Aquaculture Reports 33 (2023) 101793

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Culturing delicacies: Potential to integrate the gastropod *Babylonia areolata* into pond cultures of *Caulerpa lentillifera*

Beatrice Brix da Costa [a,b,*], Lara Elisabeth Stuthmann [b], Aaron Johannes Cordes [c], Hoang Trung Du [d], Andreas Kunzmann [b], Karin Springer [a]

[a] Marine Botany, Faculty Biology/Chemistry, University of Bremen, BIOM, James-Watt-Straße 1, 28359 Bremen, Germany
[b] Leibniz Centre for Tropical Marine Research, Fahrenheitstraße 6, 28359 Bremen, Germany
[c] Hochschule Bremerhaven, An der Karlstadt 8, 27568 Bremerhaven, Germany
[d] Institute of Oceanography, Viet Nam Academy of Science and Technology, 01, Cau Da, Nha Trang, Khánh Hòa, Viet Nam

ARTICLE INFO

Keywords:
Aquaculture
Co-culture
Macroalga
Photosynthetic performance
Sea Grapes

ABSTRACT

In Van Phong Bay, Viet Nam, the economically important macroalgae sea grapes (*Caulerpa lentillifera;* Caulerpaceae, Bryopsidales) and the spotted babylon snail (*Babylonia areolata*) are cultivated in tidal ponds in close proximity. A co-culture in the same pond could benefit farmers by saving space and mitigating potential eutrophication. The study assessed the co-culture potential of sea grapes and spotted babylon snails held within the same system. In an outdoor experiment, three different treatments (algae and snails together, algae and snails spatially separated, only algae) were assessed for their effect on water quality, biomass properties and size increase of both organisms, photosynthetic efficiency, antioxidant activity, and total phenolic content of the macroalgae and survival of the snails. In a second (indoor) experiment, the influence of different snail densities on the physiological state of the macroalgae was investigated. In both experiments, the presence of the snails raised the concentrations of nitrogen oxides (NO_x) and phosphate (PO_4). The co-culture had a positive effect on both growth and physiology of the sea grapes. Sea grapes on trays and co-cultured with *Babylonia* showed the highest increase in biomass. *Babylonia* growth and survival were not affected by spatial separation of the alga and high stocking densities could be implemented without negatively affecting the seaweed. The results are promising regarding the establishment of co-cultures and indicate the economic feasibility of integrating *B. areolata* snails into *C. lentillifera* pond cultures.

1. Introduction

Food security is one of the greatest challenges of our time, and the ever-growing world population is raising the demand for nutritious and sustainable food sources. As arable land becomes increasingly scarce, the focus must shift to aquatic food sources and their sustainable utilization. More than 50 % of the marine and coastal aquaculture products produced worldwide are macroalgae, mainly for their use in human food (directly or indirectly through food additives such as alginates or phycocolloids) (Chopin and Tacon, 2021; FAO, 2022). Global seaweed production reached a record of 35 million tonnes (t, wet weight, WW) in 2020. However, only a few genera of red and brown macroalgae dominate seaweed production, whereas green macroalgae contribute < 1 % (FAO, 2022; Moreira et al., 2022). *Caulerpa* is a genus that is gaining popularity in the last decades, with the highest average contribution to global green seaweed production in the period 1950–2019 (6404 t WW per year on average), but with declining values until 2019 (1090 t WW, Cai et al., 2021a). However, these recent production values are likely to be underestimates, based mainly on reports from The Philippines. More accurate and current data are not available.

Among the green algae of economic interest is *Caulerpa lentillifera* (Caulerpaceae, Bryopsidales), commonly known as "sea grapes" or "green caviar". This edible seaweed is popular in the Indo-Pacific regions for its unique texture and its high nutritional value (de Gaillande et al., 2017; Syakilla et al., 2022). The siphonous alga consists of a horizontal stolon with rhizomes and edible fronds that bear globular ramuli, which resemble caviar (Zubia et al., 2020; Fig. 1A). *C. lentillifera* is recognized as a rich source of antioxidants due to its high content of secondary metabolites such as ascorbic acid (vitamin C), a-tocopherol (vitamin E), and polyphenols (Matanjun et al., 2009; Nguyen et al., 2011).

* Corresponding author at: Marine Botany, Faculty Biology/Chemistry, University of Bremen, BIOM, James-Watt-Straße 1, 28359 Bremen, Germany.
E-mail address: b.brixdacosta@uni-bremen.de (B. Brix da Costa).

https://doi.org/10.1016/j.aqrep.2023.101793
Received 19 July 2023; Received in revised form 19 October 2023; Accepted 19 October 2023

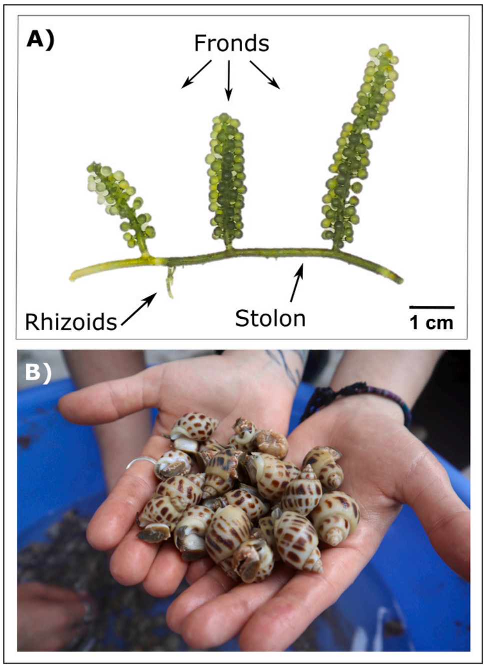

Fig. 1. A) *Caulerpa lentillifera* consists of horizontal stolons with rhizoids and the edible fronds which bear globular ramuli; B) Juvenile *Babylonia areolata* specimens with ~ 3 cm shell length.

Antioxidants play an important role as bioactive compounds due to their scavenging of reactive oxygen species (ROS), which can be harmful in high concentrations (Sharma et al., 2012). With antidiabetic, anticancer and antimutagenic activities, sea grapes have been proposed as a promising functional food ingredient (Daud et al., 2020; Manoppo et al., 2022; Syakilla et al., 2022).

Seaweeds take up dissolved nutrients from their surrounding water to grow, mainly nitrogen (N) and phosphorus (P). Sea grapes prefer nitrate (NO_3) over nitrite (NO_2) or ammonium (NH_4^+) as their inorganic nitrogen source (Liu et al., 2016). To obtain high growth rates, nutrient concentrations of 0.6 and 0.5 mmol L^{-1} NO_3 with N:P ratios of 8:1 and 5:1, respectively, have been recommended (Pariyawathee et al., 2003; Guo et al., 2015). Lower N concentrations led to growth limitation (Guo et al., 2015). P-limitation occurred at concentrations of 10 µmol L^{-1} phosphate (PO_4) and lower (Guo et al., 2015). Cai et al. (2021b) reported similar growth rates of sea grapes at different N concentrations (47, 188, 750 µmol L^{-1}) at constant P concentrations of 29 µmol L^{-1}, indicating that P might have been the limiting factor for growth.

In Viet Nam, *C. lentillifera* is increasingly cultivated in the Khánh Hòa province, in the Central South of Viet Nam. The cultivation takes place mainly in earthen tidal ponds along the coast, which are shaded with gauze material (Stuthmann et al., 2020, own observations). Algae are either planted directly into the sediment (sowing method) or grown on perforated plastic trays or nets (tray method), depending on the characteristics of the pond's substrate (Rabia, 2016). According to Rabia (2016), the sowing method results in higher biomass yields due to the high nutrient load in the sediment, which could also alter the seaweeds' nutritional composition (Long et al., 2020). Harvesting, which takes place about every two weeks, yields approximately 1000 kg WW in a 5000 m^2 pond, with farm-gate prices of USD 4.35 kg^{-1} WW (Dobson et al., 2020a).

The spotted babylon snail *Babylonia areolata* is an economically important gastropod, used for human consumption in Southeast Asia, including Viet Nam (Dobson et al., 2020a; Mai et al., 2022; Fig. 1B). The scavenging snail inhabits the sandy or muddy bottom of shallow waters. Due to high fishing pressure, the various aspects of *B. areolata* aquaculture are increasingly being studied and practiced, especially in Thailand (e.g. Chaitanawasitu et al., 2001; Kritsanapuntu et al., 2008; Sangsawangchote et al., 2010). In the Khánh Hòa province, around 30–50 t of babylon snails are produced annually (Nghia et al., 2009). *B. areolata* achieve farm-gate values of around USD 7 kg^{-1} WW when they reach marketable sizes of around 6.5–9 g per individual after approximately 6 months growth (Kritsanapuntu et al., 2006; Dobson et al., 2020a). The snails are maintained at high densities (300–400 individuals m^{-2}) and usually fed with protein-rich trash fish, resulting in suboptimal water parameters and sediment conditions, as well as nutrient rich effluents (Dobson et al., 2020b). Therefore, various co-culture approaches have been attempted in order to minimize the negative impacts of the fed species on water quality and sediment conditions by integrating bottom feeding sea cucumbers (Dobson et al., 2020a; b) or bioremediating seaweeds (Chaitanawisuti et al., 2011; Dobson et al., 2020a).

Macroalgae are known for their bioremediation potential and are therefore used as extractive species in aquaculture systems to remove nutrients and convert them into useful biomass (Chávez-Crooker and Obreque-Contreras, 2010; Abreu et al., 2011). Integrated culture of fed species and filter and/or bottom feeders with algae has been shown to be effective in reducing N and P levels in wastewater (Chopin et al., 2001; Neori et al., 2004; Crab et al., 2007). In addition to their water quality improving properties, the integration of algae also provides additional crop.

C. lentillifera is a popular co-culture species and has been studied as a bioremediator of nutrients in aquaculture effluents from various organisms including fish, shrimp, and snails (e.g. Chaitanawisuti et al., 2011; Bambaranda et al., 2019b; Anh et al., 2021). In most of the studies, presence of the seaweed had a positive effect on water quality by reducing N and P levels and also on the fed species, for example by increasing their growth rate (e.g. Anh et al., 2021; Omont et al., 2022). Some first attempts to investigate the co-culture of *C. lentillifera* and *B. areolata* have been made (Chaitanawisuti et al., 2011; Dobson et al., 2020a). Dobson et al. (2020a) investigated the co-culture in an integrated multitrophic aquaculture (IMTA) approach. Sea grapes were cultured together with *B. areolata* and sea cucumbers (*Holothuria scabra*) in Viet Nam. The results showed a positive biomass production of all organisms when cultured together in the same system. However, monoculture of only *C. lentillifera* was not studied during this approach.

Sea grapes and *B. areolata* are cultivated in close proximity in the Khánh Hòa province in Viet Nam, and the supply chain for stocking material and intermediary traders for the marketable snails appear to be in place. Therefore, the integration of snails into existing earthen sea grape ponds could be an economically advantageous endeavor for the farmers, providing an additional crop and increasing their income security (Thomas et al., 2021). *B. areolata* could potentially be integrated into the same areas as the sea grapes. However, the burrowing activity of the snails could negatively affect the growth of the seaweed through mechanical disturbances. Cultivating sea grapes on trays instead of in the sediment could potentially mitigate the negative effects of the burrowing activity. Nevertheless, spatial separation of the two organisms seems more practical, as it would be difficult to collect the snails if they were kept in the same area as the sea grapes. Since the outer areas of the sea grape ponds and the area in front of the monk (pond draining structure) are usually not shaded and sea grapes are not grown there, the snails could be easily integrated in these areas without losing cultivation area of the seaweed.

The objective of this study was to assess whether the integration of *B. areolata* snails into existing sea grape ponds could potentially increase the economic profit of sea grape farmers without reducing the yield of *C. lentillifera*. The factors of (1) sea grape culture method (tray vs. sowing), (2) co-culture approach (spatially separated vs. common bottom space), and (3) *B. areolata* stocking density were identified as key-factors, and the following research questions were derived and investigated in two separate experiments:

A. How do the (1) culture method and the (2) co-culture approach affect the growth and physiology of *C. lentillifera*?
B. What (3) stocking densities of *B. areolata* can be integrated into sea grape ponds without negative effects on the seaweed?

2. Materials & methods

This study consists of two experiments that were conducted at the Institute of Oceanography (IO) in Nha Trang, Khánh Hòa Province, Viet Nam; an outdoor terrace and an indoor laboratory experiment. Natural seawater (30 ± 1 °C, salinity of 31 ± 1, NO_x: 0.33 ± 0.2 μmol L^{-1}, PO_4: 1.19 ± 0.7 μmol L^{-1}) from the bay in front of the institute was used for the experiments. *Caulerpa lentillifera* (in the following referred to as *Caulerpa*) was collected at the sea grape farm VIJA in Van Phong Bay (12° 35′ 11.9″ N; 109° 13′ 26.2″ E) and rinsed with seawater to remove sediment and epiphytes. *Babylonia areolata* (in the following referred to as *Babylonia*) juveniles (~90 days old) were obtained from a *Babylonia* snail farm in Xuân Mỹ village (12° 57′ 38.7″ N; 109° 19′ 57.3″ E; Fig. 2).

2.1. Experimental design

2.1.1. Outdoor terrace experiment

The first experiment was conducted at the outside terrace facilities of the IO over a period of six weeks (May – July 2022). Nine 400 L glass fiber tanks (100 × 50 × 80 cm, 0.8 m^{-2}) were set up in three rows and filled with 350 L seawater and 5 cm beach sand each, allowing for complete burial of the snails. The beach sand was washed twice before filling it in the tanks to remove the coarsest impurities. Tanks were supplied with gentle aeration via air-stones. To simulate culture conditions in sea grape ponds, tanks were shaded with gauze material from natural solar irradiance to reach irradiances of 0 – 140 μmol photons m^{-2} s^{-1} during the day (Fig. 3A). Irradiance in the tanks over one experimental day was measured using a LI-1500 light sensor logger with a 2-π-flathead sensor (LI-COR Biosciences, USA) and is shown in the appendix (Fig. A.1). All organisms were acclimated for three days prior to the experiment in the shaded glass fiber tanks in natural seawater (30 ± 1 °C, salinity of 31 ± 1).

Three different treatments were assigned to the nine tanks (n = 3) in a randomized 3 × 3 design; Only *Caulerpa* – Control (C); *Caulerpa* and *Babylonia* together (T); *Caulerpa* and *Babylonia* spatially separated (S; Fig. 3B). Each tank contained *Caulerpa* with two different culture methods: 1) on perforated plastic trays and 2) directly in the sediment. Sea grape density was based on commercial stocking densities in ponds (1 kg m^{-2}), and the 4:1 seaweed to snail ratio was adapted from Chaitanawisuti et al. (2011) and Dobson et al. (2020a). 16 × 25 cm (0.04 m^2) perforated plastic trays were filled with ~100 g WW of sea grapes and tied together with fishing line before placing two of them in each tank (tray method). Additionally, two batches of *Caulerpa* (~100 g WW) were planted directly into the sediment (sowing method). Initial mean bodyweight (BW) and shell lengths of 120 *Babylonia* was 4.79 ± 0.99 g and 2.81 ± 0.21 cm, respectively. The snails were randomly allocated to the snail treatments at densities of 20 individuals per tank (~200 g m^{-2}) which is within the range of optimal stocking density for growth of the spotted babylon according to Chaitanawisuti and Kritsanapuntu (1997). Temperature and salinity in the tanks were kept constant at 30 ± 1 °C and 31 ± 1.

Babylonia snails were fed three times a week with trash fish (sardines) at a rate of 5 % total mean BW, adjusted weekly. The fish was bought at the local market and then frozen until the feeding days. Leftover food was removed after an hour or after apparent satiation was reached, as indicated by the burial of the snails. After feeding, 40 % of the seawater was exchanged in every tank.

2.1.2. Indoor laboratory experiment

The second experiment was conducted at the IO indoor laboratory facility over a period of four weeks (July 2022). 15 glass aquaria (30 × 20 × 20 cm, 0.06 m^{-2}) were filled with 10 L natural seawater taken from the bay in front of the institute, 5 cm beach sand and ~100 g (WW) *Caulerpa* in plastic trays. Four different snail densities were set up: Control, low, medium, and high with 0, 2, 4, and 8 snails, respectively which equals 0, 33, 66, and 133 snails m^{-2} (n = 5; Fig. 4). Mean initial bodyweight (BW) of 40 snails was 5.18 ± 0.99 g with shell lengths of 2.88 ± 0.22 cm. All tanks were supplied with gentle aeration via air stones and were covered with cling film. The different snail treatments were randomly assigned to the aquaria. Light was provided by light-emitting diode (LED) bars (T5 High Output Fluorescent lights, 2 × 39 W, Odyssea, China) in a 12:12 h light:dark rhythm with irradiances of 50 ± 5 μmol photons m^{-2} s^{-1}. Temperature and salinity were kept constant at 30 ± 1 °C and 31 ± 1. All organisms were acclimated for three days in glass aquaria with natural seawater (50 ± 5 μmol photons m^{-2} s^{-1}, 30 ± 1 °C, salinity of 31 ± 1).

The snails were fed every two days with trash fish as described in Section 2.1.1. In the snail tanks, 40 % of water was exchanged after feeding. Based on the results of the outdoor experiment, 100 % of the

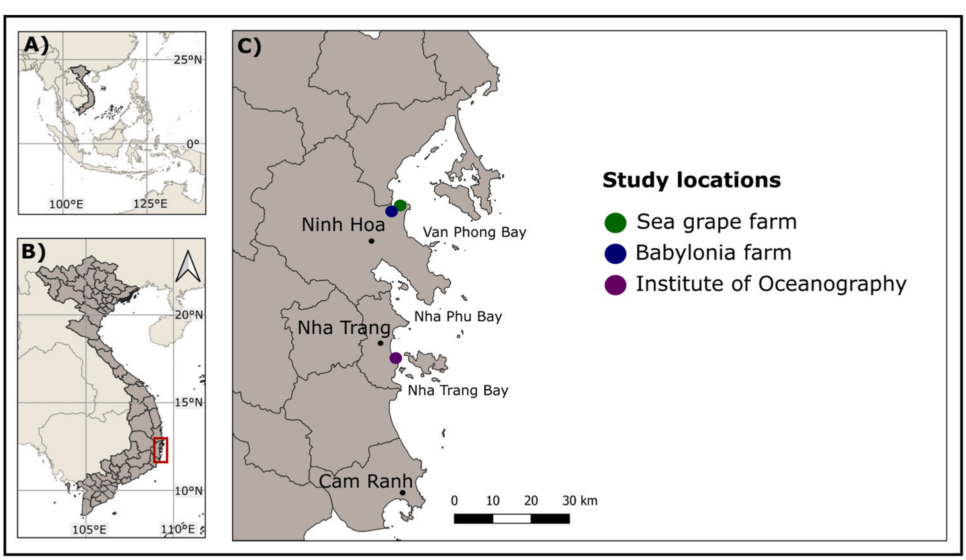

Fig. 2. A) Location of Viet Nam within Southeast Asia; B) Map of Viet Nam. The red rectangle marks the Khánh Hòa province; C) Map of study locations along the eastern coast of the Khánh Hòa province.

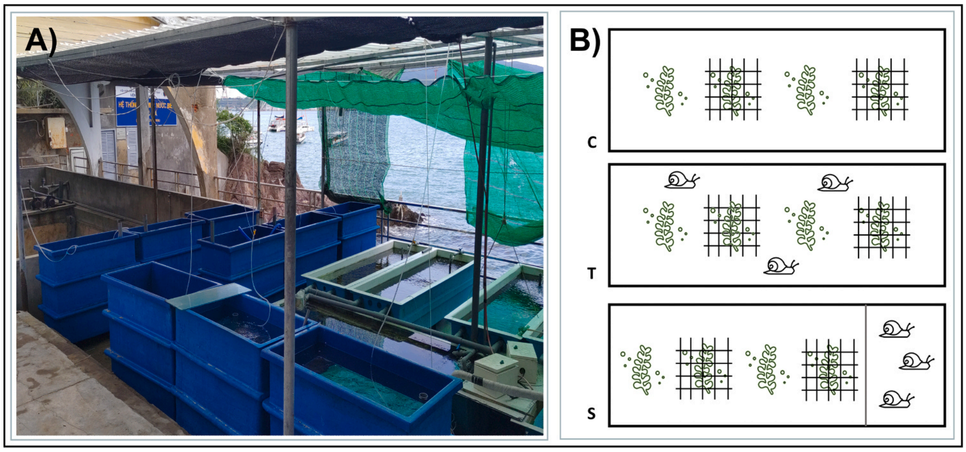

Fig. 3. A) Experimental set-up of the outdoor terrace experiment; B) Sketch of experimental design. Control treatment with only *Caulerpa lentillifera* (C); *C. lentillifera* and *Babylonia areolata* together (T); *C. lentillifera* and *B. areolata* spatially separated (S). Each tank contained *C. lentillifera* cultured with two different methods: on trays or planted directly in the sediment.

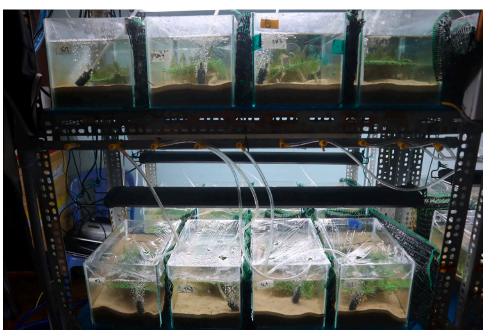

Fig. 4. Experimental set-up of the indoor laboratory experiment (not all aquaria visible). Four different treatments were investigated: Control (only *Caulerpa lentillifera*), low, medium, and high *Babylonia areolata* densities with 0, 2, 4, and 8 snails, respectively which equals 0, 33, 66, and 133 snails m^{-2}; n = 5.

water was exchanged in the "Control" treatment to ensure sufficient nutrient concentrations.

2.2. Biomass properties

For the outdoor experiment, 15 *Babylonia* snails were randomly selected from respective tanks and placed on a clean towel to allow drainage of water before measuring body weight (BW) using a digital scale with an accuracy of 0.01 g. Shell length was measured from the shell apex to the part above the siphonal notch using a vernier caliper to 0.1 mm. At the end of the experiment (after six weeks) the mean relative growth rate (RGR, % weight gain day^{-1}) was calculated as follows:

$$RGR = ((\ln BW_t - \ln BW_i)/t) \times 100$$

where BW_t is the mean final bodyweight, BW_i the mean initial bodyweight, and t the culture duration in days.

For the outdoor experiment, wet weight of *Caulerpa* was measured every week for the tray cultures and every two weeks for the sediment cultures using a digital scale. RGR was calculated as stated above. For the laboratory experiment, *Caulerpa* trays were weighed weekly. 15 % of the biomass was sampled every two weeks for further biochemical analyses. Harvestable biomass was calculated by comparing the ratio of fronds (the edible part of the alga) to stolon as follows:

$$\text{Percentage of harvestable biomass} = (W_f/W_t) \times 100$$

Where W_f is the weight of the fronds and W_t the weight of the total sample.

2.3. Chlorophyll a fluorescence measurements

Photosynthetic performance was determined by measuring chlorophyll *a* fluorescence using a portable Diving-PAM chlorophyll fluorometer (Walz, Germany). The maximum quantum efficiency of photosystem (PS) II (Fv/Fm) was measured in 7 min dark-adapted *Caulerpa* frond thallus parts.

2.4. Nutrient measurements

Prior to water exchanges 20 mL water samples were taken, filtered through 0.45 μm Minisart® syringe filters (Sartorius, Germany) and then frozen at −20 °C until analyses. Nutrient measurements were performed at the Leibniz Centre for Tropical Marine Research (ZMT, Bremen, Germany) using an Infinite® 200 Pro microtiter plate reader (TECAN, Switzerland). NO_x was determined following the procedures of García-Robledo et al. (2014). PO_4 was determined according to procedures of Ringuet et al. (2011).

2.5. Preparation of sample extracts

Sea grapes were frozen (−80 °C) directly after sampling and freeze-dried for 24 h at 1 mbar (Beta 1–8 LSCbasic, Christ GmbH, Germany). The freeze-dried samples were ground to powder for 20 s using a FastPrep-24 (MP Biomedicals, Germany). 0.05 g dry weight (DW) of the samples were dissolved in 1 mL ethanol (70 %) and extracted in a water bath (47 °C) for 4 h, being vortexed hourly. Before analyses, samples were centrifuged (2500 g, 20 °C) for 5 min.

2.6. Measurements of antioxidant activity – ABTS assay

The antioxidant activity (AOA) was determined by following the modified $ABTS^{\bullet +}$ assay (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) of Re et al. (1999). A 2.45 mM $ABTS^{\bullet +}$ stock solution was prepared by oxidising 7.0 mM ABTS with potassium disulfate ($K_2S_2O_8$) for 16 h. An $ABTS^{\bullet +}$ working solution was prepared by dilution with absolute ethanol until a consistent absorption of 0.7 ± 0.02 at 734 nm was reached (UV/VIS-spectrophotometer, Thermo Scientific Genesys 140/150, Fisher Scientific GmbH, Germany). 1 mL of the $ABTS^{\bullet +}$ working solution was added to 10 μL of sample extract and after six minutes reaction time the absorbance was measured (734 nm). Trolox was used as a standard and AOA was expressed as Trolox Equivalents (TE mmol 100 g^{-1} DW). All chemicals were purchased from Sigma

Aldrich/Merck KgaA, Germany).

2.7. *Measurements of total phenolic content – F-C assay*

The total phenolic content (TPC) was determined by following the Folin-Ciocalteu method described by Gillespie and Ainsworth (2007) with slight adaptations. 10 % (v/v) F-C-reagent and 150 µL of sample extract were mixed using a vortexer. 1.2 mL Na_2CO_3 (700 mM) was added and incubated for 45 min in the dark at room temperature. Then, samples were centrifuged (3 min, 5000 rpm, 20 °C) before reading the absorbance at 765 nm in the same UV/VIS-spectrophotometer as in Section 2.6. Gallic acid was used as a standard and results were expressed as 100 mg Gallic acid equivalents (GAE) g^{-1} DW. The source of the chemicals was as described in Section 2.6.

2.8. *Statistical analysis*

All statistical analysis and graphical outputs were conducted using R with RStudio (Wickham et al., 2019; R Core Team, 2020) and packages from the package tidyverse. Outliers were excluded from analyses using Grubbs' test using the webpage GraphPad (https://www.graphpad.com/quickcalcs/Grubbs1.cfm). QQ plots and Shapiro-Wilk test ($p > 0.05$) were used to test the datasets for normality and Levene's test to check for homogeneity of variance ($p > 0.05$). Repeated measures three-way analysis of variance (ANOVA) was conducted to investigate the effect of treatment, culture method, and time on the response variables RGR and harvestable biomass of *Caulerpa* and was followed by two-way and one-way analysis to investigate two-way interactions and the main effect. Two-way ANOVA was conducted to test the effect of treatment and culture method on the response variables Fv/Fm and AOA. One-way ANOVA was run for TPC data and for between-subject effects for each experimental day with treatment as independent variable, followed by Tukey's honestly significant difference (HSD) post-hoc test. One-way ANOVA was also used to test the biomass increase data of *Caulerpa* (indoor laboratory experiment). When the requirements for homoscedasticity and normal distribution were not met, Kruskal-Wallis tests with a following Dunn-Bonferroni post-hoc test were conducted. For the *Babylonia* weight and relative growth rate data, paired t-test and Wilcoxon tests were conducted, respectively. The confidence interval was set to 95 %, the level of significance was $\alpha = 0.05$. Summaries of the results of all statistical tests can be found in the appendix (Table A.1 & A.2).

3. Results

3.1. *Outdoor terrace experiment*

3.1.1. *Growth and harvestable biomass of Caulerpa*

Relative growth rate (RGR) of *Caulerpa* was significantly affected by treatment, culture method, and week as well as their interactions ($p < 0.001$, respectively). However, the main effect of treatment differed between the two culture methods, with significantly decreased RGRs of *Caulerpa* in monocultured trays, compared to similar RGRs for the first four weeks in sowing cultivation (Fig. 5). In the tray cultures, treatment affected the RGR of *Caulerpa* in week 2, 4, and 6 ($p < 0.001$, respectively). RGRs declined slightly in the snail treatments from 3.18 ± 0.97–1.55 ± 0.85 % day^{-1} (Separated) and from 3.81 ± 1.42–1.31 ± 0.68 % day^{-1} (Together). The growth rates of the algae in the "Control" treatment showed the highest decline, dropping to −5.52 ± 1.75 % day^{-1} in week 6. In the sowing cultures, however, the RGRs between *Caulerpa* in mono- and co-cultivation were similar until week 6. Growth rates slightly increased by 0.2–2.5 % until week 4 with no significant differences between the treatments. In week 6, growth rates declined to negative values and differences between the treatments were present with RGRs of *Caulerpa* in the monoculture being significantly lower (−7.98 ± 2.38 % day^{-1}) than in the co-culture treatments (Separated: −2.71 ± 2.06 % day^{-1}; Together: −2.28 ± 1.00 % day^{-1}).

The percentage of harvestable biomass was affected by both, treatment, and culture method ($p < 0.001$ both). The tray method resulted in overall higher harvestable biomass of sea grapes compared to the sowing method over the course of the experiment, but only when co-cultured with *Babylonia*. Treatment significantly affected the harvestable biomass in week 4 and week 6 for both culture methods (Fig. 6). The harvestable biomass did not significantly differ between the snail treatments during the course of the experiment, but was significantly lower in the "Control" treatment for both, sowing and tray cultures in week 4 and 6. Regarding the "Control" treatment, percentage of harvestable biomass was generally higher in the sowing cultures than in the tray cultures. In week 6, harvestable biomass in this treatment was 35.59 ± 9.44 % in the sowing cultures and only 8.87 ± 13.87 % in the

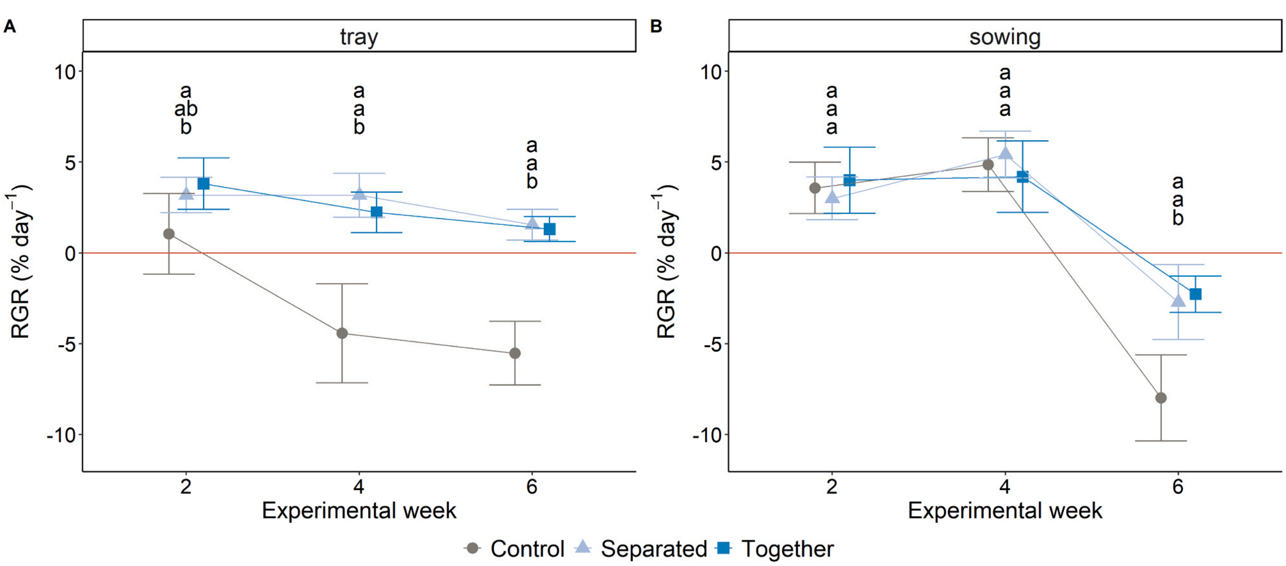

Fig. 5. Relative growth rate (RGR) of *Caulerpa lentillifera* in the tray (A) and the sowing cultures (B) over the experimental run of 6 weeks. Treatments: Control: Only *C. lentillifera*; Separated: *C. lentillifera* and *Babylonia areolata* spatially separated; Together: *C. lentillifera* and *B. areolata* together. The red line separates positive and negative growth rates. Significant differences between the treatments per day are shown by different letters (One-way ANOVA with post-hoc test, $p < 0.05$). Data are mean ± standard deviation, n = 6.

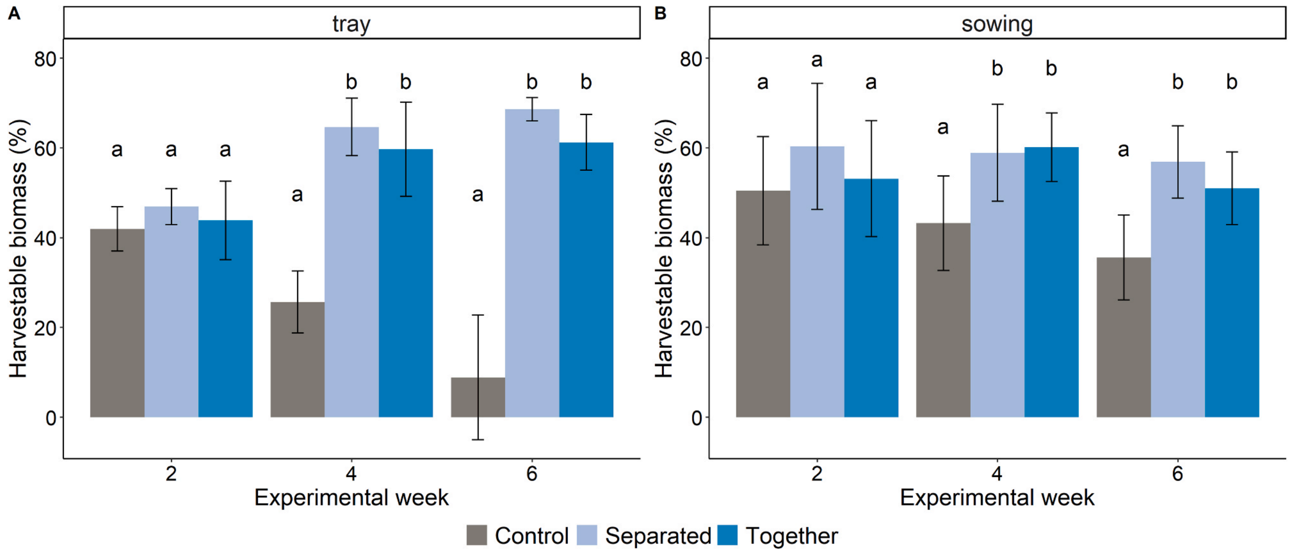

Fig. 6. Harvestable biomass (%) of *Caulerpa lentillifera* in the tray (A) and the sowing cultures (B) over the experimental run of 6 weeks. Treatments: Control: Only *C. lentillifera*; Separated: *C. lentillifera* and *Babylonia areolata* spatially separated; Together: *C. lentillifera* and *B. areolata* together. Significant differences between the snail treatments per day are shown by different letters (One-way ANOVA with post-hoc test, $p < 0.05$). Data are mean ± standard deviation, n = 4–6.

tray cultures.

3.1.2. Photosynthetic efficiency

The Fv/Fm values of monocultured sea grapes in trays and with the sowing method (0.59 ± 0.08 and 0.62 ± 0.08, respectively) decreased during the experimental run, compared to the initial values (0.71 ± 0.03), whereas Fv/Fm values of *Caulerpa* species in co-cultivation were similar (Fig. 7).

3.1.3. AOA and TPC

No differences in the AOA of *Caulerpa* were found. Values after six weeks ranged from 138.62 ± 40.07 mmol TE 100 g^{-1} DW in the "Control" treatment within the sowing cultures to 146.13 ± 38.74 mmol TE 100 g^{-1} DW in the "Together" treatment within the tray cultures (Table 1). Differences from initial values of 124.79 ± 24.82 mmol TE 100 g^{-1} DW were statistically not significant.

Total phenolic content declined in all treatments and culture methods and was significantly affected by treatment (tray: $p < 0.001$, sowing: $p = 0.043$; Table 1). In the tray cultures, TPC concentrations in the "Separated" and "Together" treatment decreased by 36.4 % and 39.1 %, respectively and significantly differed from initial values. Highest values were remained by algae in the "Control" treatment and only declined by 19.4 %. In the sowing cultures, TPC content significantly decreased in all three treatments by 23.7 % in the "Control" treatment and 40.1 % and 44.9 % in the "Separated" and "Together" treatments, respectively.

3.1.4. Growth and survival of Babylonia

The weight of the spotted babylon snails significantly increased during the six weeks from 4.79 ± 1.04 g to 8.17 ± 1.44 g in the "Separated" treatment and from 4.79 ± 0.95 g to 8.20 ± 1.40 g in the "Together" treatment (pairwise t-test, $p < 0.01$; Fig. 8). The final weight in week 6 did not significantly differ between the treatments (pairwise t-test, $p = 0.91$). Relative growth rate (RGR) of *Babylonia* did not

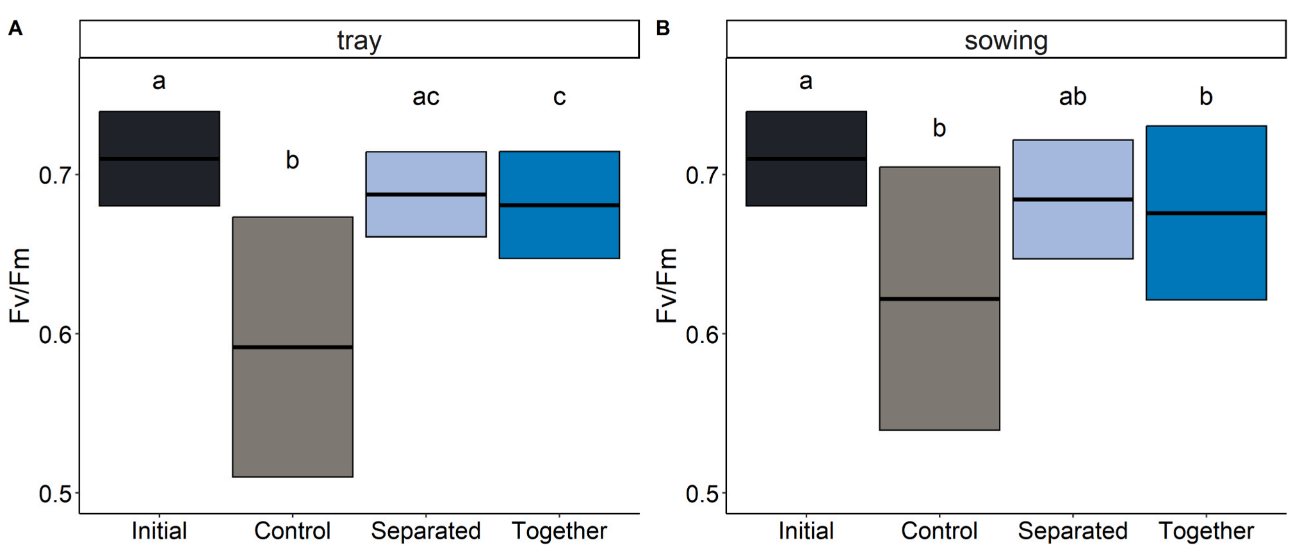

Fig. 7. Photosynthetic efficiency measured as maximum quantum yield of photosystem II (Fv/Fm) of *Caulerpa lentillifera* in the tray cultures (A) and the sowing cultures (B) after 6 weeks. Treatments: Control: Only *C. lentillifera*; Separated: *C. lentillifera* and *Babylonia areolata* spatially separated; Together: *C. lentillifera* and *B. areolata* together. Significant differences between the treatments are shown by different letters (Tray: One-way ANOVA with post-hoc test, $p < 0.05$, Sowing: Kruskal-Wallis with Dunn's post-hoc test, $p < 0.05$). Mean (middle bar) ± standard deviation (upper and lower interval), n = 4–6.

Table 1
Mean antioxidant activity (AOA; mmol Trolox Equivalents/TE 100 g^{-1} dry weight, DW) and total phenolic content (TPC; mg Gallic Acid Equivalents/GAE 100 g^{-1} DW) of *Caulerpa lentillifera* samples after 6 weeks. Treatments: Control: Only *C. lentillifera*; Separated: *C. lentillifera* and *Babylonia areolata* spatially separated; Together: *C. lentillifera* and *B. areolata* together. Significant differences are shown by different letters (tray cultures: capital letters; sowing cultures: small letters). AOA and TPC sowing data: One-way ANOVA with post-hoc test, $p < 0.05$, TPC tray data: Kruskal-Wallis with post-hoc test, $p < 0.05$). Data are mean ± standard deviation, n = 6.

Treatment	Culture method	AOA (mmol TE 100 g^{-1} DW)	TPC (mg GAE 100 g^{-1} DW)
Initial	-	124.79 ± 24.82	180.21 ± $18.43^{a,A}$
Control	tray	117.94 ± 28.26	145.26 ± 9.21^{A}
	sowing	103.02 ± 17.89	137.54 ± 14.12^{b}
Separated	tray	138.62 ± 40.07	114.66 ± 3.16^{B}
	sowing	142.74 ± 29.44	107.95 ± 28.71^{bc}
Together	tray	146.13 ± 38.74	109.83 ± 16.69^{B}
	sowing	127.13 ± 23.25	99.23 ± 13.17^{c}

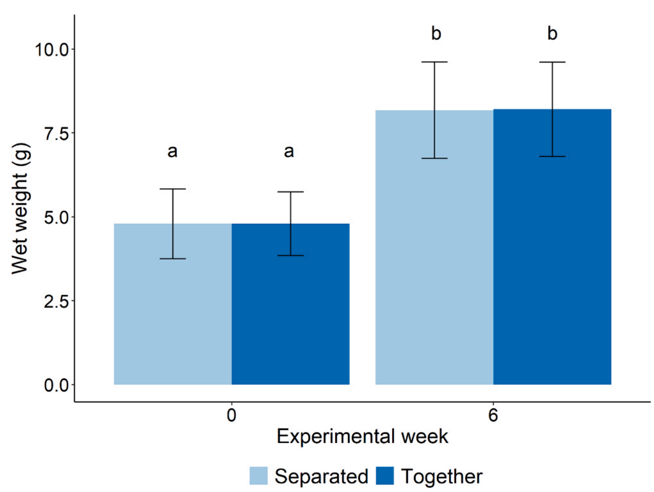

Fig. 8. Mean wet weight of *Babylonia areolata* in the different treatments (Separated: Snails spatially separated from *Caulerpa lentillifera*; Together: Snails and algae together in sediment). Significant differences are shown by different letters above bars (pairwise t-test, $p < 0.05$). Data are mean ± standard deviation, n = 20.

significantly differ between the treatments (Wilcoxon's test, $p = 0.31$). Growth rates reached maximum values of 1.31 ± 0.09 and 1.47 ± 0.17 % weight gain day^{-1} in the "Separated" and "Together" treatment, respectively, in week 5 and remained on a constant level until week 6

Table 2
Mean relative growth rates of *Babylonia areolata* over 6 weeks in the different treatments (Separated: Snails spatially separated from *Caulerpa lentillifera*; Together: Snails and algae together in sediment). Data are mean ± standard deviation, n = 15–20.

Week	Treatment	mean RGR (% weight gain day^{-1})
1	Separated	0.54 ± 0.27
2	Separated	1.13 ± 0.34
3	Separated	1.13 ± 0.35
4	Separated	1.28 ± 0.12
5	Separated	1.31 ± 0.09
6	Separated	1.27 ± 0.11
1	Together	0.86 ± 0.58
2	Together	1.50 ± 0.53
3	Together	1.04 ± 0.19
4	Together	1.33 ± 0.14
5	Together	1.47 ± 0.17
6	Together	1.28 ± 0.15

(Table 2). Survival of all organisms in all treatments was 100 %.

3.1.5. Nutrient concentrations

Treatment significantly affected the nutrient concentrations of NO_x and PO_4 in the water ($p = 0.037$ and $p = 0.018$, respectively, Fig. 9) Lowest concentrations were found in the "Control" treatment with 0.06 ± 0.08 µmol L^{-1} for NO_x and 0.48 ± 0.13 µmol L^{-1} for PO_4. Regarding NO_x, significant differences were only found between the "Control" and "Together" treatment. Phosphate concentrations only significantly differed between the "Control" and the "Separated" treatment. Overall, nutrient concentrations were higher in the snail treatments.

3.2. Indoor laboratory experiment

3.2.1. Biomass increase of Caulerpa with different snail densities

Biomass increase of *Caulerpa* was significantly affected by treatment ($p < 0.001$, Fig. 10). In the "Control" treatment, the algae decreased in weight leading to negative values after four weeks (−36.59 ± 15.83 %). In the other treatments, *Caulerpa* biomass increase ranged from 117.04 ± 7.77 % (Low) over 127.89 ± 33.88 % (Medium) to 162.00 ± 23.76 % (High). Biomass increase differed significantly between "Control" and all other treatments and between "Low" and "High".

3.2.2. Nutrient concentrations

The treatment had a significant effect on the concentrations of both, NO_x and PO_4 ($p < 0.01$). NO_x concentrations of 0.99 ± 0.47 and 5.95 ± 3.67 µmol L^{-1} in the "Control" and "Low" treatment, respectively, were significantly lower than in the other two treatments where concentrations ranged from 64.31 ± 41.08 (Medium) to 95.08 ± 14.95 µmol L^{-1} (High; Table 3). Phosphate concentrations were significantly higher than in all other treatments.

4. Discussion

4.1. Effects of co-culture approach and culture method

4.1.1. Effects on growth and photosynthesis of Caulerpa

Overall, sea grapes on trays and co-cultured with *Babylonia* showed the highest increase in biomass. The growth rate was determined by the culture method (tray vs. sowing) rather than by the co-culture approach (spatially separated vs. together). The positive RGRs of *Caulerpa* were comparable or even higher than those reported in other studies. Growth rates of 2–5 % day^{-1} have been achieved in small-scale laboratory experiments with experimental conditions similar to those in the present study (Guo et al., 2015; Tanaka et al., 2020). Chaitanawisuti et al. (2011) investigated the performance of *C. lentillifera* in a hatchery scale RAS (recirculating aquaculture system) for juvenile *B. areolata* with different seaweed biomass densities (280, 560, and 840 g WW m^{-3}). Highest growth rates of 2.58 ± 0.09 % day^{-1} were obtained at a density of 280 g m^{-3} over an experimental period of 120 days. Dobson et al. (2020a) investigated a co-culture of *Caulerpa* and *Babylonia* in comparison to a co-culture of *Caulerpa* and sandfish (*Holothuria scabra*), and all three species together in 500 L tanks. Sea grapes were cultivated on weighted trays placed vertically in the water column. During the 84-day study, growth rates of 1.87 ± 0.23 % day^{-1} were reached by *Caulerpa* (initial stocking density: 700 g WW) with *Babylonia* stocking densities of 390 ind. m^{-2} per tank (~410.16 g m^{-2}). Growth rates obtained in the present study for *Caulerpa* in the snail treatments were with 2.54 – 4.17 % day^{-1} slightly higher (except for in the last two weeks in the sowing cultures), possibly due to the lower stocking density of sea grapes at the beginning of the experiment. The continuous growth of sea grapes in the sowing cultures until week 4 may have been supported by the nutrient load in the sediment. Higher weight gain of sea grapes in sowing cultures compared to tray cultures, as a result of sediment-bound nutrients, was also reported in other studies (Rabia, 2016).

N and P are essential nutrients for algal growth. Limitations of these

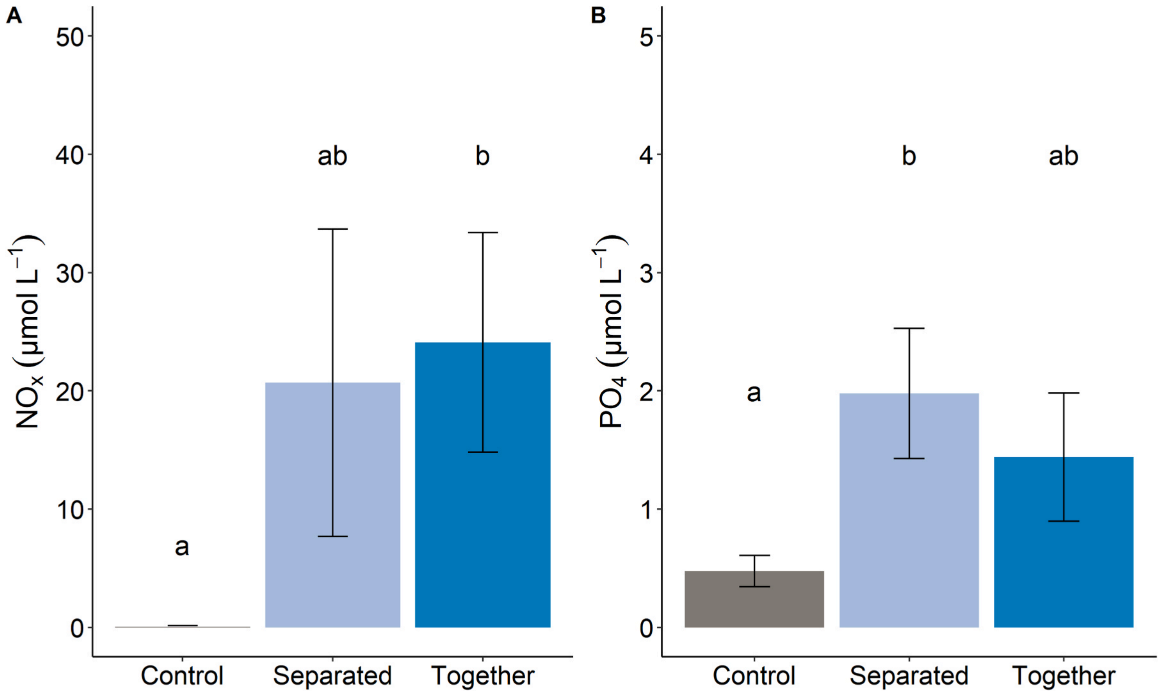

Fig. 9. Mean concentrations in μmol L^{-1} for nitrogen oxides (NO_x, A) and phosphate (PO_4, B) on the last day of the experiment. Treatments: Control: Only *Caulerpa lentillifera*; Separated: *C. lentillifera* and *Babylonia areolata* spatially separated; Together: *C. lentillifera* and *B. areolata* together. Significant differences are shown by different letters (One-way ANOVA with post-hoc test, $p < 0.05$, $n = 3$).

nutrients are known to inhibit metabolic activities of seaweeds, negatively affecting photosynthesis and growth (Qin et al., 2010; Roleda and Hurd, 2019). In the present study, NO_x concentrations in the “Control” treatment were surprisingly low, possibly due to insufficient water changes, resulting in poor growth of the sea grapes in the monocultures. The measured NO_x concentrations in the snail co-culture treatments were below the recommended nutrient concentrations reported in the literature, but the algae showed a continuous increase in biomass. The low nutrient concentrations in the water can be explained by the uptake of the algae. The nutrients that were produced by the snails were possibly taken up by the algae, which may explain the relatively low concentrations measured. As sea grapes are extremely sensitive to handling, the sudden decrease in biomass of the sea grapes in the sowing cultures after week 4 could be due to handling pressure. While the trays could be easily removed from the tanks for subsequent measurements, *Caulerpa* organisms in the sowing cultures had to be dug out and in again, which may have caused stress. In week 6, low NO_x and PO_4 concentrations of 0.06 ± 0.08 μmol L^{-1} and 0.48 ± 0.13 μmol L^{-1}, respectively, in the “Control” treatment may have led to significant differences between this treatment and the snail treatments (“Separated” and “Together”). Sea grapes in the snail treatments had higher nutrient concentration available, ranging from 7.7 to 33.7 μmol L^{-1} NO_x and 0.9–2.5 μmol L^{-1} PO_4, due to the additional organic material produced by the digesting metabolism of the *Babylonia* snails. Limited nutrients may also explain the poor growth of *Caulerpa* in the in the tray culture “Control” treatment. Since sea grapes in the “Control” treatment grew well when cultured in the sediment, it is likely that sediment-bound nutrients were the main reason for the limited growth in the tray cultures. This explanation of nutrient depletion in the “Control” treatment is supported by the values of harvestable biomass. The trend of these results is comparable to the one in the RGR data, with sea grapes in the “Control” treatment showing a significantly lower percentage of harvestable biomass than in the other treatments. For both culture methods, significant differences between the treatments start occurring from week 4 on, even in the sowing cultures when the RGR was still positive in all treatments. A lower percentage of harvestable biomass means that there was a higher proportion of stolon within the sample. Increased stolon growth in response to nutrient limitation has been reported for *C. prolifera*, and could be a strategy to overcome unfavorable environmental conditions (Malta et al., 2005). *Caulerpa* can take up nutrients via the entire thallus, including its stolon and its rhizoids, and is therefore effective at taking up nutrients in both water and sediment (Williams, 1984; Alexandre and Santos, 2020).

Nutrient limitation could also explain the differences in the maximum quantum yield of PSII (Fv/Fm). Fv/Fm is a sensitive parameter and proxy for assessing physiological stress in seaweeds (Krause and Weis, 1984), and values of ≥ 0.7 have been reported to indicate a good physiological state of sea grapes (Stuthmann et al., 2020). Regardless of the culture method, sea grapes in the “Control” treatment showed a significantly lower photosynthetic efficiency during the experimental run compared to the initial, indicating physiological stress of the photosynthetic apparatus (Higo et al., 2017). Low concentrations of phosphate and nitrate have been reported to negatively affect the photosynthetic capacity of *C. lentillifera* (Guo et al., 2015) and may therefore explain the lower values in the monoculture treatments. Nutrient limitation, such as nitrogen or phosphorus deficiency, can lead to oxidative stress in algae by altering the balance of cellular redox reactions and impairing the ability of the algae to scavenge reactive oxygen species (ROS, Sharma et al., 2012; Chokshi et al., 2017). In high concentrations, ROS are extremely harmful since they can cause oxidative damage to the photosystems and other cellular components by damaging proteins and lipids. Limitation of N and P are known to decrease photosynthetic efficiency in algae for example by decreasing the abundance of D1 proteins forming the reaction centers in the PSII (Kolber et al., 1988; Berges et al., 1996) or by decreasing cellular chlorophyll concentrations (Litchman et al., 2003), selectively inactivating the photosystems. Therefore, a decline in Fv/Fm values may indicate an inhibition of PSII, more precisely an impairment of electron transport through the photosystem. Such an inhibition can be caused and accelerated by different environmental stressors such as high light intensities or temperature (Takahashi and Murata, 2008; Goh et al., 2012). To maintain a balance between the production and scavenging of

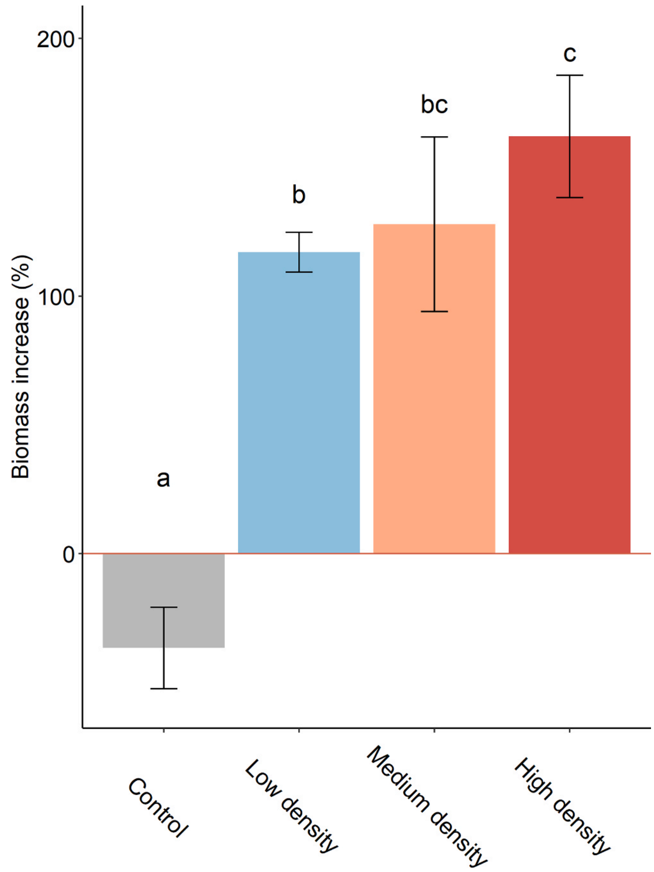

Fig. 10. Biomass increase (%) of *Caulerpa lentillifera* after 4 weeks in the indoor laboratory experiment. Treatments: Control: Only *Caulerpa*; Low: *Caulerpa* and 2 *Babylonia areolata* snails (33 m^{-2}); Medium: *Caulerpa* and 4 snails (66 m^{-2}); High: *Caulerpa* and 8 snails (133 m^{-2}). Significant differences between the treatments are shown by different letters (One-way ANOVA with post-hoc test, $p < 0.05$). Data are mean ± standard deviation, n = 5.

Table 3
Mean concentrations of nitrogen oxides (NO_x) and phosphate (PO_4) on the last day of the indoor laboratory experiment. Treatments: Control: Only *Caulerpa lentillifera*; Low: *Caulerpa* and 2 *Babylonia areolata* snails (33 m^{-2}); Medium: *Caulerpa* and 4 snails (66 m^{-2}); High: *Caulerpa* and 8 snails (133 m^{-2}). Significant differences between the treatments are shown by different letters (One-way ANOVA with post-hoc test, $p < 0.05$). Data are mean ± standard deviation, n = 5.

Treatment	NO_x (μmol L^{-1})	PO_4 (μmol L^{-1})
Control	0.99 ± 0.47^a	0.33 ± 0.08^a
Low	5.95 ± 3.67^a	0.96 ± 0.51^a
Medium	64.31 ± 41.08^b	2.45 ± 0.79^a
High	95.08 ± 14.95^b	6.59 ± 2.80^b

ROS, seaweeds have developed a complex network of antioxidants to prevent oxidative stress and damage (Sharma et al., 2012). Antioxidants inhibit oxidative processes by scavenging free ROS, that can cause cellular damage (Yu, 1994; Zampelas and Micha, 2015).

Overall, the presence of *Babylonia* had a positive effect on algal growth. A study by Williams et al. (1985) showed that burrowing activity of benthic macrofauna, such as snails, resulted in significantly lower stolon growth, biomass accumulation, and growth rate of several *Caulerpa* species. This was not observed in the present study. The better growth of *Caulerpa* in the monocultures when cultured in the sediment can be explained by the availability of nutrients in the soil. Beach sand is composed of minerals and organic material derived from the surrounding environment, such as rocks, shells, and corals. It can also contain organic matter, such as decaying plant and animal material, which is a rich source for decomposing microorganisms and therefore nutrient recycling (Leeder, 2011).

4.1.2. Effects on biochemical parameters of Caulerpa

Values for the antioxidant activity (AOA) of *Caulerpa* of ~100–150 mmol TE 100 g^{-1} DW of sea grapes were in the same range as reported in other studies (Matanjun et al., 2008; Ismail et al., 2020).

Interestingly, the total phenolic content (TPC) of the sea grapes was affected by the co-culture approach and decreased in all treatments. The initial values (180.21 ± 18.43 GAE 100 g^{-1} DW) were higher than in previous studies (e.g. Ismail et al., 2020; Stuthmann et al., 2022). Abiotic and biotic stressors are known to induce the production of secondary metabolites, including phenols (e.g. Chakraborty et al., 2015; Dixit et al., 2018; Cotas et al., 2020). As mentioned before, higher algal stress in the "Control" treatment due to nutrient limitation may explain the higher TPC content of the sea grapes in this treatment. Phenolic compounds contribute significantly to the antioxidative potential of *Caulerpa* (Nguyen et al., 2011; Ismail et al., 2020). Although a correlation between these two parameters has been reported (Stuthmann et al., 2022), no correlation between AOA and TPC was found in this study. This suggests that the two parameters are not directly related. The reduction of TPC in the algae despite constant AOA could be the result of a change in the composition of antioxidative compounds rather than a decrease in total phenolic content. This is consistent with the results of Nguyen et al. (2011) and Wichachucherd et al. (2019), who also reported no correlation between TPC and AOA.

Future studies could investigate the effect of co-culture on the proximate nutritional composition of the sea grapes. Previous studies have reported higher protein and lipid concentrations in sea grapes co-cultured with shrimp due to the additional nutrients provided by the fed species (Anh et al., 2022; Omont et al., 2022). In the present study, it was shown that the excretion of the snails acted as a natural fertilizer, enriching the water with essential nutrients. Therefore, it may be possible to increase the protein and lipid concentrations and subsequently the nutritional value of sea grapes by co-culturing the algae with *Babylonia*, similar to the results of previous studies.

4.1.3. Effects on growth and survival of Babylonia

The growth rates of *Babylonia* obtained in this study are consistent with the results reported in previous studies. In the present study, both growth and survival of *Babylonia* were not affected by spatial separation from *Caulerpa*. *Babylonia* growth was consistent in both treatments and the snails nearly doubled in weight over the course of this study. Similar results were reported in a study in which *Babylonia* was co-cultured with sandfish and *Caulerpa* and increased in biomass by approximately 300 % during the 84-day long experimental run (Dobson et al., 2020a). A co-culture study with *Caulerpa* and *Babylonia* in a RAS system showed that the presence of *C. lentillifera* did not affect growth of the snails (total shell length, body weight), but significantly increased their survival rate by 4–7 % (Chaitanawisuti et al., 2011).

4.2. Effects of different Babylonia stocking densities on growth of Caulerpa

Caulerpa weight gain was positively correlated with snail density. The "low density" treatment was with ~160 g m^{-2} comparable to the snail densities in the outdoor experiment (~200 g m^{-2}) which is also reflected in the nutrient concentrations. With increasing snail density, the nutrient concentrations of NO_x and PO_4 increased significantly, reaching values that are close to the recommended N and P concentrations for *Caulerpa* (see Section 4.1.1). Snail density in the "high density" treatment was comparable to 640 g m^{-2}, which is in the upper range of commercial stocking densities (Chaitanawisuti et al., 2002; Dobson et al., 2020c). The high increase in *Caulerpa* biomass in this treatment indicates that snails can be implemented at high densities with positive implications for the seaweed. Similar to the results of the outdoor experiment, results of the indoor experiment also show that the seaweed benefits from the additional nutrients provided by the snails, highlighting the feasibility of the co-culture approach of *Babylonia* and *Caulerpa*.

4.3. Importance of the right co-culture set-up

Many aquaculture studies of seaweeds and fed species focus either on the bioremediation properties of the investigated algae or the most beneficial densities of the cultured organisms (e.g. Anh et al., 2019; Bambaranda et al., 2019a). However, the design of the entire system and, in particular, the positioning of the organisms within it are of great importance for the successful implementation of co-cultures.

An important aspect to consider is the potential trade-offs that may occur in co-culture systems. Studies with whiteleg shrimp *Litopenaeus vannamei* and *Caulerpa* in same-tank cultures showed that *Caulerpa* improved water quality and shrimp performance, but the sea grapes also showed impaired growth with increasing shrimp density due to grazing behavior and mechanical disturbance by the shrimp (Ly et al., 2021; Anh et al., 2022). Dobson et al. (2020a) showed that the integration of sea grape trays into a sandfish culture even reduced the weight gain and subsequently the yield of sea cucumbers. The authors assumed that the floating trays have shaded the bottom of the tank and, as a consequence, reduced food sources for the sandfish by limiting the growth of microalgae and bacteria. The results of these studies highlight the importance of carefully considering the specific requirements and interactions between the organisms involved.

In the present study, no negative impacts of the co-culture were observed. Since the carnivorous snails do not feed on *Caulerpa*, spatial separation may not be necessary. However, although not observed in the present study, it is possible that the implementation of higher snail densities and the consequent increase in snail burrowing activity could lead to disturbances of sea grapes, especially if the sea grapes are cultivated using the sowing method. The use of the tray method could prevent these mechanical disturbances. Further studies are required to investigate these potential negative effects of the burrowing activity of *Babylonia* snails on *Caulerpa* in sowing cultures.

4.4. Implications and potential for the Van Phong Bay

In Van Phong Bay, *Caulerpa* and *Babylonia* are heavily cultivated and the production chains for both high-value organisms are already established. Outdoor cultivation of both organisms takes place during the dry season (March until October) due to more stable environmental conditions (Mai et al., 2022).

Co-cultivation can provide a broader employment and diversification opportunity for local people and farmers in Van Phong Bay, generating additional income by tapping into new markets (Chopin et al., 2012). However, the economic feasibility needs to be assessed and potential increases in the amount of labor and materials and equipment need to be considered. Incorporating snails into *Caulerpa* ponds provides additional nutrients to the system, so that the frequency of water exchanges can be reduced, thus saving labor. However, the snails must be fed regularly, which in turn increases labor and costs.

Sea grapes are not grown in the outer areas of the ponds, so the snails could be easily integrated into these regions without losing cultivation space of the seaweeds. Normally, spatial separation of co-cultured species increases the cost for farmers by increasing the required space/volume (Dobson, 2020a), which, however, does not apply here. Based on the results of this study, snail densities of 320–640 g m^{-2} can be recommended for an initial trial, but the implementation of even higher densities may be possible. Further large-scale experiments are needed to assess the effects of excess food on sea grape physiology. At VIJA, partial water exchanges take place daily according to the tide regime (corresponding to one full cycle every two days). In a commercial pond co-culture set-up, removal of trash fish would not be feasible and an increase in the water exchange rate might be necessary. Co-cultivation of *C. lentillifera* and *B. areolata* in Van Phong Bay shows great feasibility and potential. Even though some investment may be necessary, farmers could still make financial gains by integrating high-value *Babylonia* into existing *Caulerpa* ponds. Since sea grapes can be cultured using different methods and the co-culture approach (separated and together) with the snails did not negatively affect the organisms, a variety of co-culture set-ups is conceivable, which benefits farmers by allowing highly flexible system designs.

5. Conclusions

This study, for the first time, has shown the high potential of integrating *B. areolata* into pond cultures of *C. lentillifera*. Co-culture had a positive effect on both growth and physiology of the sea grapes. The presence of *Babylonia* snails benefited the algae and improved harvestable biomass and growth compared to the sea grape monoculture. Spatial separation from the seaweed had no effect on the snails and the indoor laboratory experiment showed that even high stocking densities of *Babylonia* can be implemented without negative effects on the seaweed. Taking this into account and the fact that the outdoor cultivation of both organisms in Viet Nam is restricted to the dry months, it seems feasible to integrate snails into existing *Caulerpa* ponds. Since the spatial separation did not affect the snails or the sea grapes, *Babylonia* could be integrated into the areas of the seaweed ponds where *Caulerpa* is not planted.

The cultivation parameters have been chosen according to real culture conditions in the field, but the experiments are only on a pilot scale in tanks and should be carried out on a larger scale in the field. The study showed that the integration of *Babylonia* into existing sea grape ponds seems feasible and that a co-cultivation of these two high-value species could increase the economic profit of sea grape farmers without reducing the yield of *Caulerpa*. Further studies should also investigate the effect on the proximate nutritional composition (i.e. proteins) of sea grapes when co-cultured with *Babylonia*.

Funding

The conduction of the research was supported by the Marine Botany working group of the University of Bremen and by Leibniz Centre for Tropical Marine Research in-house funding issued under #6073. AJ Cordes was supported by Kellner&Stoll-Stiftung.

The funding sources were not involved in study design or the preparation of the article.

CRediT authorship contribution statement

Beatrice Brix da Costa: Conceptualization, Investigation, Formal analysis, Visualization, Writing – Original Draft Preparation; **Lara Elisabeth Stuthmann**: Investigation, Writing – Review & Editing; **Aaron Johannes Cordes**: Investigation, Writing – Review & Editing; **Hoang Tru Du**: Writing – Review & Editing, Resources; **Andreas Kunzmann**: Writing – Review & Editing, Supervision, Funding acquisition; **Karin Springer**: Writing – Review & Editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data will be made openly available at figshare (DOI: 10.6084/

m9.figshare.23659683).

Acknowledgements

The authors would like to thank the staff of the Institute of Oceanography and the VIJA farm for the helpful support on-site, Mia Pribbernow for the help in the field, as well as the staff of the Chem Lab at ZMT for their technical assistance with the nutrient measurements.

Authorship contribution statement

The authorship contribution statement is included in the main text of the manuscript.

Appendix

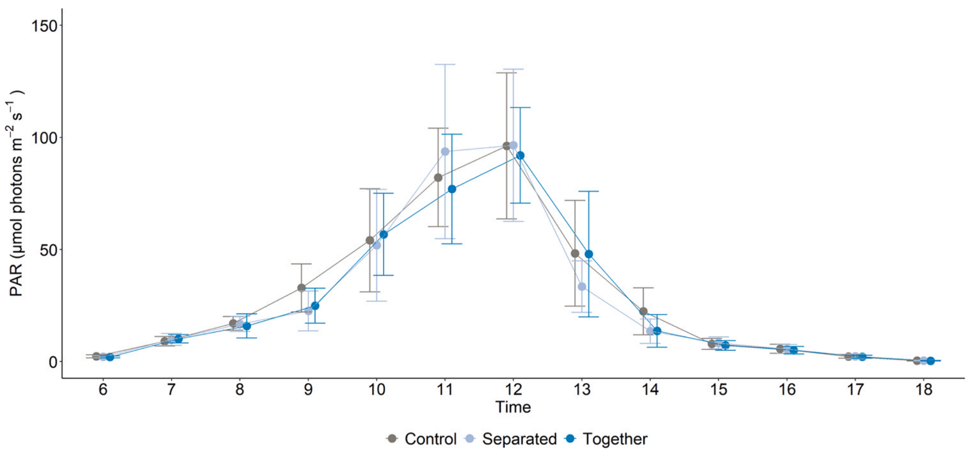

Fig A.1. Light irradiances of photosynthetically active radiation (PAR) in the experimental tanks of the outdoor terrace experiment over one day. Treatments: Control: Only *Caulerpa lentillifera*; Separated: *C. lentillifera* and *Babylonia areolata* spatially separated; Together: *C. lentillifera* and *B. areolata* together. Data are mean ± standard deviation, n = 3.

Table A.1
Summary statistics of the ANOVAs assessing the effect of the parameters treatment (T), culture method (CM), and time (W) on the relative growth rate (RGR), harvestable biomass, maximum quantum yield of photosystem II (Fv/Fm), antioxidant activity (AOA), total phenolic content (TPC) of *Caulerpa lentillifera*, and concentrations of nitrogen oxides (NO_X) and phosphate (PO_4) of the water. Statistically significant values are written in italic. Dfn: numerator degrees of freedom; DFd: denominator degrees of freedom.

Outdoor terrace experiment	**Parameter**	**DFn**	**DFd**	**F value**	**p value**
RGR *Caulerpa*	T	1.11	5.54	28.8	*<0.001*
	CM	1	5	8.12	*<0.001*
	W	1.03	5.17	123.6	*<0.001*
	TxCM	2	10	33.78	*<0.001*
	TxW	4	20	10.25	*<0.001*
	CMxW	2	10	81.65	*<0.001*
	TxCMxW	4	20	3.35	*<0.001*
Harvestable biomass	T	2	10	22.1	*<0.001*
	CM	1	5	14.12	*<0.001*
	W	2	10	4.77	*<0.001*
	TxCM	2	10	22.72	*<0.001*
	TxW	4	20	18.08	*<0.001*
	CMxW	2	10	2.09	*<0.001*
	TxCMxW	4	20	6.59	*<0.001*
Fv/Fm	T	2	186	8.26	*<0.001*
	CM	1	186	0.51	0.48
	TxCM	2	186	0.23	0.79
AOA	T	3	48	2.78	0.05
	CM	1	48	0.62	0.43
	TxCM	3	48	0.5	0.68
TPC (sowing)	T	3	25	27.99	*<0.001*
NO_X	T	2	6	5.97	*0.04*
PO_4	T	2	6	8.48	*0.02*
Indoor laboratory experiment					
Biomass increase *Caulerpa*	T	3	16	76.92	*<0.001*
NO_X	T	3	8	13.08	*<0.01*
PO_4	T	3	8	10.87	*<0.01*

Table A.2
Summary statistics of non-parametric tests assessing the effect of the parameters treatment (T), and time (W) on the total phenolic content (TPC) of *Caulerpa lentillifera*, relative growth rate (RGR), and weight of *Babylonia areolata*. Statistically significant values are written in italic. Df: degrees of freedom.

	Parameter	n	Test statistic	Df	p value	Method
TPC (tray)	T	27	21.6	3	*< 0.001*	Kruskal-Wallis
RGR *Babylonia*	T	18	129		0.3	Wilcoxon
Weight *Babylonia*	W	60	-14.4	59	*< 0.001*	Pairwise t-test
Separated	T	60	-0.01	59	0.99	
Together	W	60	-14.2	59	*< 0.001*	
	T	60	-0.11	59	0.91	

References

Abreu, M.H., Pereira, R., Yarish, C., Buschmann, A.H., Sousa-Pinto, I., 2011. IMTA with *Gracilaria vermiculophylla*: productivity and nutrient removal performance of the seaweed in a land-based pilot scale system. Aquaculture 312, 77–87. https://doi.org/10.1016/j.aquaculture.2010.12.036.

Alexandre, A., Santos, R., 2020. High nitrogen and phosphorous acquisition by belowground parts of *Caulerpa prolifera* (Chlorophyta) contribute to the species' rapid spread in Ria Formosa Lagoon, Southern Portugal. J. Phycol. 56, 608–617. https://doi.org/10.1111/jpy.12988.

Anh, N.T.N., An, B.N.T., Lan, L.M., Hai, T.N., 2019. Integrating different densities of white leg shrimp *Litopenaeus vannamei* and red seaweed *Gracilaria tenuistipitata* in the nursery phase: effects on water quality and shrimp performance. J. Appl. Phycol. 31, 3223–3234. https://doi.org/10.1007/s10811-019-01824-7.

Anh, N.T.N., Shayo, F.A., Nevejan, N., Van Hoa, N., 2021. Effects of stocking densities and feeding rates on water quality, feed efficiency, and performance of white leg shrimp *Litopenaeus vannamei* in an integrated system with sea grape *Caulerpa lentillifera*. J. Appl. Phycol. 33, 3331–3345.

Anh, N.T.N., Murungu, D.K., Khanh, L.V., Hai, T.N., 2022. Polyculture of sea grape (*Caulerpa lentillifera*) with different stocking densities of whiteleg shrimp (*Litopenaeus vannamei*): Effects on water quality, shrimp performance and sea grape proximate composition. Algal Res. 67, 102845 https://doi.org/10.1016/j.algal.2022.102845.

Bambaranda, B.V.S.A.M., Sasaki, N., Chirapart, A., Salin, K.R., Tsusaka, T.W., 2019a. Optimization of macroalgal density and salinity for nutrient removal by *Caulerpa lentillifera* from aquaculture effluent. Processes 7, 303.

Bambaranda, B.V.S.A.M., Tsusaka, T.W., Chirapart, A., Salin, K.R., Sasaki, N., 2019b. Capacity of *Caulerpa lentillifera* in the removal of fish culture effluent in a recirculating aquaculture system. Processes 7, 440.

Berges, J.A., Charlebois, D.O., Mauzerall, D.C., Falkowski, P.G., 1996. Differential effects of nitrogen limitation on photosynthetic efficiency of photosystems I and II in microalgae. Plant Physiol. 110, 689–696.

Cai J., Lovatelli A., Aguilar-Manjarrez J., Cornish L., Dabbadie L., Desrochers A., Diffey S., Garrido Gamarro E., Geehan J., Hurtado A., Lucente D., Mair G., Miao W., Potin P., Przybyla C., Reantaso M., Roubach R., Tauati M., Yuan X. (2021a) Seaweeds and microalgae: an overview for unlocking their potential in global aquaculture development. FAO Fisheries and Aquaculture Circular No. 1229, Rome, FAO. https://doi.org/10.4060/cb5670en.

Cai, Y., Li, G., Zou, D., Hu, S., Shi, X., 2021b. Rising nutrient nitrogen reverses the impact of temperature on photosynthesis and respiration of a macroalga *Caulerpa lentillifera* (Ulvophyceae, Caulerpaceae). J. Appl. Phycol. 33, 1115–1123. https://doi.org/10.1007/s10811-020-02340-9.

Chaitanawisuti, N., Kritsanapuntu, A., 1997. Laboratory spawning and juvenile rearing of the marine gastropod, spotted babylon, *Babylonia areolata* Link 1807 (Neogastropoda. Buccinidae) in Thailand. J. Shellfish Res 16, 31–39.

Chaitanawisuti, N., Kritsanapuntu, A., Natsukari, Y., Kathinmai, S., 2001. Effects of different types of substrate on growth and survival of juvenile spotted babylon, *Babylonia areolata* Link 1807, reared to marketable size in a flow-through seawater system. Asian Fish. Sci. 14, 279–284. https://doi.org/10.33997/j.afs.2001.14.3.004.

Chaitanawisuti, N., Kritsanapuntu, S., Natsukari, Y., 2002. Economic analysis of a pilot commercial production for spotted babylon, *Babylonia areolata* (Link 1807), of marketable sizes using a flow-through culture system in Thailand. Aquac. Res 33, 1265–1272. https://doi.org/10.1046/j.1365-2109.2002.00790.x.

Chaitanawisuti, N., Santhaweesuk, W., Kritsanapuntu, S., 2011. Performance of the seaweeds *Gracilaria salicornia* and *Caulerpa lentillifera* as biofilters in a hatchery scale recirculating aquaculture system for juvenile spotted babylons (*Babylonia areolata*). Aquac. Int. 19, 1139–1150. https://doi.org/10.1007/s10499-011-9429-9.

Chakraborty, K., Joseph, D., Praveen, N.K., 2015. Antioxidant activities and phenolic contents of three red seaweeds (Division: Rhodophyta) harvested from the Gulf of Mannar of Peninsular India. J. Food Sci. Technol. 52, 1924–1935.

Chávez-Crooker, P., Obreque-Contreras, J., 2010. Bioremediation of aquaculture wastes. Curr. Opin. Biotechnol. 21, 313–317. https://doi.org/10.1016/j.copbio.2010.04.001.

Chokshi, K., Pancha, I., Ghosh, A., Mishra, S., 2017. Nitrogen starvation-induced cellular crosstalk of ROS-scavenging antioxidants and phytohormone enhanced the biofuel potential of green microalga *Acutodesmus dimorphus*. Biotechnol. Biofuels 10 (1), 12. https://doi.org/10.1186/s13068-017-0747-7.

Chopin, T., Tacon, A.G.J., 2021. Importance of seaweeds and extractive species in global aquaculture production. Rev. Fish. Sci. Aquac. 29, 139–148. https://doi.org/10.1080/23308249.2020.1810626.

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G. P., Zertuche-González, J.A., Yarish, C., Neefus, C., 2001. Integrating seaweeds into marine aquaculture systems: a key toward sustainability. J. Phycol. 37, 975–986. https://doi.org/10.1046/j.1529-8817.2001.01137.x.

Chopin, T., Cooper, J.A., Reid, G., Cross, S., Moore, C., 2012. Open-water integrated multi-trophic aquaculture: environmental biomitigation and economic diversification of fed aquaculture by extractive aquaculture. Rev. Aquac. 4, 209–220. https://doi.org/10.1111/j.1753-5131.2012.01074.x.

Cotas, J., Leandro, A., Monteiro, P., Pacheco, D., Figueirinha, A., Gonçalves, A.M.M., Jorge, G., Pereira, L., 2020. Seaweed phenolics: From extraction to applications. Mar. Drugs 18, 1–47.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., Verstraete, W., 2007. Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270, 1–14. https://doi.org/10.1016/J.AQUACULTURE.2007.05.006.

Daud, D., Zainal, A.N., Nordin, M.N., Tawang, A., Ismail, A., 2020. Chelation activity and protective effect of *Caulerpa lentillifera* aqueous extract against lead acetate-induced toxicity in Sprague Dawley rats. J. Appl. Pharm. Sci. 10, 145–148. https://doi.org/10.7324/JAPS.2020.10817.

Dixit, D.C., Reddy, C.R.K., Balar, N., Suthar, P., Gajaria, T., Gadhavi, D.K., 2018. Assessment of the nutritive, biochemical, antioxidant and antibacterial potential of eight tropical macro algae along kachchh coast, india as human food supplements. J. Aquat. Food Prod. Technol. 27, 61–79.

Dobson, G.T., Duy, N.D.Q., Paul, N.A., Southgate, P.C., 2020a. Assessing potential for integrating sea grape (*Caulerpa lentillifera*) culture with sandfish *(Holothuria scabra*) and Babylon snail (*Babylonia areolata*) co-culture. Aquaculture 522, 735153. https://doi.org/10.1016/j.aquaculture.2020.735153.

Dobson, G.T., Duy, N.D.Q., Southgate, P.C., 2020b. First assessment of the potential for coculture of sandfish (*Holothuria scabra*) with Babylon snail (*Babylonia areolata)* in Vietnam. J. World Aquac. Soc. 51, 527–541. https://doi.org/10.1111/jwas.12676.

Dobson, G.T., Duy, N.D.Q., Southgate, P.C., 2020c. Preliminary assessment of large-scale co-culture of sandfish (*Holothuria scabra*) with the Babylon snail (*Babylonia areolata*) in earthen ponds and in raceways. J. World Aquac. Soc. 52, 138–154. https://doi.org/10.1111/jwas.12758.

FAO (2022) The State of World Fisheries And Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en.

de Gaillande, C., Payri, C., Remoissenet, G., Zubia, M., 2017. *Caulerpa* consumption, nutritional value and farming in the Indo-Pacific region. J. Appl. Phycol. 29, 2249–2266. https://doi.org/10.1007/s10811-016-0912-6.

García-Robledo, E., Corzo, A., Papaspyrou, S., 2014. A fast and direct spectrophotometric method for the sequential determination of nitrate and nitrite at low concentrations in small volumes. Mar. Chem. 162, 30–36.

Gillespie, K.M., Ainsworth, E.A., 2007. Measurement of reduced, oxidized and total ascorbate content in plants. Nat. Protoc. 2, 871–874. https://doi.org/10.1038/nprot.2007.101.

Goh, C.H., Ko, S.M., Koh, S., Kim, Y.J., Bae, H.J., 2012. Photosynthesis and environments: photoinhibition and repair mechanisms in plants. J. Plant Biol. 55, 93–101. https://doi.org/10.1007/s12374-011-9195-2.

Guo, H., Yao, J., Sun, Z., Duan, D., 2015. Effects of salinity and nutrients on the growth and chlorophyll fluorescence of *Caulerpa lentillifera*. Chin. J. Oceano Limnol. 33, 410–418.

Higo, S., Maung-Saw-Htoo-Thaw, Yamatogi, T., Ishida, N., Hirae, S., Koike, K., 2017. Application of a pulse-amplitude-modulation (PAM) fluorometer reveals its usefulness and robustness in the prediction of *Karenia mikimotoi* blooms: a case study in Sasebo Bay, Nagasaki, Japan. Harmful Algae 61, 63–70. https://doi.org/10.1016/j.hal.2016.11.013.

Ismail, M.F., Ramaiya, S.D., Zakaria, M.H., Mohd Ikhsan, N.F., Awang, M.A., 2020. Mineral content and phytochemical properties of selected *Caulerpa* species from Malaysia. Malays. J. Sci. 39, 115–131. https://doi.org/10.22452/mjs.vol39no3.10.

Kolber, Z., Zehr, J., Falkowski, P., 1988. Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in Photosystem II. Plant Physiol. 88, 923–929. https://doi.org/10.1104/pp.88.3.923.

Krause, G.H., Weis, E., 1984. Chlorophyll fluorescence as a tool in plant physiology - II. Interpretation of fluorescence signals. Photosynth. Res. 5, 139–157. https://doi.org/10.1007/BF00028527.

Kritsanapuntu, S., Chaitanawisuti, N., Santhaweesuk, W., Natsukari, Y., 2006. Growth, production and economic evaluation of earthen ponds for monoculture and polyculture of juveniles spotted Babylon (*Babylonia areolata*) to marketable sizes using large-scale operation. J. Shellfish Res. 25, 913–918. https://doi.org/10.2983/0730-8000(2006)25[913:GPAEEO]2.0.CO;2.

Kritsanapuntu, S., Chaitanawisuti, N., Santhaweesuk, W., Natsukari, Y., 2008. Growth performances for monoculture and polyculture of hatchery-reared juvenile spotted babylon, *Babylonia areolata* Link, 1807, in large-scale earthen ponds. Aquac. Res 39, 1556–1561. https://doi.org/10.1111/j.1365-2109.2008.02032.x.

Leeder, M.R., 2011. Sedimentology and sedimentary casins: From turbulence to tectonics, 2nd edn.,. Wiley-Blackwell, Chichester.

Litchman, E., Steiner, D., Bossard, P., 2003. Photosynthetic and growth responses of three freshwater algae to phosphorus limitation and daylength. Freshw. Biol. 48, 2141–2148. https://doi.org/10.1046/j.1365-2427.2003.01157.x.

Liu, H., Wang, F., Wang, Q., Dong, S., Tian, X., 2016. A comparative study of the nutrient uptake and growth capacities of seaweeds *Caulerpa lentillifera* and *Gracilaria lichenoides*. J. Appl. Phycol. 28, 3083–3089. https://doi.org/10.1007/s10811-016-0858-8.

Long, H., Gu, X., Zhu, Z., Wang, C., Xia, X., Zhou, N., Liu, X., Zhao, M., 2020. Effects of bottom sediment on the accumulation of nutrients in the edible green seaweed *Caulerpa lentillifera* (sea grapes). J. Appl. Phycol. 32, 705–716. https://doi.org/10.1007/s10811-019-01949-9.

Ly, K.V., Murungu, D.K., Nguyen, D.P., Nguyen, N.A.T., 2021. Effects of different densities of sea grape *Caulerpa lentillifera* on water quality, growth and survival of the whiteleg shrimp *Litopenaeus vannamei* in polyculture system. Fishes 6, 19.

Mai, M.D., Nguyen, Q.N., Tran, B.T.T., Vu, B.D.T., 2022. Growth performance of babylon snails (*Babylonia areolata* Link, 1807) fed formulated diet in ponds and recirculating aquaculture system. Agric. Fish. 11, 180–185. https://doi.org/10.11648/j.aff.20221105.15.

Malta, E.J., Ferreira, D.G., Vergara, J.J., Pérez-Lloréns, J.L., 2005. Nitrogen load and irradiance affect morphology, photosynthesis and growth of *Caulerpa prolifera* (Bryopsidales: Chlorophyta). Mar. Ecol. Prog. Ser. 298, 101–114. https://doi.org/10.3354/meps298101.

Manoppo, J.I.C., Nurkolis, F., Pramono, A., Ardiaria, M., Qhabibi, F.R., Yusuf, V.M., Amar, N., Rico, M., Karim, A., Subali, A.D., Natanael, H., Rompies, R., Halim, R.F., Sam, A., Bolang, L., Joey, G., Novianto, C.A., Permatasari, H.K., 2022. Amelioration of obesity-related metabolic disorders via supplementation of *Caulerpa lentillifera* in rats fed with a high-fat and high-cholesterol. Front. Nutr. 9, 1010867 https://doi.org/10.3389/fnut.2022.1010867.

Matanjun, P., Mohamed, S., Mustapha, N.M., Muhammad, K., Ming, C.H., 2008. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. J. Appl. Phycol. 20, 367–373. https://doi.org/10.1007/s10811-007-9264-6.

Matanjun, P., Mohamed, S., Mustapha, N.M., Muhammad, K., 2009. Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. J. Appl. Phycol. 21, 75–80. https://doi.org/10.1007/s10811-008-9326-4.

Moreira, A., Cruz, S., Marques, R., Cartaxana, P., 2022. The underexplored potential of green macroalgae in aquaculture. Rev. Aquac. 14, 5–26. https://doi.org/10.1111/raq.12580.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M., Yarish, C., 2004. Integrated aquaculture: Rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231, 361–391. https://doi.org/10.1016/j.aquaculture.2003.11.015.

Nghia, N.D., Lunestad, B.T., Trung, T.S., Son, N.T., Maage, A., 2009. Heavy metals in the farming environment and in some selected aquaculture species in the Van Phong Bay and Nha Trang Bay of the Khanh Hoa Province in Vietnam. Bull. Environ. Contam. Toxicol. 82, 75–79. https://doi.org/10.1007/s00128-008-9561-z.

Nguyen, V.T., Ueng, J.P., Tsai, G.J., 2011. Proximate composition, total phenolic content, and antioxidant activity of seagrape (*Caulerpa lentillifera*). J. Food Sci. 76, 950–958. https://doi.org/10.1111/j.1750-3841.2011.02289.x.

Omont, A., Peña-Rodríguez, A., Terauchi, S., Matsui, A., Magallón-Barajas, F., Torres-Ochoa, E., Endo, M., 2022. Growth performance and mineral composition of the white shrimp *Penaeus vannamei* and the sea grape *Caulerpa lentillifera* in a co-culture system. Aquac. Res. 1–13. https://doi.org/10.1111/are.16118.

Pariyawathee S., Songsangjinda P., Deraxbudsarakom S., Tuntichodok P., Chiayvareesajja S. (2003) Optimum condition of environmental factors for growth of sea grape (*Caulerpa lentillifera*: J. Agardh). Warasan Kanpramong (Thai Fish Gaz).

Qin, C.X., Liu, C.F., Zhang, L.Y., 2010. Removal of nitrate and phosphorus by *Ulva pertusa* and *Chondrus ocellatus* Holmes and biochemical compositions of macroalgae cultured at different concentration of nitrate. J. Hydroecol. 3, 41–46.

R Core Team (2020) A Language And Environment for Statistical Computing.

Rabia, M.D.S., 2016. Cultivation of *Caulerpa lentillifera* using tray and sowing methods in brackishwater pond. Environ. Sci. 4, 23–29. https://doi.org/10.12988/es.2016.51012.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med 26, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.

Ringuet, S., Sassano, L., Johnson, Z.I., 2011. A suite of microplate reader-based colorimetric methods to quantify ammonium, nitrate, orthophosphate and silicate concentrations for aquatic nutrient monitoring. J. Environ. Monit. 13, 370–376.

Roleda, M.Y., Hurd, C.L., 2019. Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. Phycologia 58, 552–562. https://doi.org/10.1080/00318884.2019.1622920.

Sangsawangchote, S., Chaitanawisuti, N., Piyatiratitivorakul, S., 2010. Reproductive performance, egg and larval quality and egg fatty acid composition of hatchery-reared Spotted Babylon (*Babylonia areolata*) broodstock fed natural and formulated diets under hatchery conditions. Int. J. Fish. Aquac. Sci. 1, 49–57.

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot. 1–26. https://doi.org/10.1155/2012/217037.

Stuthmann, L.E., Springer, K., Kunzmann, A., 2020. Cultured and packed sea grapes (*Caulerpa lentillifera*): effect of different irradiances on photosynthesis. J. Appl. Phycol. 33, 1125–1136. https://doi.org/10.1007/s10811-020-02322-x.

Stuthmann, L.E., Achuthan, R., Pribbernow, M., Du, H.T., Springer, K., Kunzmann, A., 2022. Improving the nutritional value of edible *Caulerpa lentillifera* (Chlorophyta) using high light intensities. A realistic tool for sea grape farmers. Algal Res. 66, 102785 https://doi.org/10.1016/j.algal.2022.102785.

Syakilla, N., George, R., Chye, F.Y., Pindi, W., Mantihal, S., Wahab, N.A., Fadzwi, F.M., Gu, P.H., Matanjun, P., 2022. A review on nutrients, phytochemicals, and health benefits of green seaweed, *Caulerpa lentillifera*. Foods 11, 2832. https://doi.org/10.3390/foods11182832.

Takahashi, S., Murata, N., 2008. How do environmental stresses accelerate photoinhibition? Trends Plant Sci. 13, 178–182. https://doi.org/10.1016/j.tplants.2008.01.005.

Tanaka, Y., Ashaari, A., Mohamad, F.S., Lamit, N., 2020. Bioremediation potential of tropical seaweeds in aquaculture: low-salinity tolerance, phosphorus content, and production of UV-absorbing compounds. Aquaculture 518, 734853. https://doi.org/10.1016/j.aquaculture.2019.734853.

Thomas, M., Pasquet, A., Aubin, J., Nahon, S., Lecocq, T., 2021. When more is more: taking advantage of species diversity to move towards sustainable aquaculture. Biol. Rev. 96, 767–784. https://doi.org/10.1111/brv.12677.

Wichachucherd, B., Pannak, S., Saengthong, C., Rodcharoen, E., Koodkaew, I., 2019. Correlation between growth, phenolic content and antioxidant activity in the edible seaweed, *Caulerpa lentillifera* in open pond culture system. J. Fish. Environ. 43, 66–75.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., et al., 2019. Welcome to the Tidyverse. J. Open Source Softw. 4, 1686. https://doi.org/10.21105/joss.01686.

Williams, S.L., 1984. Uptake of sediment ammonium and translocation in a marine green macroalga Caulerpa cupressoides. Limnol. Oceanogr. 29, 374–379. https://doi.org/10.4319/lo.1984.29.2.0374.

Williams, S.L., Breda, V., Anderson, T., Nyden, B., 1985. Growth and sediment disturbances of *Caulerpa spp*. (Chlorophyta) in a submarine canyon. Mar. Ecol. Prog. Ser. 21, 275–281. https://doi.org/10.3354/meps021275.

Yu, B.P., 1994. Cellular defenses against damage from reactive oxygen species. Physiol. Rev. 74, 139–162. https://doi.org/10.1152/physrev.1994.74.1.139.

Zampelas A., Micha R. (eds) (2015) Antioxidants in health and disease. 1st edn. CRC Press, Boca Raton.

Zubia, M., Draisma, S.G.A., Morrissey, K.L., Varela-Álvarez, E., De Clerck, O., 2020. Concise review of the genus *Caulerpa* J.V. Lamouroux. J. Appl. Phycol. 32, 23–39. https://doi.org/10.1007/s10811-019-01868-9.