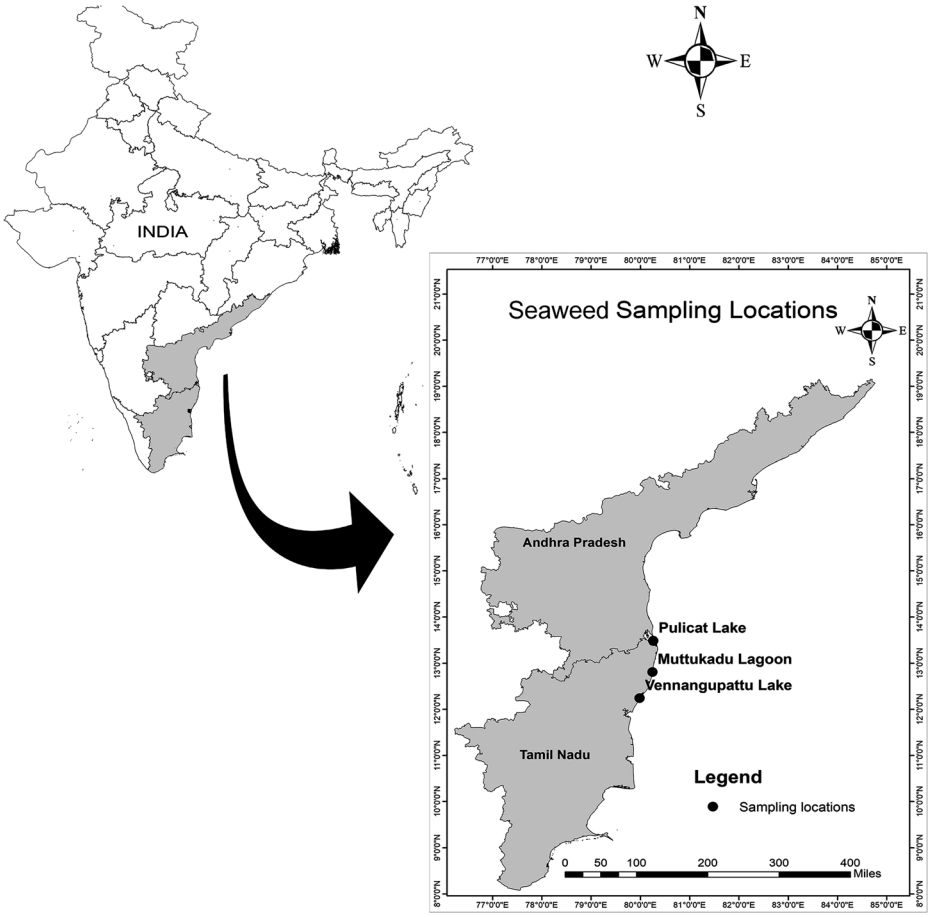

Fig. 1 Map showing collection locations of Gracilariacean seaweeds for this study

Bioremediation efficiency

Experiment design

The experimental system was placed inside a poly-house with a natural photoperiod and the photon flux density of sunlight inside the room was ranged between 57 and 76 μmol photons m^{-2} s^{-1} during the daytime. Besides, temperature was simulated in the poly house with the help of a cellulose cooling pad, and the overall room temperature was maintained between 27 and 30 °C during the daylight period. A factorial design was employed analysing Factor 1 (two seaweeds species from three different locations) and Factor 2 (biomass density) in the following treatments: 0 g L^{-1} (without any seaweed), 1.5 g L^{-1} (310 g m^{-2}), 2.5 g L^{-1} (517 g m^{-2}), 3.5 g L^{-1} (724 g m^{-2}) and 4.5 g L^{-1} (931 g m^{-2}) as control, T1, T2, T3 and T4, respectively. Thirty plastic tanks each having a capacity of 30 L (triplicate) were filled with filtered sea water, and adequate amount of fresh water was added into it to maintain a constant salinity of 25 g L^{-1}. Air stones were placed in the bottom of each tank to provide continuous

aeration, and DO level was maintained between 5.5 and 6 mg L^{-1} ranges during the experiment. Initially, 5 mg L^{-1} of NH_4-N and 2 mg L^{-1} of PO_4-P level were administered to the treatments with the help of $(NH_4)_2SO_4$ and KH_2PO_4 salts respectively to determine the bioremediation efficiency of the algae. The experiment was continued up to 96 h from the time of the addition of seaweed in the tanks.

Sample collection and analytical methods

Water pH and dissolved oxygen (DO) were determined and recorded using probes (Orion 9107 BNMD for pH and Lutron DO-5510 for dissolved oxygen) at every 12 h interval for 24 h. The water samples (triplicate) were collected and analysed for NH_4-N (phenol hypochlorite method), NO_2-N (sulphanilamide NED method), NO_3-N (sulphanilamide NED method after reduction of NO_3 to NO_2 with cadmium) and PO_4-P (phosphomolybdic acid-ascorbic acid method) for the same duration (APHA 1998).

Nutrient removal percentage (NR %) for ammonium (NH_4-N) and orthophosphate (PO_4-P) in the systems was estimated after Zhou et al. (2006):

$$\mathrm{NR}\% = 100 \times \left(C_{\mathrm{cnl}} - C_{\mathrm{p}}\right)/C_{\mathrm{cnl}} \tag{1}$$

where C_{cnl} is nutrient concentration in the control treatment (mg L^{-1}) and C_{p} is nutrient concentration in the seaweed treatment (mg L^{-1}) at a particular time since beginning.

At the end of the experiment (96 h), the seaweed in each tank was weighed and their growth was estimated as below (Rosenberg et al. 1984):

$$\text{Specific growth rate } \left(\mathrm{SGR}\%\mathrm{d}^{-1}\right) = \ln\left(W_{\mathrm{f}}/W_0\right)/t \times 100 \tag{2}$$

where W_0 is the initial wet weight of algae (g); W_{f} is the final wet weight of algae (g) at time t since the beginning; and t is the number of days between initial and final sampling.

Statistical analysis

The parameters were computed and expressed as mean along with the standard error. Data concerning nutrient removal percentage and SGR were analysed by two-way ANOVA to determine the effect of species and biomass density on the treatments. The level of significance ($\alpha = 0.05$) was used. Statistical analyses were performed with SPSS version 17.0 and graphics generated by GraphPad Prism version 5.

Results

Seaweeds identification

Based on morphological and anatomical features (Figs. 2 and 3, Table 1), algae from Muttukadu lagoon and Vennangupattu Lake were identified as *Agarophyton tenuistipitatum* (= *Gracilaria tenuistipitata* C. F. Chang & B.-M. Xia) and that from Pulicat Lake was identified as *Hydropuntia edulis* (= *Gracilaria edulis* (S. G. Gmelin) P. C. Silva). This identification was confirmed by genetic analysis (Fig. 4; Table 2).

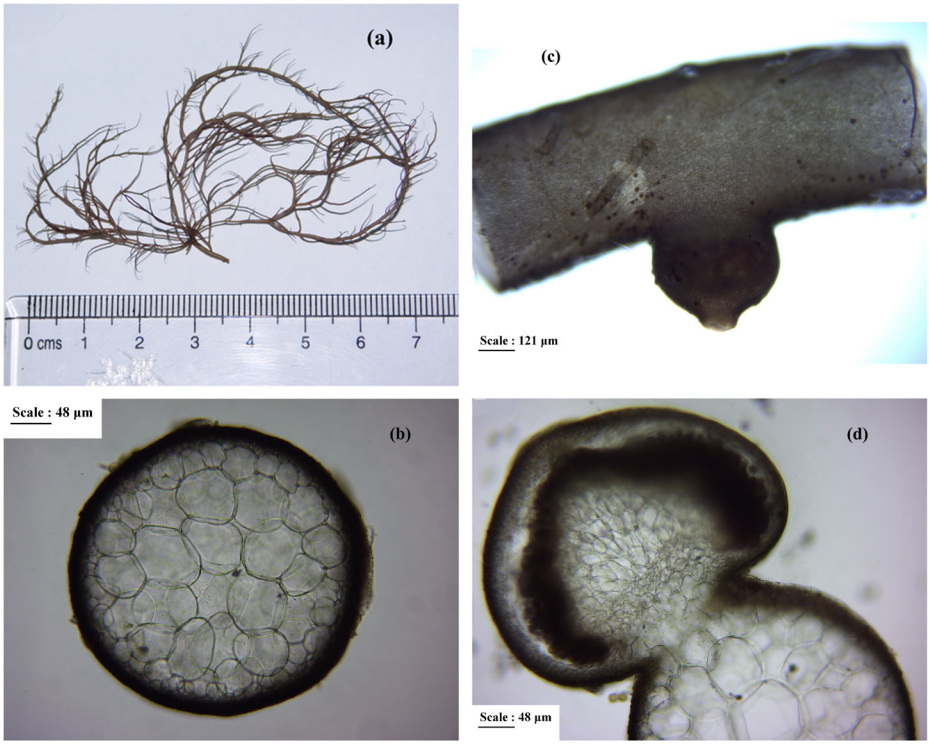

Fig. 2 *Agarophyton tenuistipitatum* thallus, collected from southern brackishwater zone of Chennai coast (**a**); thallus cross-section of main axis (**b**); external view of cystocarp (**c**); and thallus cross-section of cystocarp (**d**)

Bioremediation efficiency

Abiotic parameters

The average water temperature ranged between 28.1 and 30.6 °C in all treatments during the study (Table 3). The average pH was insignificant between different biomass for both species during 0 h and 12 h duration, but it was significantly affected during 24 h of study. It was found higher as biomass of seaweed increased. Average pH ranged from 7.95 to 8.19 among seaweed treatments whereas it was 7.75 in control (Table 3). Initial water quality revealed that source water contained both the NO_2-N and NO_3-N. The variation with respect to NO_2-N and NO_3-N during the experimental trial is depicted in Table 3. Mean NO_2-N concentration was 0.001 mg L^{-1} initially in all treatments for both species during the entire experimental period (Table 3). It was observed that mean NO_3-N concentration was 0.30 mg L^{-1} initially in all treatments and in control during 12 h and 24 h. Mean NO_3-N concentration reduced significantly for both species, and reduction was significantly lesser at 1.5 g L^{-1} than other higher biomass density during the experiment. Optimum NO_3-N sequestration observed at 3.5 and 4.5 g L^{-1} biomass density was approximately 0.1 mg L^{-1} at 12 h and < 0.1 mg L^{-1} 24 h for both species (Table 3).

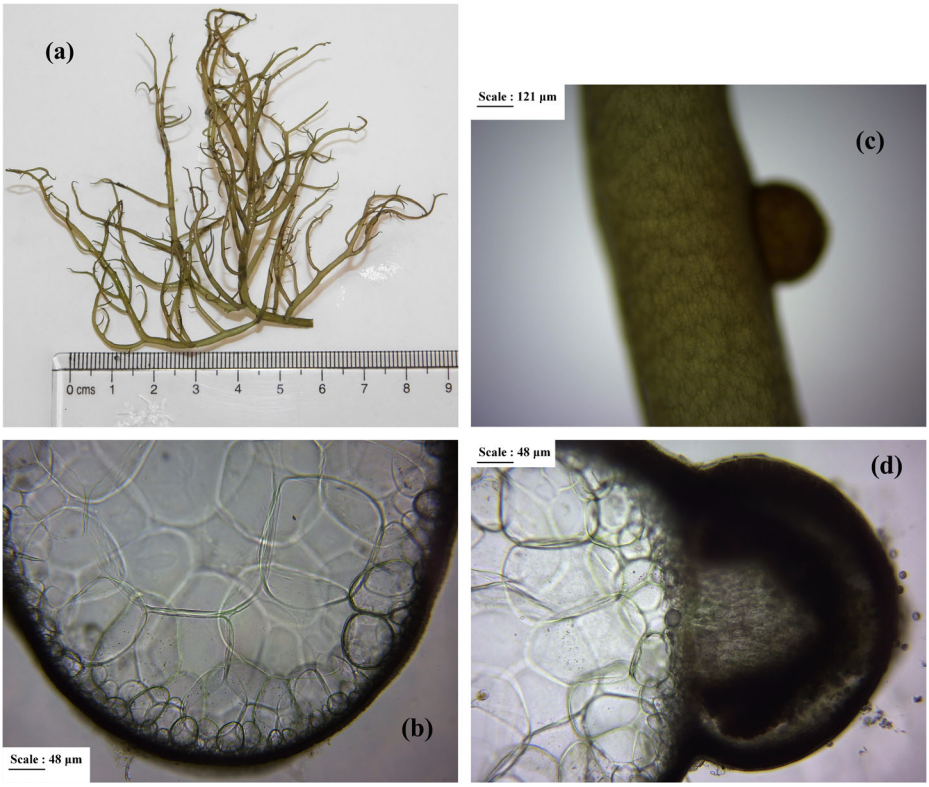

Fig. 3 *Hydropuntia edulis* thallus, collected from northern brackishwater zone of Chennai coast (**a**); thallus cross-section of main axis (**b**); external view of cystocarp (**c**); and thallus cross-section of cystocarp (**d**)

Macroalgal performance

H. edulis and *A. tenuistipitatum* showed a significant difference with respect to mean NH_4-N removal percentage in the water at 12 h (Table 4; Fig. 5a). Higher removal efficiency was observed in *A. tenuistipitatum* than *H. edulis*. However, at 24 h, there was no difference between the species in terms of mean NH_4-N removal percentage (Table 4; Fig. 5b).

With respect to different biomass densities, it was observed that the mean ammonia removal was significantly higher after 24 h than after 12 h (Table 4). Mean ammonia removal increased with biomass increase, reaching > 85% after 12 h (Fig. 5a) and 95% after 24 h (Fig. 5b) at highest biomass (4.5 g L^{-1}).

Phosphate removal % did not reveal a difference between species both at 12 h and 24 h time intervals (Table 4; Fig. 6a, b). Similarly, with respect to different biomass densities, it was observed that mean phosphate removal was significantly higher after 24 h than after 12 h (Table 4). Mean phosphate removal increased with biomass increase, reaching 11–12% after 12 h (Fig. 6a) and about 30% after 24 h (Fig. 6b) at highest biomass (4.5 g L^{-1}).

The SGR of both the species was significantly different with *A. tenuistipitatum* revealing a higher SGR than *H. edulis* at the experimental system (Table 4; Fig. 7). With respect to biomass density, a higher SGR was observed at 3.5 and 4.5 g L^{-1} treatment groups compared to 1.5 and 2.5 g L^{-1} treatment groups, but there was no significant difference between 3.5 and

Table 1 Morphological and anatomical traits of seaweed specimens from different locations

Species name and location	Identification traits	References
Agarophyton tenuistipitatum from Muttukadu lagoon and Vennangupattu Lake	Colour: purplish-brown, greenish-brown or purplish-red Form of Thallus: thallus ramiform, cylindrical, arising from a more or less small disc-like holdfast Thallus dimension: 5 to 30 cm tall, 0.2–1 mm in diameter Branching: dichotomous or lateral, produced in all directions in the terete forms and branches up to 3rd to 4th order Constriction of branches: branches slightly constricted or non-constricted at the base Branch apex: apiculate, sometimes alternate (Fig. 2a). Cross section of frond: medulla of large parentchymatous cells, 225–390 μm at the centre, surrounded by 1–2 layers of small cortical cells, 10–20 μm in diameter, with an abrupt transition from medulla to cortex (Fig. 2b). Characteristics of cystocarp: prominently protrude, globose, rostrate, constricted at base (Fig. 2c). Anatomy of cystocarp: pericarp thick with distinct ostiole and the outmost layer cells are round or ovoid with a distinct cell wall (Fig. 2d).	FAO/NACA (1996), Desikachary et al. (1998) and Jha et al. (2009)
Hydropuntia edulis from Pulicat Lake	Colour: dark green to yellowish or brownish red Form of Thallus: thallus erect, cylindrical and highly branched, arising from an irregularly discoid base, 2 mm in thickness, thinning to 1.5 mm at middle of the axis and then attenuating to the apex Thallus dimension: 6–27 cm tall, main axis 1–1.5 mm in diameter Branching: branches lateral, sub-dichotomous, opposite or sub-opposite, often alternate, highly polymorphic. 1.5 mm at base, gradually increasing to 2 mm towards middle and becoming thinner, to 0.3 mm towards the tip; branching up to 3rd or even 4th order Constriction of branches: branches slightly constricted or non-constricted at the base Branch apex: attenuate, often curved like tendrils (Fig. 3a). Cross section of frond: consisting of roundish thin walled medulla cells, 100–300 μm in diameter, 1–2 rows of small cortical cells, 5–15 μm in diameter, with an abrupt transition from medulla to cortex (Fig. 3b). Characteristics of cystocarp: globose 0.5–1.0 mm in diameter with rostrate tips and constricted at base (Fig. 3c).	

Table 1 (continued)

Species name and location	Identification traits	References
	Anatomy of cystocarp: pericarp thick with distinct ostiole and the outmost layer cells are oval with a distinct cell wall and inner cells horizontally compressed (Fig. 3d).	

The comparison between *Agarophyton tenuistipitatum* and *Hydropuntia edulis* at a glance

4.5 g L^{-1} and also between 1.5 and 2.5 g L^{-1} biomass density (Fig. 7). The results showed that the optimum SGR achieved for *A. tenuistipitatum* was 1.43% day^{-1} whereas it was 0.43% day^{-1} for *H. edulis* at minimum 3.5 g L^{-1} biomass density.

Discussion

Studied species

The characterisation of the species is largely based on gross plant morphology and the developmental morphologies of vegetative and reproductive structures (Withell et al. 1994; Womersley 1996). In a genus like *Gracilaria* which is morphologically diverse, it is inevitable

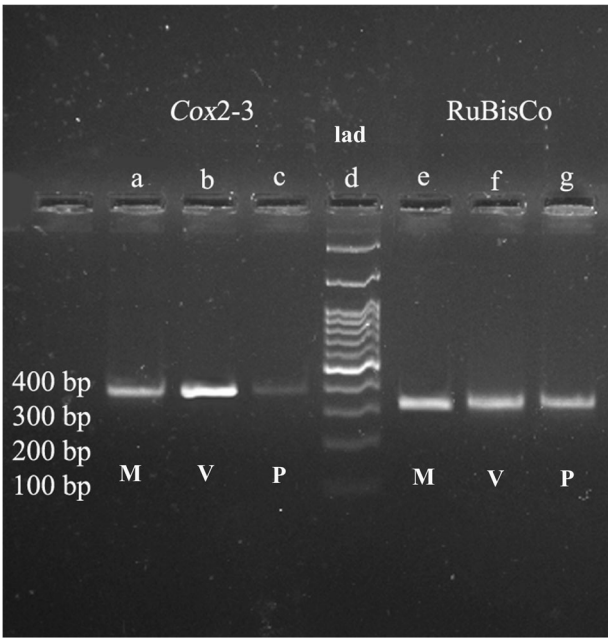

Fig. 4 Amplified *Cox*2–3 (left) and RuBisCo spacer region (right) separated by agarose gel electrophoresis (1.5%) and visualised following ethidium bromide staining. Lane d, molecular weight ladder. Left side of ladder shown amplified *Cox*2–3 spacer region for *A. tenuistipitatum* from Muttukadu (M) lagoon (lane a), Vennangupattu (V) Lake (lane b) and *H. edulis* from Pulicat (P) Lake (lane c). Right side of ladder shows amplified RuBisCo spacer region of *A. tenuistipitatum* from Muttukadu (M) lagoon (lane e), Vennangupattu (V) Lake (lane f) and *H. edulis* from Pulicat (P) Lake

Table 2 Online analysis for statistical significance of matches for sequenced database of three specimens using BLASTN programme in GenBank database

Sample location	Intergenic region studied	Query cover (%)	*E* value	Percent identity (%)	GenBank Accession No.	Organism
Muttukadu Lagoon	RuBisCo spacer	74	2e−144	99.31	AY673996.1	*Agarophyton tenuistipitatum*
	*Cox*2–3 spacer	84	2e−154	97.84	AY131317.1	*Agarophyton tenuistipitatum*
Vennangupattu Lake	RuBisCo spacer	90	1e−140	100	KY429207.1	*Agarophyton tenuistipitatum*
	*Cox*2–3 spacer	88	2e−163	100	KY429206.1	*Agarophyton tenuistipitatum*
Pulicat Lake	RuBisCo spacer	–	–	–	–	–
	*Cox*2–3 spacer	85	1e−156	99.68	MK461149.1	*Hydropuntia edulis*

that the relatively few consistent features that classically distinguish the species overlap and ambiguity arises, particularly in cases where field populations are seldom represented by all life history stages. The ambiguous natures of the populations make the genus *Gracilaria* as well as the genera *Hydropuntia* and *Agarophyton*, recently segregated from *Gracilaria* (Gurgel and Fredericq 2004; Gurgel et al. 2018) difficult for identification throughout the world (Byrne et al. 2002). Often, cylindrical Gracilariacean algae in Asia were reported as *Gracilaria verrucosa* or were not identified, similarly to algae from Muttukadu lagoon and Pulicat Lake (Bharathan 1987; Kaliaperumal et al. 1995, Jayasankar et al. 2006). To exclude any ambiguities, we used combinations of morphological and molecular methods for identification of algae in this study. The analysis revealed the identity of the species as *A. tenuistipitatum* from Muttukadu lagoon and Vennangupattu Lake and *H. edulis* from Pulicat Lake region. Both species are widely distributed in the tropical zones. *A. tenuistipitatum* grows mainly in brackishwater conditions (Haglund and Pedersen 1993, Chaoyuan et al. 1993; Lee et al. 1999). In India, in brackishwater lagoons such as Muttukadu lagoon and Vennangupattu Lake, *A. tenuistipitatum* could be found throughout the year. In contrast, *Hydropuntia edulis* usually occurs in seawater salinity regime (> 30 g L^{-1}) having a better growth under these conditions (Kaladharan et al. 1996; Jayasankar and Ramamoorthy 1997; Raikar et al. 2001; Ganesan et al. 2011). In the present study, it was found that Pulicat Lake has an average salinity of more than 30 g L^{-1} throughout the year (Dhinamala et al. 2015; Basuri et al. 2020).

Bioremedial potential and growth

In general NH_4-N is cited as the preferred form of nitrogen for seaweed growth because NH_4-N is the most reduced form of inorganic nitrogen, but simultaneously seaweeds also absorb other inorganic nitrogenous substances (Lobban and Harrison 1994). This observation corroborated the findings of the study of Marinho-Soriano et al. (2009), in which it was shown that *G. caudata* absorbed both NH_4-N and NO_3-N simultaneously, but removal of NO_3-N was lower than that of removal of NH_4-N. Different biomass densities of the seaweed was efficient to remove nitrite and nitrate as evident by the results on nitrite and nitrate concentration which were at a very negligible level throughout the experiment, i.e. below the harmful level for aquaculture practices. The study also revealed that irrespective of the seaweed biomass density, nitrite and nitrate concentration was found to be insignificant with different biomass

Table 3 Physico-chemical parameters of water in the experimental tanks for *H. edulis* and *A. tenuistipitatum* for different biomass densities during the study intervals

Seaweeds species		*H. edulis*					*A. tenuistipitatum*				
Time (h)	Parameters	Control (0 g L^{-1})	1.5 g L^{-1}	2.5 g L^{-1}	3.5 g L^{-1}	4.5 g L^{-1}	Control (0 g L^{-1})	1.5 g L^{-1}	2.5 g L^{-1}	3.5 g L^{-1}	4.5 g L^{-1}
0	Temperature (°C)	30.47 ± 0.03	30.23 ± 0.23	30.53 ± 0.07	30.33 ± 0.03	30.6 ± 0.00	30.33 ± 0.09	30.6 ± 0.06	30.33 ± 0.09	30.4 ± 0.1	30.43 ± 0.09
	pH	7.77 ± 0.02	7.77 ± 0.00	7.78 ± 0.00	7.76 ± 0.01	7.76 ± 0.01	7.75 ± 0.01	7.75 ± 0.01	7.75 ± 0.00	7.77 ± 0.02	7.77 ± 0.01
	NO_2-N (mg L^{-1})	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00
	NO_3-N (mg L^{-1})	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.01
12	Temperature (°C)	28.67 ± 0.03	28.63 ± 0.03	28.57 ± 0.03	28.53 ± 0.09	28.53 ± 0.03	28.6 ± 0.0	28.5 ± 0.06	28.53 ± 0.03	28.47 ± 0.07	28.1 ± 0.25
	pH	7.78 ± 0.00	7.82 ± 0.01	7.82 ± 0.02	7.82 ± 0.02	7.83 ± 0.00	7.75 ± 0.02	7.86 ± 0.06	7.84 ± 0.03	7.79 ± 0.09	7.84 ± 0.02
	NO_2-N (mg L^{-1})	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00
	NO_3-N (mg L^{-1})	0.3 ± 0.01a	0.24 ± 0.01ab	0.18 ± 0.02bc	0.13 ± 0.01cd	0.1 ± 0.02d	0.3 ± 0.01a	0.23 ± 0.01b	0.15 ± 0.01c	0.11 ± 0.00d	0.09 ± 0.01d
24	Temperature (°C)	30.47 ± 0.03	30.43 ± 0.09	30.43 ± 0.07	30.43 ± 0.03	30.23 ± 0.09	30.57 ± 0.03	30.57 ± 0.07	30.53 ± 0.07	30.43 ± 0.03	30.47 ± 0.12
	pH	7.75 ± 0.01a	7.97 ± 0.00b	7.95 ± 0.00b	7.97 ± 0.00b	8.09 ± 0.02c	7.76 ± 0.01a	8.06 ± 0.00b	8.08 ± 0.01b	8.13 ± 0.01bc	8.19 ± 0.04c
	NO_2-N (mg L^{-1})	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00
	NO_3-N (mg L^{-1})	0.3 ± 0.01a	0.2 ± 0.01b	0.14 ± 0.01c	0.11 ± 0.01cd	0.08 ± 0.01d	0.3 ± 0.01a	0.18 ± 0.01b	0.13 ± 0.01c	0.1 ± 0.01c	0.07 ± 0.01d

Data represents mean ± SE ($n = 3$ replicates). Values with different lowercase letters in the same row are significantly different ($p < 0.05$)

Table 4 Analysis of variance (two-way ANOVA) examining the effects of biomass density and different species on nutrients removal and SGR

Factors	*df*	SS	MS	*F*	*P*
12 h AR					
Biomass density	3	6506.743	2168.914	181.803	< 0.0001
Species	1	206.996	206.996	17.351	< 0.001
Interaction	3	9.981	3.327	0.279	0.8400
24 h AR					
Biomass density	3	5582	1861	48.71	< 0.0001
Species	1	9.652	9.652	0.253	0.6220
Interaction	3	19.51	6.504	0.170	0.9149
12 h PR					
Biomass density	3	480.4	160.1	637.0	< 0.0001
Species	1	0.597	0.597	2.376	0.1428
Interaction	3	0.838	0.279	1.111	0.3738
24 h PR					
Biomass density	3	1574	524.6	80.89	< 0.0001
Species	1	0.089	0.089	0.014	0.9082
Interaction	3	4.965	1.655	0.255	0.8565
SGR					
Biomass density	3	2.018	0.673	17.19	< 0.0001
Species	1	2.094	2.094	53.52	< 0.0001
Interaction	3	0.651	0.217	5.545	0.0084

densities whereas the reduction efficiency of ammonium and phosphorus was raised with biomass increase. A similar observation was reported in clam and mussel culture by Mao et al. (2009).

Average ammonium reduction efficiency was found to be higher in *A. tenuistipitatum* than in *H. edulis* at 12 h of experiment whereas at 24 h, both species performed equally with respect to NH_4-N absorption. Higher extracellular concentration of NH_4-N primarily triggers the downhill transport and thus resultant nutrient uptake rate is proportional to the external concentration. Passive uptake has been shown to occur for ammonium uptake in *Macrocystis*, *Gracilaria tikvahiae* and *Agardhiella subulata* at ammonium concentrations greater than 25 μM (Lobban and Harrison 1994). At used in the experiments ammonia concentration more than 250 μM, the algae mainly uptake nutrients by passive transport. The factors affecting the nutrient uptake between different species are enigmatic. Although, the above-mentioned higher removal efficiency for *A. tenuistipitatum* may be explained according to its higher growth rate compared to *H. edulis* because increase of growth rate may increase the nutrient uptake (Lobban et al. 1985). Yu et al. (2013) reported a significantly higher growth rate for *A. tenuistipitatum* than *H. edulis*. Besides, for passive transport, the rate of diffusion varies with chemical potential gradient across the plasmalemma (Lobban et al. 1985). Ammonium and phosphate removal % increased proportionately to biomass density, indicating that the thallus density positively affected the capacity of nitrogen and phosphorus uptake. Overall studies regarding ammonium and phosphate removal indicated that biomass density of 3.5 g L^{-1} can efficiently remove optimum levels of nitrogen and phosphorus from the system. Higher seaweed biomass density increases the potential for nutrient uptake, mainly due to the increased biomass and the higher surface area of macroalgae (Samocha et al. 2015). Besides, the effect of different seaweed biomass density on nutrient uptake may be explained by the specific growth rate because growth has a direct effect on nutrient absorption, i.e. increase the growth rate and thus increase the nutrient uptake (Lobban et al. 1985). Biomass density at a

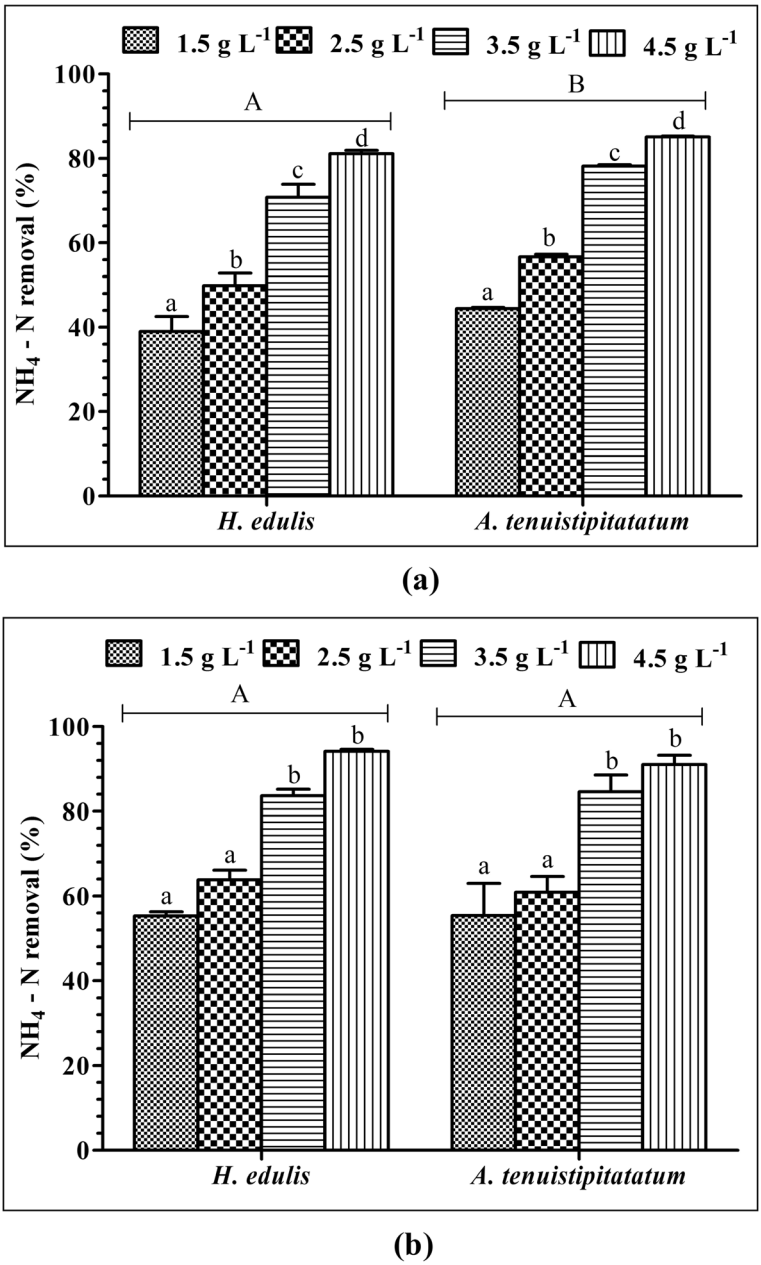

Fig. 5 Ammonia reduction efficiency during 12 h (**a**) and 24 h (**b**) of *H. edulis* and *A. tenuistipitatum* for different biomass densities. Data represents mean ± SE ($n = 3$ replicates). Different small and capital letters indicate statistical significance ($p < 0.05$) among different seaweed biomass densities and species, respectively

minimum of 3.5 g L^{-1} was found to be optimum with respect to specific growth rate; hence, this biomass density could be considered appropriate for optimum nutrient absorption. A similar observation was reported by Sarkar et al. (2019a) for IMTA experiment with *A. tenuistipitatum*.

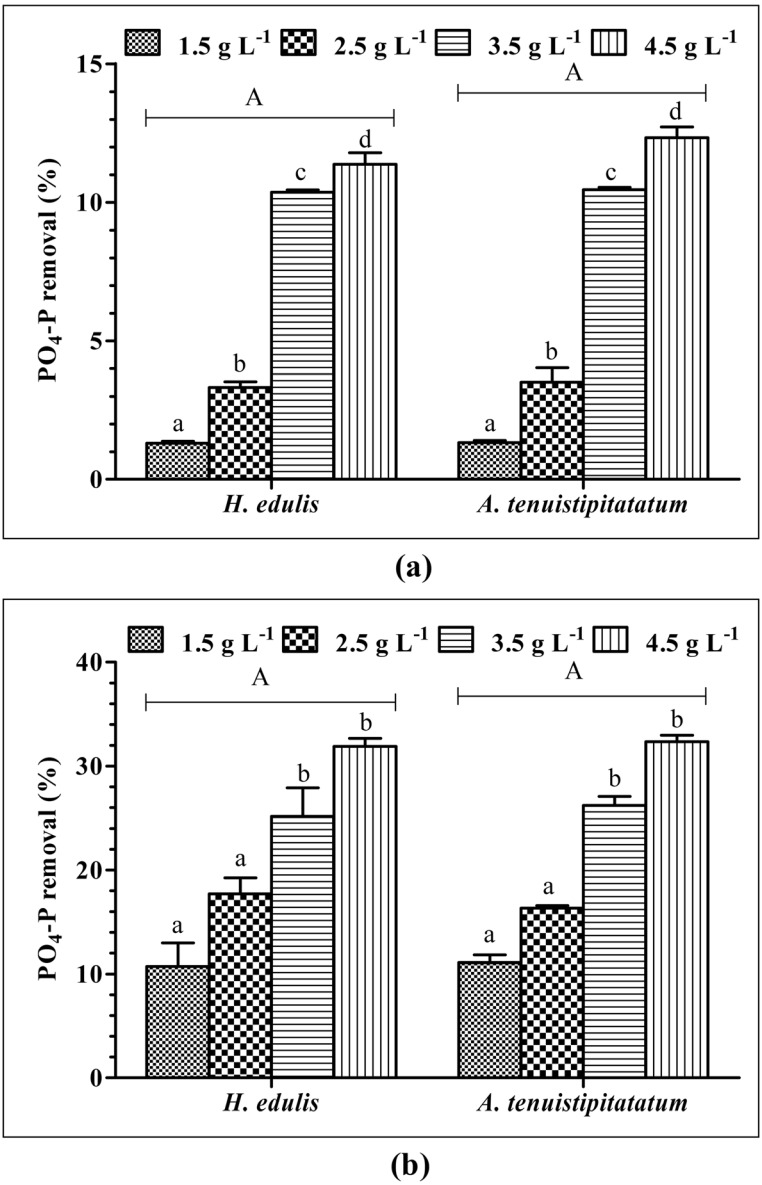

Fig. 6 Phosphate reduction efficiency during 12 h (**a**) and 24 h (**b**) of *H. edulis* and *A. tenuistipitatum* for different biomass densities. Data represents mean ± SE ($n = 3$ replicates). Different small and capital letters indicate statistical significance ($p < 0.05$) among different seaweed biomass density and species, respectively

The bioremediation efficiency of NH_4-N in this study was > 80% at 3.5 g L^{-1} algal density after 24 h. The NH_4-N uptake was reported as 87% for *H. edulis* in a shrimp farm effluent water at 20 g L^{-1} density into an integrated system with oyster after 24 h (Jones et al. 2001); whereas, it was 59.5% with *G. caudata* at density of 5 g L^{-1} in a shrimp farm wastewater system within 4 h (Marinho-Soriano et al. 2009) and 60% with *Gracilaria lemaneiformis* in 8 days into a fed fish culture system in coastal waters (Zhou et al. 2006). Sarkar et al. (2019a)

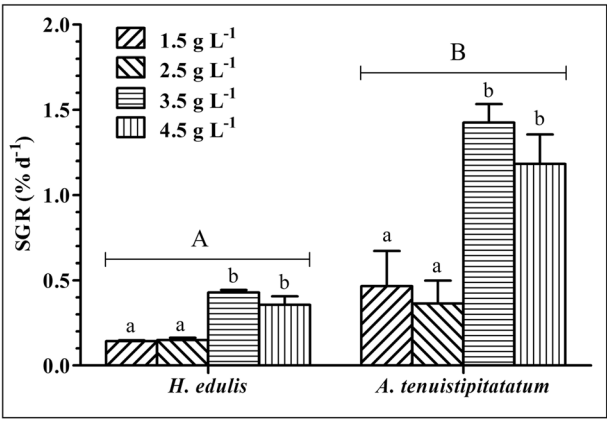

Fig. 7 Specific growth rate (SGR) of *H. edulis* and *A. tenuistipitatum* among different biomass densities kept for 96 h. Data represents mean ± SE ($n = 3$). Different small and capital letters indicate statistical significance ($p < 0.05$) among different seaweed biomass densities and species, respectively

also reported a 95.71% removal for *A. tenuistipitatum* at 3.5 g L^{-1} density in shrimp culture system after 21 days of culture. The PO_4-P removal % also followed a similar trend as that of NH_4-N removal % for different seaweed biomass densities, but reduction efficiency was lower compared to NH_4-N removal %. The 3.5-g L^{-1} algal density was found to be optimal for removal of PO_4-P (> 20%). A reduction of PO_4-P concentration of 12.3% in 4 h with *G. caudata* at 5 g L^{-1} density was reported by Marinho-Soriano et al. (2009) in a shrimp farm effluent system; whereas, a figure of 32% was recorded by Buschmann et al. (1996) in *Gracilaria chilensis* at 1.5 kg m^{-2} density from fish culture effluent during the cultivation cycle of 13 months; Troell et al. (1997) recorded 27% for *G. chilensis* in a co-culture system with fish during 2 months of cultivation; Jones et al. (2001) reported a 35% reduction for *H. edulis* in a shrimp farm effluent water at 20 g L^{-1} density after 24 h into an integrated system with oyster, and Sarkar et al. (2019a) also reported a 95.74% removal for *A. tenuistipitatum* at 3.5 g L^{-1} density in shrimp culture system after 21 days of cultivation. In our study, the removal efficiency was close to these earlier reported values, but these slight differences observed are a characteristic of different culture systems and may also be related to other factors such as light, temperature, nutritional status, algal density, duration of culture, nutrient uptake rate, associated epiflora, type of tissue and age of the alga (DeBoer 1981).

Seaweed-specific growth rate was found to be significantly higher in *A. tenuistipitatum* compared to *H. edulis* throughout the study. SGR is directly related to the concentration of extracellular substrate (Lobban et al. 1985). Therefore, significantly higher uptake of NH_4-N during 12 h period could be a triggering factor for higher growth rate in the case of *A. tenuistipitatum*. Besides, salinity might be a factor for this difference because several authors from India viz. Kaladharan et al. (1996), Raikar et al. (2001) and Ganesan et al. (2011) demonstrated that seawater salinity regime is better for the growth of *H. edulis* in Indian climate whereas *A. tenuistipitatum* is euryhaline in nature in Indian water and grows well in brackishwater salinity regime (Sarkar et al. 2019b). Moreover, *H. edulis* was collected from Pulicat Lake which has an average salinity of more than 30 g L^{-1} throughout the year (Dhinamala et al. 2015; Basuri et al. 2020). Although the physiological studies of Yu et al. (2013) showed that *H. edulis* could be adapted in brackishwater salinity regime, according to our studies, a selection of

A. tenuistipitatum would be a better choice for brackishwater aquaculture system (Sarkar et al. 2019b). Other observations have been reported by Luhan (1992) for *Gracilaria heteroclada* in the Philippines and by Msuya and Neori (2002) for *Eucheuma denticulatum* in Tanzania with loss of biomass and growth due to decrease of salinity. Studies with different biomass densities indicated that optimum specific growth rate was found at a density of 3.5 g L^{-1} after which it decreased. Zhou et al. (2006) reported that thallus density negatively affected growth. A similar observation was recorded by Mao et al. (2009) who opined that static water, lower temperature and light limitation may also affect seaweed growth. A possible reason for lower growth at greater biomass density (> 3.5 g L^{-1}) could partly be attributed to limitation of light due to higher biomass density.

Conclusion

The accelerated development of high-density brackishwater shrimp farming necessitates the importance of bioremediation. In India, the methods for treating effluents from brackishwater mariculture systems with macroalgae could be initiated and simultaneously can be utilised to reduce the risk of eutrophication of the environment by introducing culture systems. Our results expressed that *A. tenuistipitatum* could be utilised as a potential candidate species to improve water quality at a biomass density ranging from 3.5 to 4.5 g L^{-1} as this species showed better growth in brackishwater salinity regime. Besides, *A. tenuistipitatum* is potentially a fast-growing species with higher SGR compared to *H. edulis* in brackishwater culture system and hence, multiple crops may be obtained from an IMTA system with lesser input. Therefore, this study gave a preliminary idea for its IMTA potential in brackishwater condition and further association between biomass production and agar content needs to be investigated in order to check the economic feasibility of *A. tenuistipitatum*. Currently, seaweed farming is gaining momentum along the southeast Indian coast owing to constant depletion of fishing resources and fish catches over the years. This will help fetch additional income besides supplying continuous source of raw material to the seaweed-based industries.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with animals performed by any of the authors.

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