



## Carbon capture by macroalgae *Sarcodia suae* using aquaculture wastewater and solar energy for cooling in subtropical regions



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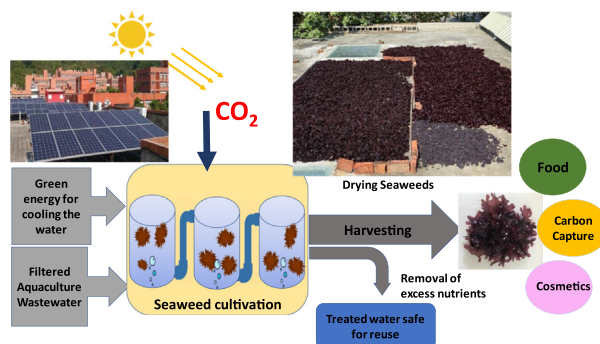
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### HIGHLIGHTS

- *Sarcodia suae* is an excellent carbon catcher when temperature can be controlled during the summer.
- Nutrients are replete in aquaculture wastewater and they can be assimilated by *Sarcodia suae*.
- *Sarcodia suae* could help mitigate the impacts of wastewater on coastal eutrophication.
- Carbon fixation by *Sarcodia suae* can be conducted with an easy and low-cost facility.
- The mean carbon fixation rate of *Sarcodia* is 3-fold higher than the average global forest trees.

### GRAPHICAL ABSTRACT

Schematic illustration of carbon fixation of *Sarcodia suae* utilizing wastewater and green energy.



### ARTICLE INFO

Editor: Kuishuang Feng

#### Keywords:

Carbon catch and sequestration (CCS)

Wastewater

Nutrient removal

Seaweed

Carbon neutrality

Taiwan

### ABSTRACT

Rapid growth in the aquaculture industry and corresponding increases in nutrient and organic carbon levels in coastal regions can lead to eutrophication and increased greenhouse gas emissions. Macroalgae are the organisms primarily responsible for the capture of CO<sub>2</sub> and removal of nutrients from coastal waters. In the current study, we developed a novel wastewater treatment system in which the red macroalga, *Sarcodia suae*, is used to capture CO<sub>2</sub> under thermodynamic conditions in subtropical regions. In 2020 (without temperature control), the carbon capture rate (CCR) of *Sarcodia suae* varied considerably with the season: winter/spring (2.1–3.9 g-C m<sup>-2</sup> d<sup>-1</sup>) and summer (0.09 g-C m<sup>-2</sup> d<sup>-1</sup>). In 2021, solar powered cooling reduced summer seawater temperatures from 31 to 33 °C to 23–25 °C with a corresponding increase in the mean CCR: winter/spring (2–7 g-C m<sup>-2</sup> d<sup>-1</sup>) and summer (1.33 g-C m<sup>-2</sup> d<sup>-1</sup>). The proposed aquaculture wastewater system proved highly efficient in removing nitrogen (20.7 mg-N g<sup>-1</sup> DW d<sup>-1</sup>, DW = dry weight) and phosphorus (4.4 mg-P g<sup>-1</sup> DW d<sup>-1</sup>). Furthermore, the high density of *Sarcodia* (1.10 ± 0.03 g cm<sup>-3</sup>) would permit the harvesting and subsequent dumping of *Sarcodia* in deep off-shore waters. This study demonstrated a low-cost land-based seaweed cultivation system for capturing CO<sub>2</sub> and excess nutrients from aquaculture wastewater year-round under temperature controlled environments in subtropical regions.

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<http://dx.doi.org/10.1016/j.scitotenv.2022.158850>

Received 12 June 2022; Received in revised form 6 September 2022; Accepted 14 September 2022

Available online 19 September 2022

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## 1. Introduction

Macroalgae (seaweed) is the most common algae in the shallow coastal waters adjacent to continental shelves. These simple organisms, lacking true roots, stems, and leaves, provide a rich food source for humans and animals (Fleurence et al., 2012), raw material for the manufacture of cosmetics and biomedical products (Vonthron-Sénécheau, 2016), and biofertilizer for the agriculture industry (Thirumaran et al., 2009). Meanwhile, macroalgae have been used as feedstock to produce various bionergy forms in sustainable circular economies (Devadas et al., 2021; Leong et al., 2021; Chia et al., 2022; Kumar et al., 2020). They have also attracted considerable attention for the sequestration of carbon dioxide (CO<sub>2</sub>) and dissolved organic carbon (POC and DOC) compounds in the deep ocean and oceanic sediments (Hill et al., 2015; Krause-jensen et al., 2018).

Seaweed can be harvested from wild sources, farmed in coastal waters, or from land-based aquaculture installations. Despite its relatively low value (Rusekwa et al., 2020), intensive culturing has made seaweed the second largest aquaculture product in terms of volume (114.5 million t in 2018) (FAO, 2020). Chung et al. (2011) posited the use of seaweed in carbon capture and the amelioration of CO<sub>2</sub> emissions. Duarte et al. (2017) suggested a potential role for seaweed farming in mitigating climate change. *Saccharina japonica*, *Euchema*, *Porphyra*, *Pyropia*, *Undaria pinnatifida*, *Kappaphycus*, and *Sargassum* (Chopin and Tacon, 2021) have undergone cold-water cultivation for food and medical purposes (Tacon and Metian, 2013). Note however that the year-round cultivation of individual seaweeds is limited by seasonal variations in water temperature, light intensity, salinity, nutrient availability, and geographic features (Chopin and Tacon, 2021). In the current study, we sought to establish a year-round seaweed cultivation system for subtropical regions.

In 2018, 96.4 million t of fish was caught; however, even this was insufficient to satisfy consumer demand (Wu et al., 2015). It is crucial therefore to develop sustainable farming practices for coastal marine waters. Rapid expansion in finfish and shellfish aquaculture (Wilfart et al., 2013) contributed >30.8 million t of product in 2018 (FAO, 2020); however, it also had alarming adverse environmental effects on coastal marine ecosystems (Anh et al., 2010). Intensive marine aquaculture inevitably increases the concentrations of organic matter, residual feed products, and feces as well as inorganic pollutants, such as ammonia, nitrites and nitrates (Krasaesueb et al., 2019; Chopin et al., 2001). Heavy nutrient discharge can result in eutrophication, hypoxia, red tides and green tides in receiving waters (Abreu et al., 2011; Hsieh et al., 2021). This situation has prompted research into integrated multi-trophic aquaculture (IMTA) to facilitate the removal of excess nutrients and carbon from aquaculture wastewater (Abreu et al., 2011; Xu et al., 2011). Nonetheless, the practical applicability of these methods is curtailed by high summer seawater temperatures (>28 °C), which are unfavorable to the growth of many seaweed species. Elevated spring/summer temperatures affect numerous subtropical regions (China, Indonesia, India, Taiwan, Philippines, Vietnam, Sri Lanka) as well as regions at higher latitudes (China, Korea, Japan). For example, kelp has tremendous potential for carbon capture in high latitude regions; however, it is unsuitable for most subtropical regions.

The coastal regions of Taiwan are home to intensive aquaculture (Liao et al., 2019); however, many of the small facilities lack wastewater treatment systems (Chen and Qiu, 2014; Yeh et al., 2017). Note that the limited availability of usable land in Taiwan also hinders the implementation of ecologically friendly wastewater treatment systems combining fish aquaculture with seaweed aquaculture.

Taiwan produced 290 million t of carbon dioxide in 2020 (EPA in Taiwan, [www.epa.gov.tw](http://www.epa.gov.tw)); however, plans are underway for carbon sequestration systems to achieve carbon neutrality by 2050. Researchers have developed a wide range of open and closed carbon capture systems involving the cultivation of microalgae and nanochloropsis algae (Chiu et al., 2011; Kao et al., 2014). Note that microalgae are easily cultivated in small-scale closed systems (several hundred liters); however, those methods are not easily implemented at larger scales (several thousand liters). To our knowledge, no large-scale outdoor microalgae carbon capture system has been established in Taiwan.

Seaweed farming has been proposed as an alternative approach to carbon sequestration. The carbon captured in seaweed is transported out of coastal waters for long-term disposal in the deep ocean (Duarte et al., 2017; Krause-Jensen et al., 2018; Ortega et al., 2019; Chen and Xu, 2020). The red seaweed *Sarcodia suae* is easily cultured under artificial conditions in the coastal waters of Taiwan (20–28 °C) (Su, 2012; Lee et al., 2019). The specific density of *Sarcodia* exceeds that of seawater means that the associated detritus provides a natural carbon sink. Nonetheless, there has been little research on the cultivation of *Sarcodia* for carbon capture.

Our objective in the current study was to establish a year-round *Sarcodia suae* cultivation system to capture CO<sub>2</sub> and excess nutrients from aquaculture wastewater. Note that this necessitated the development of a solar powered water cooling system. We compared the blue carbon capture capacity of *Sarcodia suae* with that of other seaweed species and marine phytoplankton from various marine environments. We also compared the proposed system with existing systems in terms of carbon emissions and carbon sequestration in Taiwan.

## 2. Materials and methods

### 2.1. Seaweed cultivation

The proposed land-based multi-trophic mariculture system was established at the Department of Oceanography, National Sun Yat-sen University, Kaohsiung, Taiwan. The proposed system combined an aquaculture system for fauna (e.g., shrimp, lobsters, and groupers) and one specific macroalgae (*Sarcodia suae*). Culturing was performed in continuously aerated natural seawater in fiber reinforced polymer (FRP) tanks.

As shown in Fig. 1, *Sarcodia suae* was cultured from January 2020 to December 2021 in three FRP tanks, each measuring 6000 L (~6 m<sup>2</sup> × 1 m) linked serially under continuous aeration conditions. Each tank contained the same seaweed stock density of 1.4 kg m<sup>-2</sup>. In 2020, the aquaculture wastewater was from shrimp fed artificial shrimp food pellets. In 2021, the aquaculture wastewater was from grouper fed natural coarse fish (including mackerel, mackerel scad, and small skipjack tuna). The wastewater was filtered through a high-density polyester fiber filter (commonly used in aquarium filters; pore size ~150 μm) to remove large suspended particles, before being added to seaweed tanks at a flow rate of 20 L min<sup>-1</sup>. Clean effluent from seaweed tanks was re-introduced to the seaweed tanks (hereafter referred to as treated water). A black screen shaded the seaweed tanks, resulting in light intensity of 50 to 250 μE m<sup>-2</sup> s<sup>-1</sup> during the summer (May to October). Note that these optimal values were based on previous studies on the incubation of *Sarcodia suae* (Su, 2012; Lee et al., 2019). In 2021, we also implemented a solar-powered seawater temperature control system (1 MkW; <https://oga.nsysu.edu.tw/p/403-1005-4720.php?Lang=zh-tw>). *Sarcodia suae* was also cultured in triplicate at the same stock density using normal seawater with a nitrite (potentially detrimental to seaweed) concentration of <2 μM as a control.

### 2.2. Measurements of seawater temperature, pH, salinity, and solar irradiance

Seawater temperature and surface irradiance in the seaweed culturing tanks were measured using HOBO (ONSET 1-800-LOGERS) sensors at intervals of 30 min. Irradiance was recorded as LUX and converted to μE m<sup>-2</sup> s<sup>-1</sup> at a conversion factor of 0.024. The pH of the seawater was measured using a pH meter (WTW pH 3110, Germany and 987C2\_PD, Taiwan). A conductivity meter (HANNA HI 98192 USA) was used to measure the salinity of the seawater. Hydrographic data are reported as monthly mean values in the text and figs.

### 2.3. Analysis and calculation of nutrient content and nutrient flux

Every day between January 2020 and December 2021 except for several important holidays (no >10 days), seawater samples were collected using a falcon tube and filtered through GF/F filters to determine NO<sub>2</sub><sup>-</sup> concentrations. Note that nitrate (NO<sub>3</sub><sup>-</sup>) is the primary nitrogen nutrient; however,

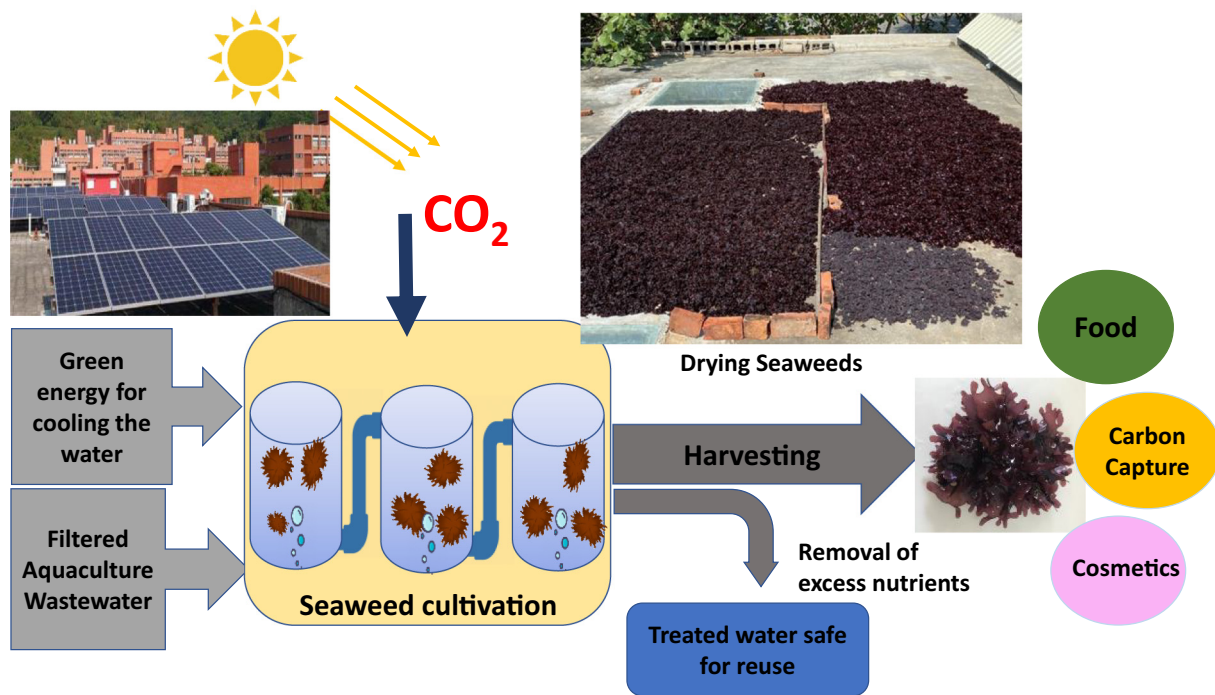


Fig. 1. Schematic illustration of proposed carbon sequestration scheme using seaweed, aquaculture wastewater, and solar energy system on the campus of Sun Yat-sen University (1 M-kw/year). Raw *Sarcodia* can be used for the production of food and cosmetics, but also for direct storage in deep waters or on the deep sea floor resulting in a potential carbon sink.

nitrite ( $\text{NO}_2^-$ ) is more toxic for shrimp and fish (Chen and Chen, 1992; Ciji and Akhtar, 2019). We therefore focused on nitrite as a long-term indicator of nitrogen accumulation. Between December 16, 2020 and April 08, 2021, samples of aquaculture wastewater and treated water were collected at intervals of 7 days to assess nutrient removal rates and efficiency. The concentrations of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , and  $\text{PO}_4^{3-}$  were derived from colorimetric measurements (Hung et al., 2000) obtained using a UV/VIS SP-80001 spectrophotometer.

The nutrient removal rate (NRR) was calculated as follows:

$$\text{NRR} = (C_i - C_o) \times V / \text{DW} / t$$

The nutrient removal efficiency (NRF) was calculated as follows:

$$\text{NRF} = 100 - (100 \times C_o / C_i)$$

where  $C_i$  and  $C_o$  respectively indicate the concentration of nutrients in the wastewater and treated water ( $\mu\text{mol l}^{-1}$ ) throughout the experiment period, DW indicates the dry weight of the seaweed, and V is the volume of seaweed.

#### 2.4. Deriving growth parameters

Seaweed (*Sarcodia suae*) from the culturing tanks was harvested once a month. The biomass was weighed on a load balance (EXCELL, Taiwan) after draining for 20 min. The tanks were then cleaned and restocked at the same initial culture density ( $1.43 \text{ kg m}^{-2}$ ). Specific growth rates and changes in mass (dry weight) were calculated every day using the methods outlined by Yong et al., 2013.

$$\text{Specific growth rate (\%)} = ((W_f - W_i) / W_i) / t \times 100\%$$

$$\text{Change in dry weight (g m}^{-2} \text{ d}^{-1}) = (\text{DW}_f - \text{DW}_i) / A / t$$

where  $W_f$  (g) and  $W_i$  (g) respectively indicate the final and initial wet weight in grams, whereas  $\text{DW}_f$  (g) and  $\text{DW}_i$  (g) respectively indicate the

final and initial dry weight in grams, “A” refers to the surface area of the tank ( $\text{m}^2$ ), and “t” indicates the cultivation time.

#### 2.5. Measuring the dry weight, density, total carbon content, and nitrogen content of seaweed

Seaweed samples were carefully washed using RO water to remove surface salt and attached epiphytes. Some of the cleaned *Sarcodia* were used to measure the density of the seaweed in terms of weight and volume ( $\text{g cm}^{-3}$ ). After undergoing drying in an oven at  $60^\circ \text{C}$  for 24 h, seaweed samples were weighed using an analytical balance (AUW220D, Japan) with an accuracy of  $\pm 0.01 \text{ mg}$  (for measuring dried weight). The dry seaweed was then ground to a fine powder and immediately stored in glass bottles until analysis. Concentrations of total carbon (TC) and total nitrogen (TN) in *Sarcodia suae* powder were obtained using an element analyzer (Elementar Vario EL cube, Germany) by the methods outlined by Shih et al. (2015).

$$\begin{aligned} \text{Net carbon capture by seaweed (g C m}^{-2} \text{ day}^{-1}) \\ = (\text{Dry weight increase in g m}^{-2} \text{ day}^{-1}) \times (\text{carbon\%/100}) \end{aligned}$$

$$\begin{aligned} \text{Net nitrogen capture by seaweed (g N m}^{-2} \text{ day}^{-1}) \\ = (\text{Dry weight increase in g m}^{-2} \text{ day}^{-1}) \times (\text{nitrogen\%/100}) \end{aligned}$$

#### 2.6. Statistical analysis

Statistical analysis was performed using Sigma Plot 14.0 with a significance value of 0.05 for all statistical tests. The normality and homogeneity of variance of the datasets were respectively tested using the Shapiro-Wilk normality test and Levene's test. Data in tables and figures are expressed as mean  $\pm$  SE (standard error).

### 3. Results and discussion

#### 3.1. Hydrographic settings during seaweed cultivation

Fig. 2A–D detail the mean salinity, nitrite concentration, pH, and surface light intensity each month from January 2020 to December 2021. Mean salinity ranged from 25.39 to 33.38 (i.e., normal for seawater) throughout both years, with only two periods of low salinity during March 2020 (following shrimp pond water exchange) and August 2021 (following heavy precipitation) (Fig. 2A). Note that the variation in salinity had no effect on the growth of *Sarcodia suae*, because it remained within the range identified as conducive to growth (20 to 40) by Lee et al. (2019).

Mean monthly nitrite concentrations in aquaculture wastewater ranged from 1.8 to 20.5  $\mu\text{M}$  in 2020, with lower values in the winter season (Nov. to Feb.), due to reduced feeding activity. Nitrite concentrations rapidly increased in May (16.9  $\mu\text{M}$ ) and remained at higher levels until September (5.6  $\mu\text{M}$ ), whereupon they decreased after October (Fig. 2B). In 2021, nitrite concentrations were low in winter, increasing to 20  $\mu\text{M}$  in March, where they remained until December. In 2021, nitrite concentrations in aquaculture wastewater were higher than those in 2020 due to the use of nutrient-rich coarse fish as feed. In 2020, the mean pH in the culturing tanks oscillated between 8.19 and 8.45. In 2021, the mean pH values were 8.2–8.3 from January to April. Still they dropped to 8.0 in May,

where they remained until December (Fig. 2C). The lowering of pH in the culture tanks between August to December could be from decomposition of microorganisms or some *Sarcodia suae* clogged in the discharged and/or aerated tubes. Meanwhile, tiny organic matter particles and feces from aquaculture wastewater could also decrease the pH during decomposition of DOC, because groupers have a better ingestion activity in the summer, getting bigger in the winter and then generated more wastewater. This lowering of the pH might be worthy for further study.

As shown in Fig. 2B, nitrite concentrations in grouper aquaculture wastewater from May to December 2021 were 2 to 20 higher than those during the same period in 2020, indicating an elevated supply of nutrients (i.e., abundant organic input). We also observed a corresponding gradual decrease in pH values after May 2021, following the decomposition of organic matter. The same phenomena have previously been reported for lagoons or estuarine systems (Chou et al., 2018; Hsieh et al., 2021). Note that nitrite was not a major N-nutrient in our seaweed cultivation system. Other sources of nitrogen, including nitrate and ammonium (Table 1), were also generated from feed, the release of fecal pellets, and/or the decomposition of organic matter. The fact that nitrate and ammonium concentrations were at times far higher than nitrite suggests that the nitrogen levels were sufficient for the growth of seaweed, which means that the system could be expanded by installing additional seaweed tanks. Nitrite levels were generally far lower than nitrate levels; however, even moderate  $\text{NO}_2^-$  levels could have deleterious effects on shrimp or fish.

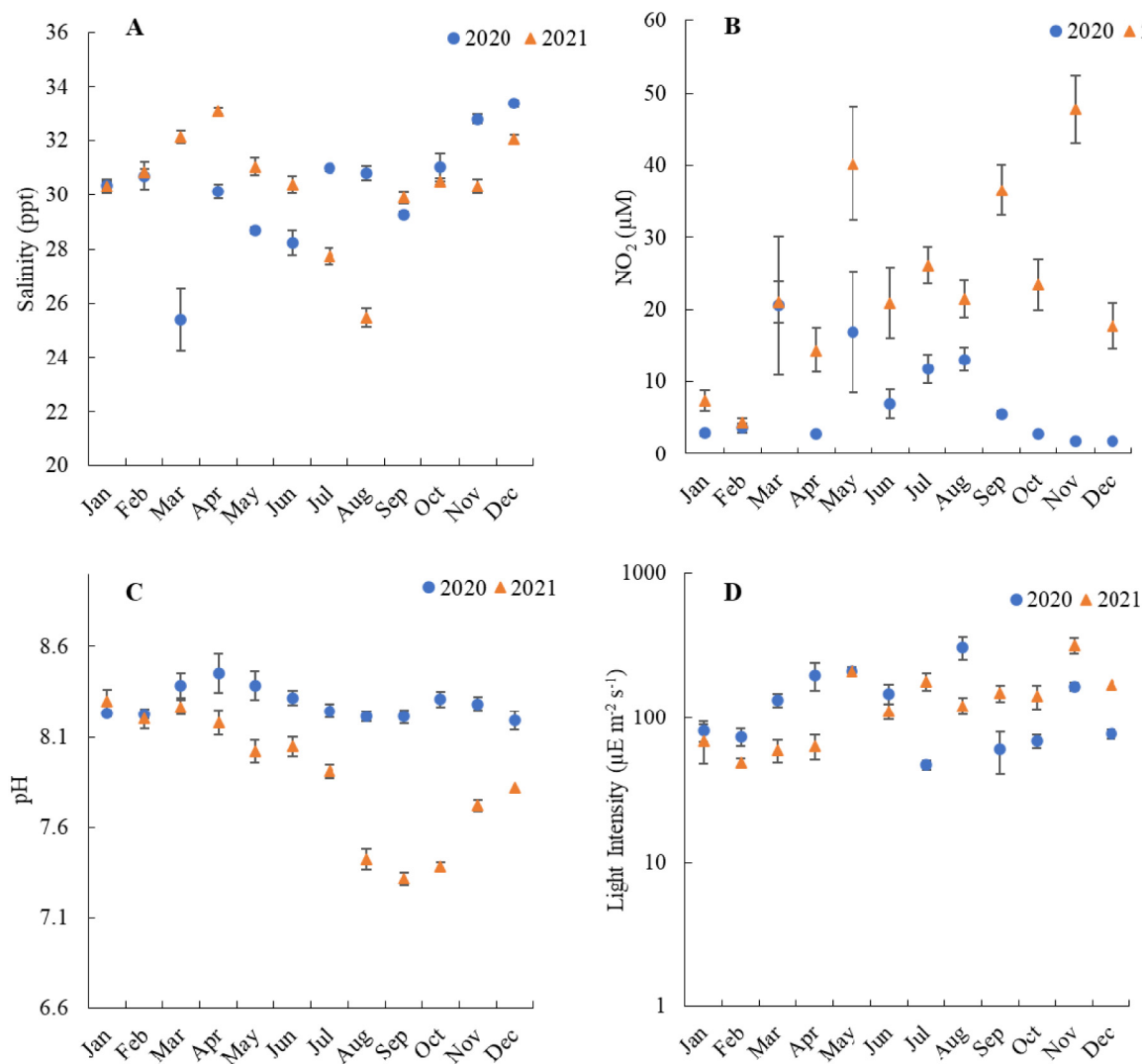


Fig. 2. Temporal distribution of (A) salinity; (B) nitrites; (C) pH; and (D) light intensity in culture tanks in 2020 and 2021 ( $\pm$  SE).

**Table 1**

Average concentrations of nutrients in aquaculture wastewater and treated water and associated removal rates of various nutrients through seaweed cultivation (mean  $\pm$  SE,  $n \geq 30$ ).

Nutrient	Wastewater ( $\mu\text{M}$ )	Treated water ( $\mu\text{M}$ )	Removal rate ( $\text{mg g}^{-1} \text{DW d}^{-1}$ )	Removal efficiency
$\text{NH}_4^+$	$15.6 \pm 4.1$	$9.8 \pm 3.0$	$11.9 \pm 2.8$	$(37.7 \pm 6.4 \%)$
$\text{NO}_2^-$	$5.8 \pm 0.2$	$2.2 \pm 0.1$	$7.3 \pm 0.3$	$(59.7 \pm 1 \%)$
$\text{NO}_3^-$	$354.3 \pm 74.0$	$332.1 \pm 74.4$	$45.5 \pm 13.2$	$(8.7 \pm 2.5 \%)$
$\text{PO}_4^{3-}$	$6.4 \pm 2.0$	$5.4 \pm 1.9$	$4.4 \pm 1.1$	$(20.9 \pm 4.2 \%)$

Mean monthly surface irradiance in the seaweed tanks ranged from 60 to 210  $\mu\text{E m}^{-2} \text{s}^{-1}$  in 2020 and 49 to 315  $\mu\text{E m}^{-2} \text{s}^{-1}$  in 2021 (Fig. 2D). The shaded light intensity at our seaweed tanks was similar to that reported by Ashkenazi et al. (2019) and slightly higher than that (80  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) reported by Lee et al. (2019). Bidwell et al. (1985) reported that the optimal light intensity for seaweed Irish moss (*Chondrus crispus* Stackh) was roughly 0.05 % of the surface irradiance (at a depth of roughly 0.5 m). In our seaweed rearing system, irradiance of 10  $\mu\text{E m}^{-2} \text{s}^{-1}$  at a depth of 60 cm was insufficient for photosynthesis; however, the aeration used in our culture system caused the *Sarcodia* to continuously flow from the depths to the shallows and vice versa, thereby exposing all of the seaweed to sufficient sunlight.

### 3.2. Temperature variation and temperature control methods

Mean monthly seawater temperatures ranged from 18.3 °C to 32.0 °C with typical seasonal variations in 2020; i.e., higher temperatures in the summer (May to September) and lower temperatures in the winter (November to February) (see Fig. 3; detailed dataset in Supplementary Materials). The carbon capture rate (CCR) was inversely proportional to temperature, with higher CCR in the winter and lower (or negative) CCR in the summer. Thus, we were not surprised to observe an extremely low CCR value of 0.1  $\text{g-C m}^{-2} \text{d}^{-1}$  in the summer (without temperature control). Previous studies have recommended that *Sarcodia suae* be maintained at temperatures of <28 °C (Lee et al., 2019). Seaweed can be categorized according to temperature tolerance as eurythermal seaweed (i.e., tolerance for a broad range of temperatures) or stenothermal (i.e., tolerance for a narrow range of temperatures). Researchers previously identified *Sarcodia suae* as eurythermal (18–28 °C). Lee et al. (2019) reported that *Sarcodia suae* could be grown in water as cool as 15 °C. In the current study, *Sarcodia suae* did not flourish under warm temperature conditions (>28 °C) (Fig. 4). We observed that

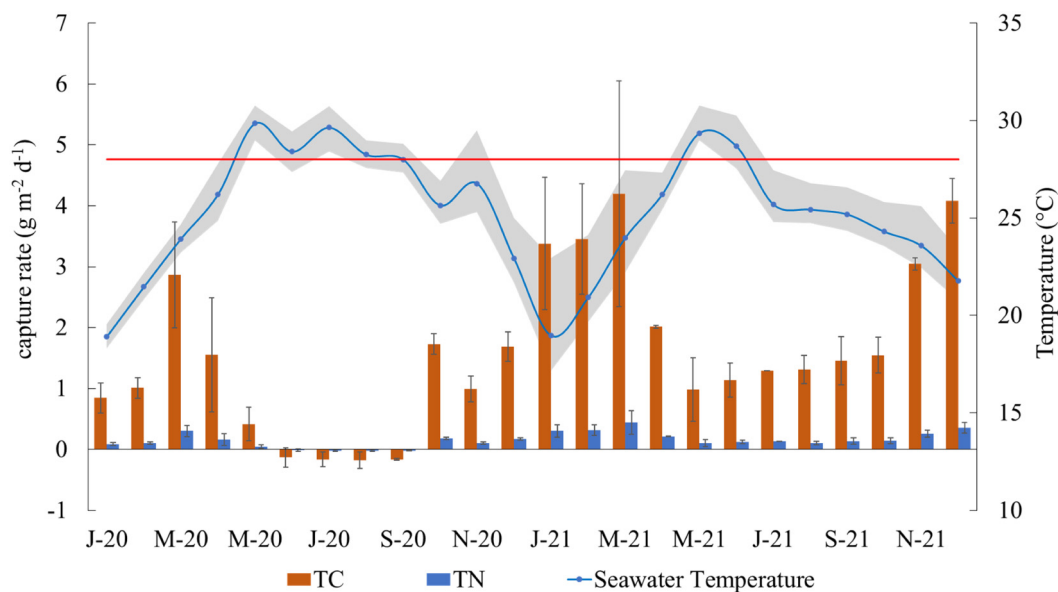
when the seawater temperatures increased to 28–29 °C for an extended period (>24 h) in the summer, the color of *Sarcodia suae* changed to green. At temperatures of >33 °C, the youngest parts of the thallus began decomposing, as indicated by white patches (Fig. 4). Lee et al. (2019) reported that the color of *Sarcodia suae* can be affected by arsenic species in cultured seawater under limited phosphorous availability; however, we can probably rule this out in the current study because the total phosphorous concentration was not a limiting nutrient (see discussion below).

In 2021, seawater temperatures in seaweed tanks ranged from 17.2 °C to 30.9 °C, with elevated temperatures occurring in May and June. Note that in the study, summer seawater temperatures were well controlled ( $26.3 \pm 0.82$  °C) by a solar powered cooling system (Fig. 1), which operated from July to October 2021. This cooling system reduced the mean seawater temperature in July 2021 from 30 °C (without cooling in July 2021) to 26 °C (with cooling). Controlling the summer temperatures increased the carbon capture rates (CCR; 1.33  $\text{g-C m}^{-2} \text{d}^{-1}$ ) by roughly 15-fold higher compared to the summer of 2020 (Fig. 3). Note that the CCR values are discussed in greater detail below.

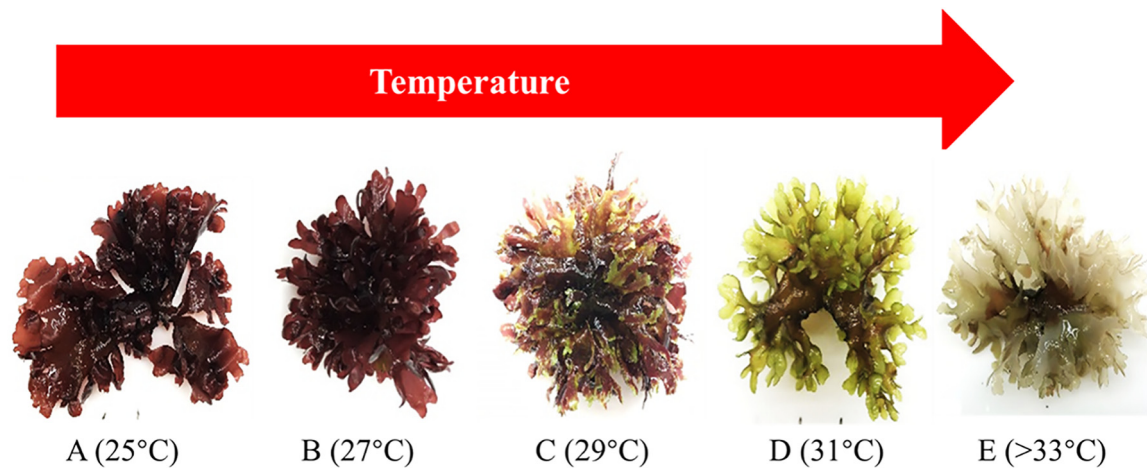
Our results suggest that *Sarcodia suae* can be cultured year-round at large-scales as long as the seawater temperatures are controlled in the summer. Amortizing the cost of a conventional (non-green) cooling system capable of cooling 3 six-ton seaweed tanks (US\$ 1500) over the expected lifespan of the components (five years) would be roughly US\$ 300/yr. With energy consumption of 29  $\text{kW d}^{-1}$  (=NT\$ 138  $\text{d}^{-1}$  = US\$ 4.6  $\text{d}^{-1}$ ), cooling costs would be roughly US\$ 138  $\text{month}^{-1}$  (5-months power + cooling system), such that the total cost of cooling the proposed seaweed cultivation system would be US\$ 940 per year, which is far from insignificant. Furthermore, the consumption of this much energy would generate 435 kg of  $\text{CO}_2$ . Clearly, any feasible cooling system must employ green energy to cost down.

One obvious approach to cooling down seawater during the summer is to use solar power, particularly in areas with sunlight exposure, such as southern Taiwan ([www.cwb.gov.tw](http://www.cwb.gov.tw)). National Sun Yat-sen University established a solar power system in 2019 capable of generating roughly 2903–4317  $\text{kW d}^{-1}$  between April and October. A single air conditioner (model FT\_42DYSR/R-42DYSR, Tatung, Taiwan) is able to cool approximately 20-tons of seawater (i.e., 3 six-ton seaweed tanks).

Another approach is to use liquified natural gas (LNG) cooling seawater. In fact, the firm Yongan LNG (CPC cooperation, 2022) has cultured small-scale *Sarcodia suae* for the production of food ([gvm.com.tw/artic/90198](http://gvm.com.tw/artic/90198)), and been providing cooling water to local aquaculture facilities for several years (<https://www.cpc.com.tw/>). There are also plans to build another



**Fig. 3.** Temporal distribution of seawater temperatures (°C) and total capture rates of carbon and nitrogen ( $\text{g m}^{-2} \text{d}^{-1}$ ) by *Sarcodia suae* in 2020 and 2021.



### Raising temperature will be leading to spoiling *Sarcodia suae*

Fig. 4. Changes in the color of *Sarcodia suae* as a function of temperature: (A) 25 °C; (B) 27 °C; (C) 29 °C; (D) 31 °C; and (E) >33 °C.

LNG station in northern Taiwan, the cooling water of which could be used in seaweed cultivation. Finally, it should be possible to use low-temperature seawater pumped up from deep ocean areas; however, this is beyond the scope of the current investigation.

#### 3.3. Nutrient removal by *Sarcodia suae*

Table 1 summarizes the average nutrient concentrations ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ ) in aquaculture wastewater and treated water from seaweed cultivation tanks between December 2020 and April 2021. All nutrient concentrations in *Sarcodia suae* tanks remained at a reasonable level throughout the study period. The seaweed tanks reduced the average nutrient concentrations in aquaculture wastewater as follows:  $\text{NH}_4^+$  (from 15.6  $\mu\text{M}$  to 9.8  $\mu\text{M}$ ; 37.8 %),  $\text{NO}_2^-$  (from 5.8 to 2.2  $\mu\text{M}$ ; 59.8 %),  $\text{NO}_3^-$  (from 354 to 332  $\mu\text{M}$ ; 8.8 %), and  $\text{PO}_4^{3-}$  (from 6.4 to 5.4  $\mu\text{M}$ ; 20.9 %) (see details in Supplementary materials). We can see that among the various sources of nitrogen, the preference of *Sarcodia suae* for  $\text{NO}_2^-$  far exceeded the preference for  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . This nutrient species uptake order is similar to other seaweeds and marine phytoplankton (*Gracilaria* species, D'Elia and DeBoer, 1978; Jones et al., 2001).

The rates of nutrient removal by *Sarcodia suae* were as follows:  $\text{NH}_4^+$  (11.9  $\text{mg g}^{-1} \text{d}^{-1}$ ),  $\text{NO}_2^-$  (7.3  $\text{mg g}^{-1} \text{d}^{-1}$ ),  $\text{NO}_3^-$  (45.5  $\text{mg g}^{-1} \text{d}^{-1}$ ), and  $\text{PO}_4$  (4.4  $\text{mg g}^{-1} \text{d}^{-1}$ ) (dry weight) with a total nitrogen removal rate of 64.7  $\text{mg-N g}^{-1} \text{d}^{-1}$ . In comparison, Braga and Yoneshigue-Valentin (1996) reported that *Laminaria japonica* can take up 2.4  $\text{mg-N g}^{-1} \text{d}^{-1}$  and 0.6  $\text{mg-P g}^{-1} \text{d}^{-1}$ , respectively. Nutrient removal by *Laminaria japonica* in the coastal waters of Sungo Bay was as follows: N (0.2–270  $\text{mg-N g}^{-1} \text{DW d}^{-1}$ ) (Xu et al., 2011). Nutrient removal by *Laminaria saccharina* was as follows:  $\text{NH}_4\text{-N}$  (3.0  $\text{mg-N g}^{-1} \text{DW d}^{-1}$ ) and  $\text{NO}_3\text{-N}$  (3.6  $\text{mg-N}$

$\text{g}^{-1} \text{DW d}^{-1}$ ) (Ahn et al., 1998). Overall, the N and P capture rates of *Sarcodia suae* were several times higher than those of *Laminaria*.

As shown in Table 2, it is also possible to estimate the capture of dissolved inorganic nitrogen (DIN) and phosphate based on the difference between concentrations in wastewater and treated water as a function of water residence time (0.63  $\text{d}^{-1}$ , based on flow rate = 1.2-ton  $\text{h}^{-1}$ ). Accordingly, DIN removal by *Sarcodia suae* was 0.71  $\text{g-N m}^{-2} \text{d}^{-1}$ , which exceeded the mean value (0.31  $\text{g-N m}^{-2} \text{d}^{-1}$ ) and was close to the maximum nitrogen capture rate in winter (0.65  $\text{g-N m}^{-2} \text{d}^{-1}$ ) (detailed in the Supplementary Materials). Despite the growth of some epiphytes and other macro- and micro-fauna on the walls of the tanks, our results indicate the efficient use of DIN by *Sarcodia suae*. These results also indicate that nitrogen could be over-supplied, considering that phosphorus concentrations appeared not to be a limiting factor, because phosphate concentrations were several times higher than those in seawater and the DIN/P ratio (58.7) far exceeded the Redfield ratio. Thus, it would be reasonable to expect that coupling 5–7 tanks in series would increase the nitrogen removal capacity even further.

#### 3.4. Specific growth rates and carbon and nitrogen capture rates

Table 2 lists the average specific growth rates (SGR), carbon capture rates (CCR), and nitrogen capture rates (NCR) of *Sarcodia suae* as a function of season. In 2020, the SGR values were relatively high (1.1–2.7 %) in the spring, fall, and relatively low (even negative) in the summer. Note that the negative SGR values can be attributed to the decomposition of young thallus parts due to elevated temperatures (Fig. 3). The same trend was observed in the other measures during cooler months: CCR (1.32–1.61  $\text{g-C m}^{-2} \text{d}^{-1}$ ) and NCR (0.13–0.17  $\text{g-N m}^{-2} \text{d}^{-1}$ ), (Fig. 3 and

Table 2

Specific growth rate (SGR%), carbon capture rate (CCR,  $\text{g-C m}^{-2} \text{d}^{-1}$ ) and nitrogen capture rate (NCR,  $\text{mg-N m}^{-2} \text{d}^{-1}$ ) by *Sarcodia suae* as a function of season in 2020 and 2021.

Year		Winter			Spring			Summer			Fall		
		SGR (%)	CCR ( $\text{g m}^{-2} \text{d}^{-1}$ )	NCR	SGR (%)	CCR ( $\text{g m}^{-2} \text{d}^{-1}$ )	NCR	SGR (%)	CCR ( $\text{g m}^{-2} \text{d}^{-1}$ )	NCR	SGR (%)	CCR ( $\text{g m}^{-2} \text{d}^{-1}$ )	NCR
2020	Average	2.1	1.3	0.13	1.7	1.6	0.17	-0.3	-0.2	-0.02	1.6	1.4	0.14
	Max	82	2.1	0.22	3.5	3.9	0.41	0.1	0.1	0.01	2.3	2	0.21
	Min	0.6	0.4	0.04	0.1	0.1	0.01	-0.8	-0.5	-0.05	1	0.8	0.08
2021	Average	4.4	3.4	0.31	6.9	2.8	0.28	1.9	1.3	0.14	2.9	2.3	0.24
	Max	13.7	6.1	0.65	11	7.9	0.83	2.7	2.6	0.28	4.3	3.3	0.35
	Min	1.1	0.9	0.09	2.2	0.5	0.05	1.3	0.9	0.09	1.5	1	0.11

Max and Min: indicating maximum and minimum values, respectively. Winter (Dec-Feb), Spring (Mar-May), Summer (Jun-Sep), and Fall (Oct, Nov).

Table 2). The negative CCRs and NCRs during summer months could be attributed to higher temperatures and/or strong irradiance inhibiting the growth of *Sarcodia suae*.

In 2021, the average SGRs were as follows: winter (4.4 %), spring (6.9 %), summer (1.9 %), and fall (2.9 %). The fact that all of these values were higher than those in 2020, indicates that the supply of nutrients and other elements by grouper aquaculture wastewater (fed by natural coarse fish) exceeded that of shrimp wastewater (fed by artificial pellets) (Fig. 2A). Between January and March 2021, the three 6-ton tanks ( $\sim$  culture area = 18 m<sup>2</sup>) yielded 100–114 kg per *Sarcodia suae* per month. In 2021, the maximum SGR of *Sarcodia suae* reached 13.7 in the winter and 11 % in the spring. These results indicate that excellent *Sarcodia suae* growth rates could be achieved year round provided the availability of cold water with sufficient nutrients and suitable light intensity (Fig. 3). The highest SGR of *Sarcodia suae* in the current study exceeded that of similar species, including the red algae *Gracilaria chouae* in a sea cage aquaculture system (7.4–8.5 % d<sup>-1</sup>) (Wu et al., 2015).

The SGR of *Sarcodia suae* during the summer of 2021 reached 1.9 %, which is roughly 15 times higher than during the summer of 2020 (−0.8–0.13 %). This is a clear demonstration of the efficacy of the proposed cooling system. In 2021, CCR (1.3–4.5 g-C m<sup>-2</sup> d<sup>-1</sup>) and NCR (0.14–0.31 g-N m<sup>-2</sup> d<sup>-1</sup>) remained positive through all four seasons (Fig. 3 and Table 2). As shown in Fig. 5, the CCR of *Sarcodia suae* (2–7 g-C m<sup>-2</sup> d<sup>-1</sup>) exceeded that of other macroalgae species (1–6 g-C m<sup>-2</sup> d<sup>-1</sup>) (Ben-Ari et al., 2014; Neori et al., 2004; Abreu et al., 2011; Wei et al., 2017; Samochoa et al., 2015; Vicente et al., 2006; Schuenhoff et al., 2006). The other species at slower growth can be attributed to the fact that wild varieties are found mainly in rocky coastal waters or intertidal zones, wherein spores are produced only when the currents are favorable. By contrast, *Sarcodia suae* can be cultivated via asexual reproduction without the need for anchoring on rocky outcrops.

Fig. 6 compares the CCR of *Sarcodia suae* with that of other blue carbon catchers and marine phytoplankton (tonnes-CO<sub>2</sub> km<sup>-2</sup> d<sup>-1</sup>). The carbon sequestration capacity of *Sarcodia suae* in the current study, far exceeded that of marine phytoplankton in the East China Sea and South China Sea (Chen, 2005; Gong et al., 2003; Shih et al., 2021) and mangroves with associated benthic microalgae in Thailand (Inoue, 2019) as well as Eelgrass beds, *Sargassum* beds, and warm kelp beds in Japan (Yoshida et al., 2019) (Fig. 6).

Note that the above rates of carbon fixation by phytoplankton and blue carbon catchers were treated as carbon capture under the assumption that

the products (i.e., seaweed) would be transported to deep waters. However, one can note that if the harvested seaweed were consumed as food or used as raw material, then the credit for carbon fixation would be lost. Although *Sarcodia* can be used in the production of food and cosmetics, the current study focused primarily on its use in carbon capture. The measured density of *Sarcodia* in this study was 1.07–1.13 g cm<sup>-3</sup> (average = 1.10 ± 0.03 g cm<sup>-3</sup>), which exceeds that of normal seawater ( $\sim$ 1.025 g cm<sup>-3</sup>), allowing it to sink naturally into the depths after being dumped at the surface.

As with the marine carbon pumps driven by marine phytoplankton and blue carbon, *Sarcodia* could be stored in deep waters or as bottom sediment for hundreds of years. Easy access to deep waters (>3000 m) from southern or eastern Taiwan would also ensure that harvested *Sarcodia* remain fresh until the point at which it is dumped. We plan to test the sinking of raw *Sarcodia* in open oceans soon.

Raw *Sarcodia* as food or raw material must be cleaned and sanitized; however, it can be stored in the deep ocean without further processing. The enormous dilution capacity of the open oceans in conjunction with the dark and chilly deep waters means that the effects of decomposing *Sarcodia* on marine biodiversity and eutrophication can largely be disregarded. *Sarcodia* currently growing at Siao Liouciou island would no doubt lead to the transport of DOC and POC into the northern South China Sea; however, we have seen no reports pertaining to deleterious effects of seaweed detritus on marine ecosystems in the areas adjacent to Siao Liouciou Island.

Note that the average N content in *Sarcodia suae* cultivated in wastewater (3.70 ± 0.2 % dry weight) exceeded that of *Sarcodia suae* cultured in natural seawater (2.16 ± 0.1 %). The C/N ratio of the *Sarcodia suae* during the culture period was calculated by the measuring the percentages of carbon and nitrogen in the thallus (yearly average of 9.91 ± 0.05). C/N ratios were higher in the middle of the winter (13.32 ± 0.35) with little variation during the other seasons (9.60 ± 0.02). Wei et al. (2017) reported the absorption of large quantities of nitrogen and phosphorous from nutrient-rich water by *Gracilaria lemaneiformis* under optimum growth conditions in Yangtian Bay, China.

### 3.5. Implications of carbon sequestration using seaweed

Researchers have proposed seaweed farming for the capture of carbon in coastal waters, and then dumping the resulting particulate and dissolved organic matter (Krause-Jensen et al., 2018) into the world's largest carbon sink; i.e., the deep ocean (Duarte et al., 2017; Krause-Jensen et al., 2018;

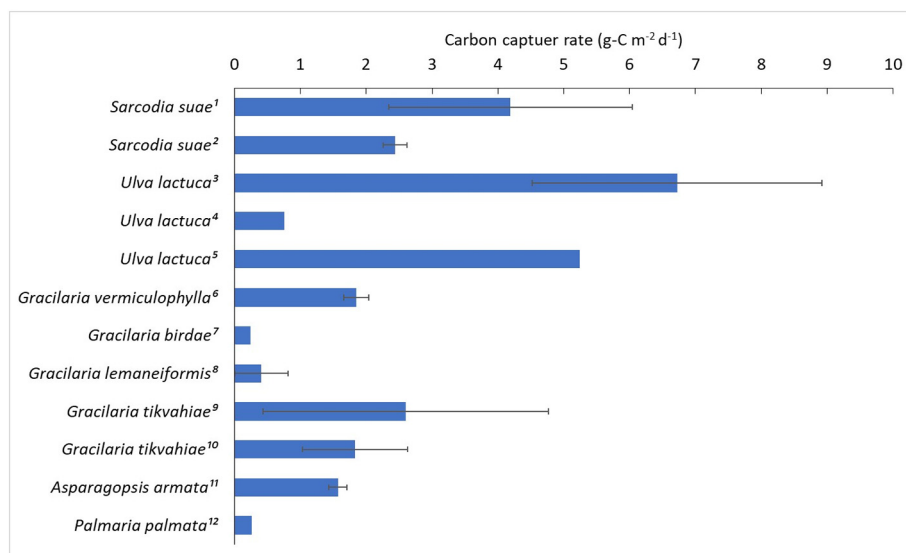


Fig. 5. Carbon capture rates of red seaweed in tropical and subtropical areas in land-based aquaculture systems suggesting *Sarcodia suae* with strong carbon capture rate. *Sarcodia suae* growth in 1: high nutrient aquaculture wastewater (winter) and 2: growth in seawater, 3: Ben-Ari et al., 2014, 4: Neori et al., 2004, 5: Neori et al., 2003, 6: Abreu et al., 2011, 7: Marinho-Soriano et al., 2009, 8: Wei et al., 2017, 9: Samochoa et al., 2015, 10: Vicente et al., 2006, 11: Schuenhoff et al., 2006, 12: Kim et al., 2013.

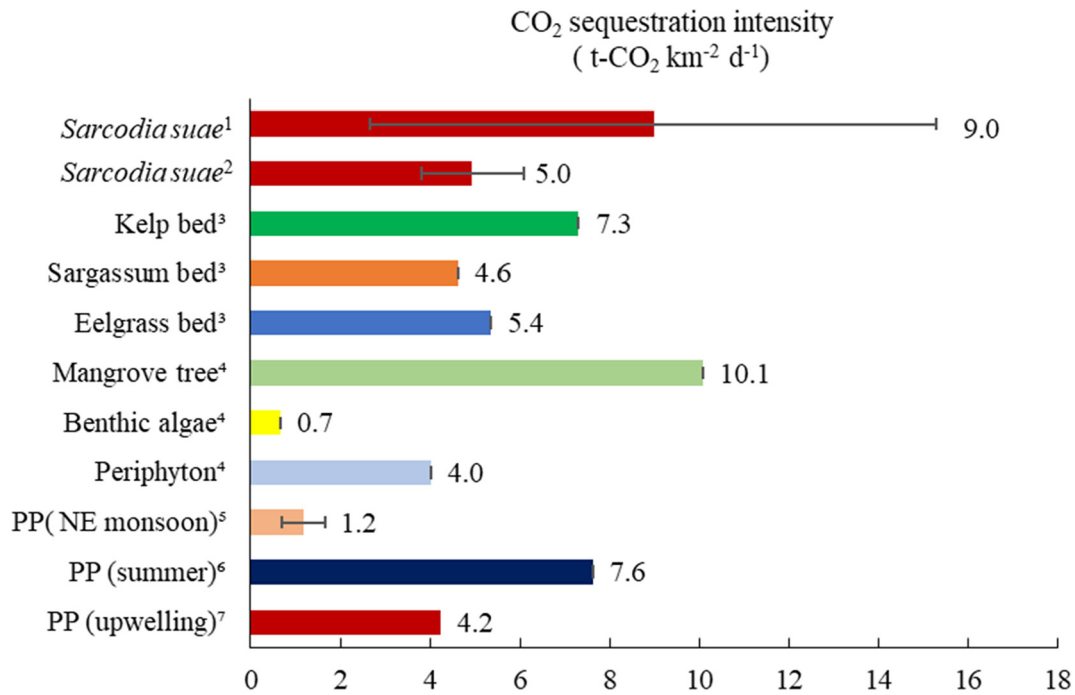


Fig. 6. Comparison of candidate marine species for CO<sub>2</sub> sequestration. *Sarcodia suae* growth in <sup>1</sup> high nutrient aquaculture wastewater (winter) and <sup>2</sup>seawater. <sup>3</sup>Moderate temperature kelp bed, Sargassum bed and warm Eelgrass bed in Japan (Yoshida et al., 2019). <sup>4</sup> Periphyton on Mangrove aerial roots, Benthic algal in a mangrove forest and Mangrove trees in Thailand (Inoue, 2019). <sup>5</sup> In situ primary production in South China sea (Shih et al., 2021), Integrated primary production and <sup>6</sup>East China Sea (Gong et al., 2003) and <sup>7</sup>Primary production in Taiwan coastal upwelling in East China Sea (Hung et al., 2000).

Ortega et al., 2019). Taiwan produced 290 million t of carbon dioxide ([https://unfccc.saveoursky.org.tw/nir/tw\\_nir\\_2021.php](https://unfccc.saveoursky.org.tw/nir/tw_nir_2021.php)). Reaching the projected carbon zero balance by 2050 will require reducing carbon dioxide emissions through the adoption of green energy sources (e.g., solar, wind, hydroelectric, geothermal, green hydrogen) and electric vehicles as well as increasing carbon sequestration in the forests and seas. There are currently no official reports pertaining to carbon sequestration in Taiwan. Researchers have determined that the forests in Taiwan could sequester roughly 20–40 mt-CO<sub>2</sub> y<sup>-1</sup> (Fig. 7, Liaw, 2009; EPA in Taiwan, [www.epa.gov.tw](http://www.epa.gov.tw)).

Hung and his colleagues reported that the marine carbon pumps (i.e., transporting particulate organic carbon to the bottom of the euphotic zone) in the seas (the East China Sea, the northern South China Sea, the Kuroshio and the Northwest Pacific) around Taiwan could sequester 59–130 mt-CO<sub>2</sub> y<sup>-1</sup> (Fig. 7) (Hung and Gong, 2010, 2011; Hung et al., 2010, 2012, 2013, 2016; Chen et al., 2013; Shih et al., 2013, 2015, 2020a, 2020b). Thus, the combined potential carbon sequestration capacity of Taiwan is approximately 80–170 mt-CO<sub>2</sub> y<sup>-1</sup>. Blue carbon catchers are particularly important in light of the difficulties in expanding forests on a small island and the need for agricultural land. Estimating carbon sequestration intensity based on an average CCR of 2.4 g-C m<sup>-2</sup> d<sup>-1</sup> for land-based aquaculture systems, it appears that *Sarcodia suae* could be used to sequester 9 t of CO<sub>2</sub> km<sup>-2</sup> d<sup>-1</sup> (~2990 t of CO<sub>2</sub> km<sup>-2</sup> y<sup>-1</sup> = 30 t of CO<sub>2</sub> ha<sup>-1</sup> y<sup>-1</sup>, where carbon capture rate of 1 g-C is equal to 3.67 g-CO<sub>2</sub> with 330 working days per year). In other words, the potential CCR of *Sarcodia* exceeds that of forests (15 t of CO<sub>2</sub> ha<sup>-1</sup> y<sup>-1</sup>) by roughly 2-fold in Taiwan (EPA, Taiwan) and 3-fold global mean value.

In 2001, Taiwanese aquaculture farmers using roughly 1000-ha grew 16,000 t of *Gracilaria tenuistipitata* as feed for Abalone (*Haliotis diversicolor*) cultivation (Su et al., 2009); however, the production of *Gracilaria* has declined due to a shrinking of the Abalone aquaculture market. In cultivating *Gracilaria* for a co-cultured system involving fish and shrimp (~16 t-wet weight ha<sup>-1</sup> y<sup>-1</sup>, assuming a minimum of 200 working days per year), the CCR would be 0.34 g-C m<sup>-2</sup> d<sup>-1</sup>. Further research will be required to explore other seaweeds for carbon sequestration. Taiwan has land-based aquaculture farms covering >50,000 ha as well as numerous sea cage aquaculture farms. Taiwan is also developing offshore wind power systems. It would be interesting to determine whether offshore windfarms could be combined with seaweed aquaculture systems.

#### 4. Conclusions

This study demonstrated that *Sarcodai suae* could be cultured throughout the year in subtropical regions simply by controlling summer seawater temperatures via solar powered cooling systems. The land-based

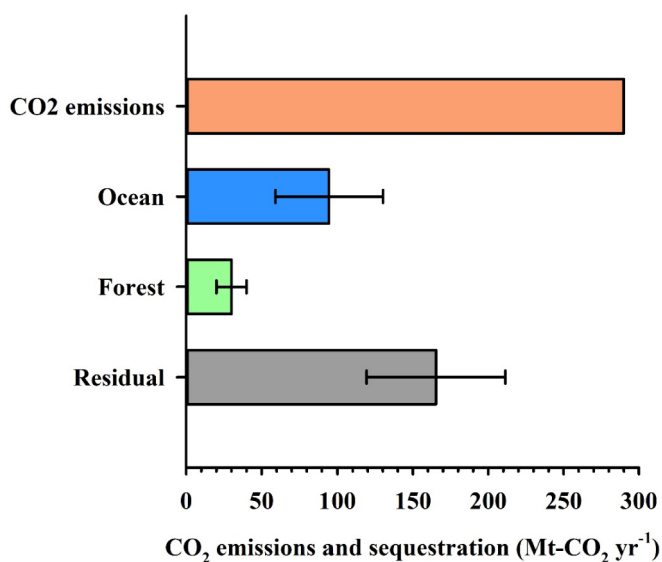


Fig. 7. CO<sub>2</sub> emissions in Taiwan and estimated carbon sequestration rates in forests and surrounding seas. The residual represents potential carbon sequestration due to a reduction in CO<sub>2</sub> emissions or an increase in the quantity of sequestered CO<sub>2</sub> with the aim of achieving neutral carbon generation in Taiwan.



cultivation of *Sarcodia suae* could mitigate the environmental impact of aquaculture wastewater, while capturing CO<sub>2</sub>. The average CCR of *Sarcodia suae* is roughly 2.4 g-C m<sup>-2</sup> d<sup>-1</sup> (= 30 t-CO<sub>2</sub> ha<sup>-1</sup> y<sup>-1</sup>), which is 2-fold higher than that of forests (15 t-CO<sub>2</sub> ha<sup>-1</sup> y<sup>-1</sup>) in Taiwan. Nonetheless, further research will be required to mitigate the effects of epiphytes, plan pumping systems, and determine the optimal stocking density for large-scale land-based culturing systems and sea cage farms in coastal regions.

### CRedit authorship contribution statement

**W. Sanjaya:** Experiment, Methodology, Investigation, data analysis, Writing – original draft. **Kahin Ling, Hsueh-Han Hsieh, Vincente G Abedneko:** Experiment, Methodology, nutrient analysis. **Jeng-Feng Shyu** Seaweed identification, Methodology, Writing – review & editing. **Tse-Min Lee:** Seaweed identification, Methodology, Writing – review & editing. **Yung-Yen Shih:** Writing – review & editing. **R.R.M.K.P. Ranatunga:** Writing – review & editing. **Peter H. Santschi:** Writing – review & editing. **Chin-Chang Hung:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

### Data availability

The detailed datasets are attached in the Supplementary file.

### 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.

### Acknowledgements

The authors would like to thank Ming-Hsiu Chuang, Yan-Ting Wang, Ting-Chu Lin, and the team at Carbon Search Lab at NSYSU for the extended help in gathering the relevant data in conducting this study. We also thank two anonymous reviewers for providing valuable comments on our manuscript. This research was supported by National Sun Yat-sen University and the Ministry of Sciences and Technology (MOST 108-2611-M-019-MY3, and MOST 110-2927-I-110-001, MOST 110-2634-F-019-002, MOST 111-2119-M-019-002) of Taiwan.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.158850>.

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