



Comparison of two cultivation methods for domesticating wild red algal eucheumatoids for use in the seaweed industry

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Received: 5 April 2024 / Revised: 4 July 2024 / Accepted: 20 July 2024 / Published online: 14 August 2024
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Abstract

Eucheumatoid cultivation is an important alternative livelihood for small-scale farmers in coastal areas in east Malaysia. Over the last 50 years, the same clones of the red eucheumatoid *Kappaphycus alvarezii* and *Kappaphycus striatus* have been vegetatively propagated for cultivation resulting in low genetic diversity. This has made the cultivars more vulnerable to pests and diseases, which has been further exacerbated by climate change. There is an urgent need, therefore, to develop new cultivars that are resilient to pests and diseases, as well as environmental change. In this study, wild eucheumatoids were collected from four regions in Semporna, East Malaysia: Kerindingan, Sibuhun, Sebangkat and Omadal. These eucheumatoids were then grown at a commercial seaweed farm in Silungun, Semporna using two methods: i) tie-tie and ii) basket net for 60 days and their performance was evaluated based on growth and survival rates. A higher mean specific growth rate (SGR) was recorded by wild-collected *Kappaphycus* spp. ($2.15 \pm 1.90\%$ day⁻¹) and *Eucheuma denticulatum* ($2.83 \pm 3.27\%$ day⁻¹) when tie-tie was used. However, survival of the wild-collected eucheumatoids was better with the basket net, which led to a reduction in grazing and prevented detachment from culture lines. Of 212 original individuals, 72 survived throughout the study, despite the prevalence of pests and ice-ice disease. Of these, 46.15% *Kappaphycus* spp. and 50.75% *E. denticulatum* survived when cultivated using the basket net and 17.20% *Kappaphycus* spp. and 30.77% *E. denticulatum* survived when cultivated using tie-tie. The mean SGR and survival rate were significantly correlated with temperature, salinity, pH, current velocity and rainfall ($p < 0.05$). The surviving wild-collected *Kappaphycus* spp. and *E. denticulatum* have the potential to be developed into new cultivars for possible commercial use.

Keywords Basket net · *Eucheuma* · Growth · *Kappaphycus* · Rhodophyta · Survival · Tie-tie

Introduction

Seaweeds are widely used in food and non-food industries, such as food additives, animal feeds, pharmaceuticals, nutraceuticals, cosmetics, textiles, biofertilizers, bio-packaging and biofuel (McHugh 2003; FAO 2018; Cai et al. 2021). Malaysia is the world's third largest producer of eucheumatoid seaweeds, including the red carrageenophytes *Kappaphycus* spp. and *Eucheuma* spp., after Indonesia and the Philippines (Cai et al. 2021). *Kappaphycus* and *Eucheuma* species are widely cultivated on the east coast of Sabah, which has a suitable ecosystem, including long coastlines, an extensive continental shelf and clean water (Sade et al. 2006; FAO 2018; Tan et al. 2022a).

The production of eucheumatoids in Malaysia is inconsistent and has faced a decreasing trend from 2012 to 2021 (DOFM 2022). Low quality of seedlings, difficulties in

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generating healthy and vigorous seed stock, disease problems and climate change are among the factors that have contributed to the decline in the production (Sulistiani et al. 2012; Porse and Rudolph 2017; FAO 2018; Kumar et al. 2020; Parenrengi et al. 2020; Ward et al. 2021). To date, local farmers still rely on conventional farming methods, including the use of clones from the original *Kappaphycus alvarezii* and *Kappaphycus striatus* cultivars, which have been vegetatively propagated for the past 50 years (Parker 1974; Tan et al. 2022a). Vegetative culture reduces genetic variability and leads to a slowing in the growth rate of stocks, deterioration in the carrageenan quality and reduced gel strength (Luhan and Sollesta 2010; Sulistiani et al. 2012). Changes in environmental conditions, as a consequence of climate change, further impact the cultivars and increase their susceptibility to pest and disease outbreaks (Bernard 2018; Kumar et al. 2020; Msuya et al. 2022).

There is an urgent need, therefore, to develop new, more resilient cultivars from wild eucheumatoid populations to improve the quality of cultivars currently used by the Malaysian seaweed industry. Wild eucheumatoids with high genetic diversity from the east coast of Sabah have been identified as having good potential for the development of new cultivars (Tan et al. 2022b). This study therefore aims to establish the most suitable culture method for wild eucheumatoids by comparing the efficacy of a basket net method with the commercially used tie-tie method for growth and survival of wild-collected eucheumatoids in Semporna, Sabah.

Materials and methods

Study area

The study was conducted in the eastern part of Sabah at Semporna. Wild eucheumatoids were collected at Kerindangan, Sibuhun, Sebangkat and Omodal which have been identified as having *Kappaphycus* spp. and *Eucheuma* spp. with high genetic diversity (Tan et al. 2022b). The wild-collected eucheumatoids were then transferred to a commercial seaweed farm at Silungun (4° 19.278' N, 118° 27.545' E) (Fig. 1). To keep the eucheumatoids in the best condition prior to putting them in the commercial farm, the eucheumatoids were placed in fishing nets immediately after collection from the wild and kept submerged in the sea overnight prior to their transfer to the farm the following day. Care was taken to ensure that the wild eucheumatoids were not subjected to prolonged desiccation, temperature or light stress during the transit from the collection site to the farm. Silungun was selected as the location in which to culture the wild eucheumatoids, as seaweed cultures are known to grow well there and the site is typically free from sea turtles

grazing, given that it is not within their migration route or foraging grounds.

Wild eucheumatoid collection and on-growing

The wild eucheumatoids, including *Kappaphycus striatus* and *K. malesianus* (hereafter *Kappaphycus* spp.) and *Eucheuma denticulatum*, were found growing among other seaweeds and corals at a depth of 0.5 – 3 m and both reproductive and non-reproductive thalli were observed. To minimise the impact on the natural populations and habitat, only visibly healthy (characterized by the absence of diseases and pests) eucheumatoids with a main axis longer than ≈ 15 cm were collected. Smaller sized thalli were left untouched to ensure conservation of the wild population (Yahya et al. 2023). Care was taken to ensure that the number of individuals collected did not exceed 30% of the wild populations in each sampling location. Permission to access these biological resources was obtained from the Sabah Biodiversity Centre (<https://sabc.sabah.gov.my/>). Two methods were tested in this study: i) the commercially used tie-tie and ii) the basket net method (Fig. 2). A total of 212 wild-collected eucheumatoids individuals comprising a mixture of *Kappaphycus* spp. (132 individuals) and *E. denticulatum* (80 individuals), each weighing > 20 g, were used in this study. The unequal number of individuals used for the two genera reflects the availability of wild eucheumatoids during the time of sampling, whereby *Kappaphycus* spp. are present in greater abundance than *E. denticulatum*. For the tie-tie method, 106 of the mixed wild-collected *Kappaphycus* spp. and *E. denticulatum* individuals were selected at random and tied onto the cultivation line made by 100 m long and 12 mm diameter polyethylene (PE) rope with a distance of 35 cm between each individual (Lim et al. 2021). Another 106 mixed wild-collected *Kappaphycus* spp. and *E. denticulatum* individuals were cultivated using cylindrical basket nets made of polyvinyl chloride (PVC) net and pipe material, which were 43 cm length by 30 cm diameter and had a mesh size of 6 mm. The basket nets were tied onto the cultivation line horizontally with 1 m interval between each net. Fifteen individuals of mixed wild-collected *Kappaphycus* spp. and *E. denticulatum* were put into each basket net, which ensured that they had sufficient space to grow. Each individual of the wild-collected eucheumatoids was weighed, tagged and photographed before being tied onto the cultivation lines or put into the basket net. The growth of the wild-collected eucheumatoids was recorded every two weeks for 60 days for both methods by recording individual seaweed weight from 19 October to 17 December 2022. The presence of any pests and ice-ice disease was recorded during the study period. Any infected part of the thallus or those with pests were removed from each eucheumatoid during sampling.

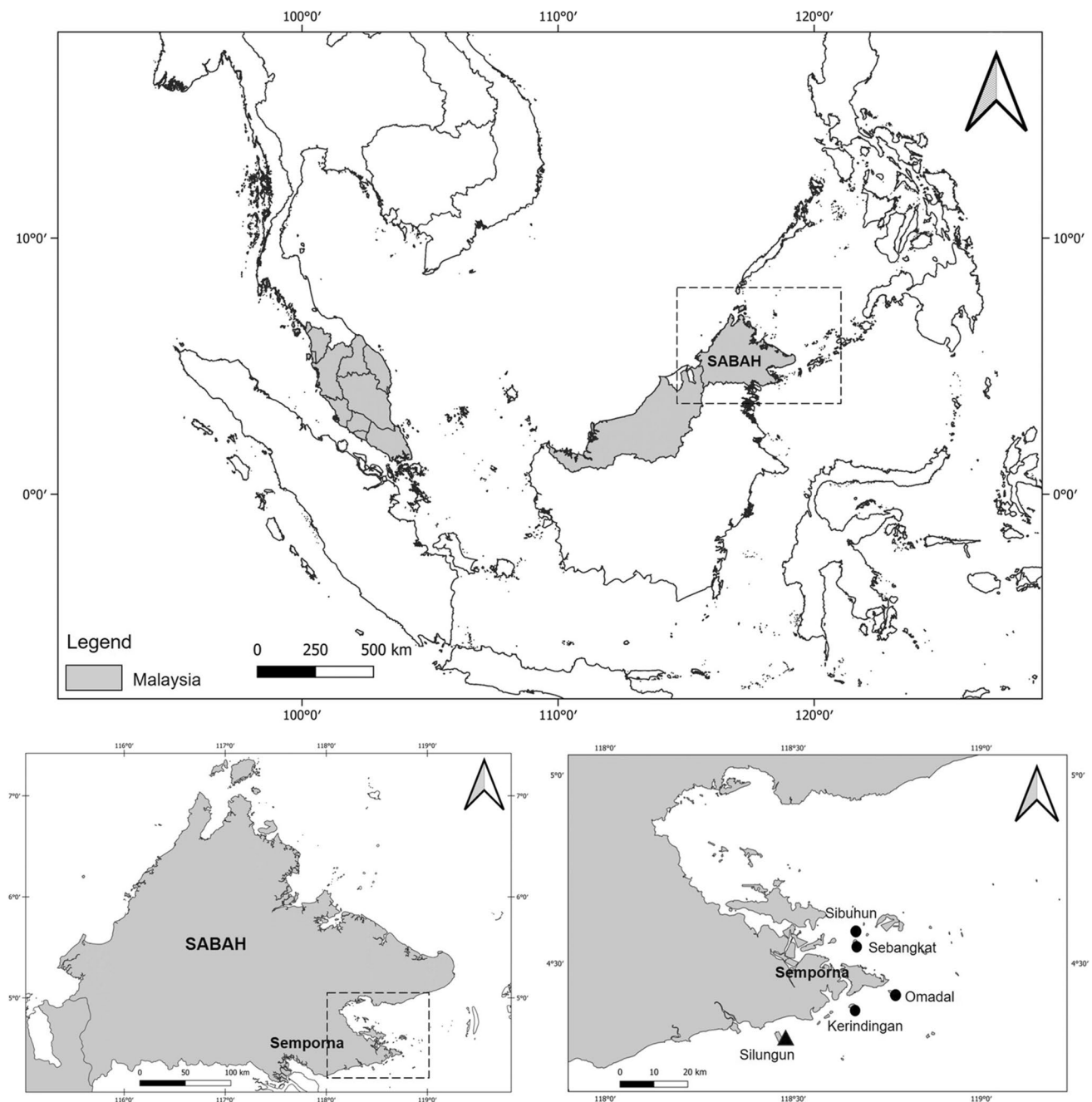


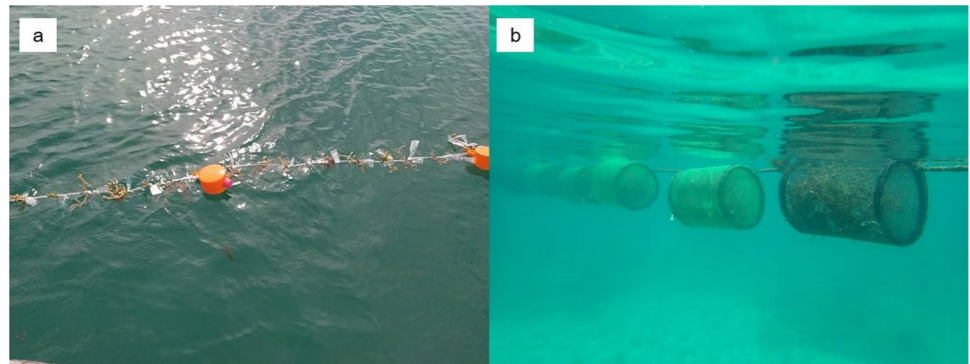
Fig. 1 Collection areas for the wild eucheumatoids (circles) and cultivation area (triangle) at the farm at Silungun, Semporna, Sabah

Environmental parameters

Every two weeks, at the same time as the seaweed monitoring, the following environmental parameters were recorded at 0.3 m depth from three scattered points surrounding the farm using a multiparameter meter (YSI ProQuatro, USA) per monitoring: temperature, salinity and pH. Current circulation data with a spatial resolution of $0.083^{\circ} \times 0.083^{\circ}$ was accessed from the freely available Copernicus Marine

Environment Monitoring Service (CMEMS; <https://data.marine.copernicus.eu/product>). Daily rainfall data were obtained for the Semporna Agriculture Station, which was chosen as the nearest station to the study area, from the myMETdata website (<https://mymetdata.met.gov.my/>) developed by the Malaysian Meteorological Department (METMalaysia). The monthly rainfall data from 2013 to 2022 was also obtained from METMalaysia as a reference to the rainfall trends at the Semporna area.

Fig. 2 Methods used in this cultivation study of wild eucheumatoids: **a.** tie-tie method, **b.** basket net method



Data analysis

The specific growth rates (SGR) of the wild-collected eucheumatoids were calculated using the formula below (Luhan and Sollesta 2010):

$$SGR(\% \text{ day}^{-1}) = (\ln W_t - \ln W_0) / t \times 100$$

where: *SGR*: specific growth rate (% day⁻¹); *W_t*: weight at the end of the study (g); *W₀*: weight at the beginning of the study (g); *t*: number of observation days.

The mean specific growth rates (SGR) were calculated based on the SGR of the surviving wild-collected eucheumatoids for each study period. The percentage survival of wild-collected eucheumatoids was calculated by:

$$\text{Survival}(\%) = \frac{N_s}{N_T} \times 100$$

where *N_s* = number of surviving eucheumatoid individuals for each method per genus, and *N_T* = total number of eucheumatoid individuals used for each method per genus.

One-way ANOVA (analysis of variance) by IBM SPSS Statistics 27 (Statistical Package for the Social Sciences) was used to determine any significant differences in environmental parameters data, survival rate and mean SGR with the study period. Two-way and three-way ANOVA were run to determine whether there were any statistically significant differences in survival rate and mean SGR of the two genera of wild-collected eucheumatoids when using tie-tie compared with the basket net and across the study period.

Principal component analysis (PCA) was performed in XLSTAT 2021.2.2 (Addinsoft 2023) to determine the correlation between the environmental parameters (salinity, temperature, pH, rainfall and current velocity), mean SGR and survival rate of the wild-collected eucheumatoids.

Results

Survival

All the wild-collected eucheumatoids (*n* = 106) survived using the basket net method up until the fourth sampling period (Day 30 – 39). Nonetheless, only 46.15% *Kappaphycus* spp. and 50.75% *E. denticulatum* survived by the end of the study period (Day 60) (Fig. 3). In comparison, the survival of wild-collected eucheumatoids cultivated using the tie-tie method started to decrease by the third sampling (Day 20 – 29) and only 17.20% *Kappaphycus* spp. and 30.77% *E. denticulatum* survived by Day 60. The result of a two-way ANOVA showed a significant effect of the cultivation method (*p* < 0.05) and study period (*p* < 0.05) respectively on the survival, and the interaction of both factors on the survival was similarly statistically significant (*p* < 0.05) (Table S1). There was an insignificant effect of the different genera on the survival (*p* > 0.05). The result of a three-way ANOVA showed insignificant effect of the interaction between cultivation method, study period and genus (*p* > 0.05). Despite a decreasing trend of survival against time for both cultivation methods, wild-collected eucheumatoids showed better survival when cultivated using the basket net compared to the tie-tie method.

Specific growth rates

The wild-collected eucheumatoids recorded the highest mean SGR on Day 10 – 19 with the tie-tie method (Table 1). Results of the two-way ANOVA analysis (Table S2) showed an insignificant effect of the cultivation method (*p* > 0.05) and genus (*p* > 0.05) on SGR, whilst the study period (*p* < 0.05) had a significant effect on SGR. The interaction of the cultivation method and study period

Fig. 3 Survival (%) of wild-collected *Kappaphycus* spp. and *Eucheuma denticulatum* cultivated using the tie-tie and basket net methods. Different letters indicate significant differences ($p < 0.05$) between study periods for each cultivation method

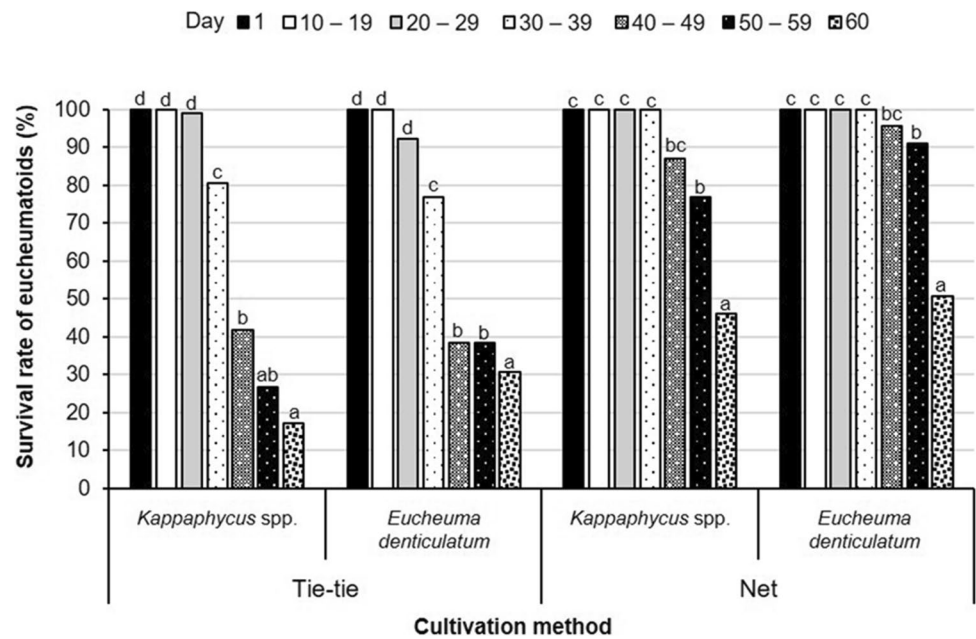


Table 1 Specific growth rate (SGR % day⁻¹; mean $\pm$ SD) of wild-collected *Kappaphycus* spp. and *Eucheuma denticulatum* when using the tie-tie and basket net methods at the farm at Silungun, Sabah. Different superscript letters indicate significant differences ($p < 0.05$) between study periods for each method

Study period (day)	Tie-tie		Basket net	
	<i>Kappaphycus</i> spp.	<i>E. denticulatum</i>	<i>Kappaphycus</i> spp.	<i>E. denticulatum</i>
10 – 19	2.15 $\pm$ 1.90 ^b	2.83 $\pm$ 3.27 ^b	1.52 $\pm$ 2.25 ^c	2.62 $\pm$ 3.83 ^c
20 – 29	1.56 $\pm$ 1.95 ^b	2.60 $\pm$ 2.16 ^b	0.72 $\pm$ 1.30 ^b	1.06 $\pm$ 2.21 ^b
30 – 39	0.72 $\pm$ 1.81 ^a	-0.21 $\pm$ 2.75 ^a	0.61 $\pm$ 0.84 ^b	1.01 $\pm$ 1.33 ^b
40 – 49	0.29 $\pm$ 1.81 ^a	-0.02 $\pm$ 1.47 ^a	0.18 $\pm$ 1.31 ^{ab}	0.83 $\pm$ 1.10 ^{ab}
50 – 59	0.18 $\pm$ 1.67 ^a	-0.27 $\pm$ 1.64 ^a	0.16 $\pm$ 0.91 ^a	0.71 $\pm$ 1.24 ^a
60	-0.40 $\pm$ 1.59 ^a	-0.31 $\pm$ 0.98 ^a	-0.07 $\pm$ 1.19 ^a	0.37 $\pm$ 1.08 ^a

on SGR is similarly statistically significant ($p < 0.05$). A Tukey post hoc test revealed that the mean SGR for tie-tie method for the wild-collected eucheumatoids were significantly different ($p < 0.05$) on Day 10 – 19 and Day 20 – 29 compared to other study periods. The mean SGR for basket net method of the wild-collected eucheumatoids were significantly different on Day 10 – 19 ($p < 0.05$). The result of a three-way ANOVA showed insignificant interactive effect of the cultivation method, study period and genus ($p > 0.05$) on the mean SGR. The mean SGR for both cultivation methods and for both genera decreased over the study period. The wild-collected *Kappaphycus* spp. recorded higher mean SGR using tie-tie compared to basket net, but this is reversed for *E. denticulatum*.

Pest and ice-ice disease incidence

The wild-collected *Kappaphycus* spp. and *E. denticulatum* were affected by biofilms, pests (e.g., epiphytes, colonial tunicates, barnacles) and ice-ice disease in both the tie-tie and basket net cultivation methods (Fig. 4). The earliest

recorded appearances of biofilm, pest and ice-ice disease were during Day 10 – 19 for the basket net method compared with Day 30 – 39 using the tie-tie method. The occurrence of the biofilm, pest and ice-ice disease were recorded for both wild-collected *Kappaphycus* spp. and *E. denticulatum*. The biofilms and pests typically covered the thalli, triggered the ice-ice disease and eventually caused the thalli to break. Consequently, the infected wild-collected eucheumatoids were lost, particularly when using the tie-tie method.

Environmental parameters

There was a statistically significant difference in the environmental parameters (i.e., salinity, temperature, pH) recorded at the farm in Silungun during the study period as determined by one-way ANOVA ($p < 0.05$) (Fig. 5, Table S3). However, a Tukey post hoc test revealed that only temperature was significantly different throughout the 7 study periods ($p < 0.05$) with the mean temperature ranging from 28.70 ± 0.00 to 30.23 ± 0.12 °C. The current velocity was stronger in November and December 2022 (0.10 m s^{-1}) than

Fig. 4 Examples of the biofilms and pests (red arrow) and ice-ice disease (white arrow) affecting the thallus of the wild-collected *Kappaphycus* spp. and *Eucheuma denticulatum* at the farm at Silungun, Sabah



in October 2022 (0.03 m s^{-1}) (Fig. 6), correlating with the inter-monsoon season (October) and the northeast monsoon season (November and December). In addition, there was a significantly higher mean rainfall in 2022 compared to the 2013 to 2021 period in Semporna ($p < 0.05$) (Fig. 7a). The mean rainfall data in 2022 was the highest reported in the last 10-years. The rainfall in October and November 2022 was more than 100 mm higher compared to the mean rainfall from 2013 to 2021 and approximately 100 mm lower in December (Fig. 7b). There was also a significant difference in the daily rainfall in Semporna recorded from 19 October to 17 December 2022 (Fig. 7c) ($p < 0.05$). From the rainfall data, the area received the highest amount of rain during the period Day 10 – 19 (133.5 mm) and Day 30 – 39 (157.3 mm) compared with Day 40 – 49 and Day 50 – 59, which received 52.7 and 80.7 mm of rain, respectively.

The principal component analysis (PCA) performed on the environmental parameters (salinity, temperature, pH, current velocity and rainfall), mean SGR and survival rate during the study period presented three principal components (PCs) with eigenvalues more than 1, representing 47.91% (PC1), 25.22% (PC2) and 16.67% (PC3) of the total variance (Table S4). Fig. S1 presented the biplot of the first two principal components, which accounted for 73.13% of the variability of the experimental data. The PC1 was positively correlated with temperature, pH, current velocity, SGR and survival rate (Table S4). PC2 was positively correlated with rainfall and PC3 positively correlated with salinity. The results from this study showed that the SGR and survival rate increase with higher rainfall which in turn reduced the temperature and salinity during the study period.

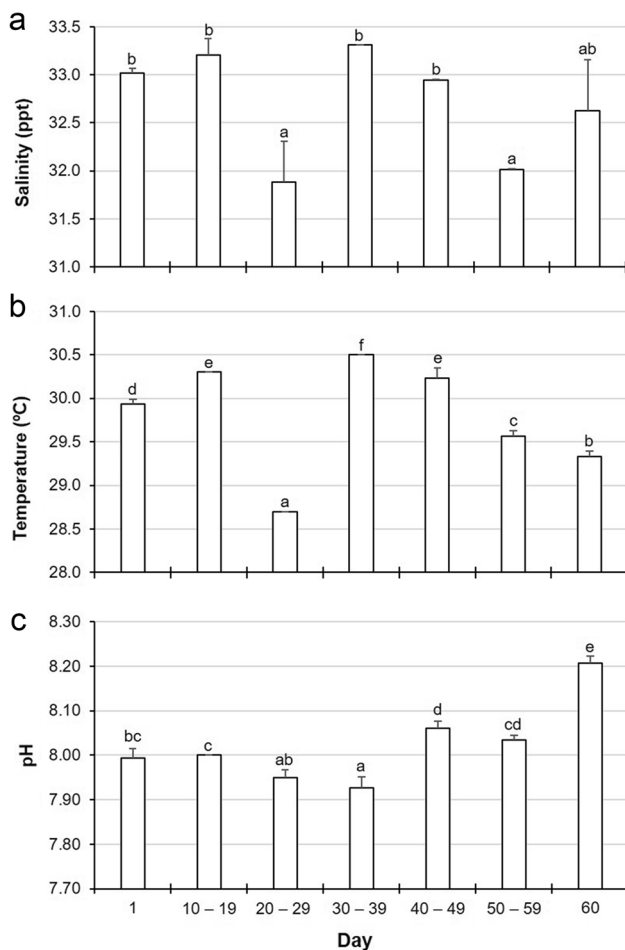


Fig. 5 Environmental parameters (a. salinity, b. temperature and c. pH) recorded during the data collection at the farm in Silungun, Sabah (n=21, mean ± SD). Different letters indicate significant differences ($p < 0.05$) between study periods for each parameter

Discussion

The cultivation of wild-collected *Kappaphycus* spp. and *E. denticulatum* was challenging, as the individuals required time to adapt to their new growing conditions. In their natural environment, they are typically attached to the substratum on the sea bottom at approximately 3 m depth during high tide, whilst commercial cultivation methods, such as tie-tie require their suspension near the sea surface on long-lines. Using the commercial cultivation methods will expose the wild-collected eucheumatoids to higher light intensity, a shift in the spectral light quality and intensity of ultraviolet radiation, and higher temperatures than experienced in the deeper water where they grow. This might have led, therefore, to the significant declines in survival rate, particularly using tie-tie method, and in growth rates of the wild-collected eucheumatoids for both cultivation methods from Day 20 – 29 of the study periods.

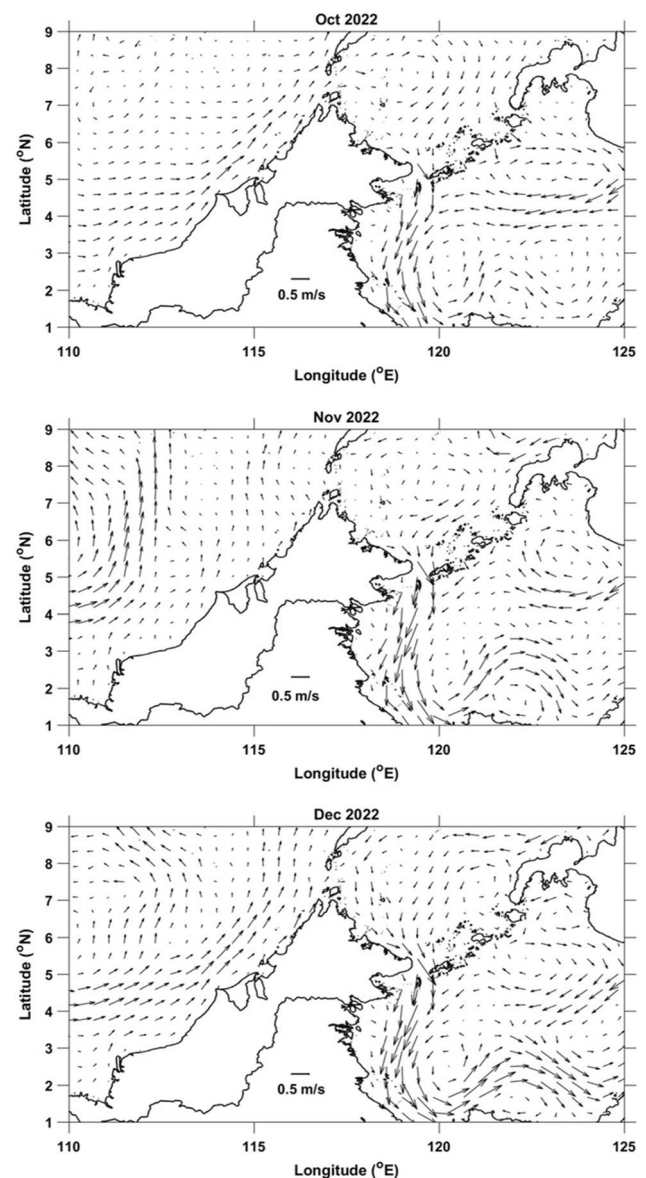
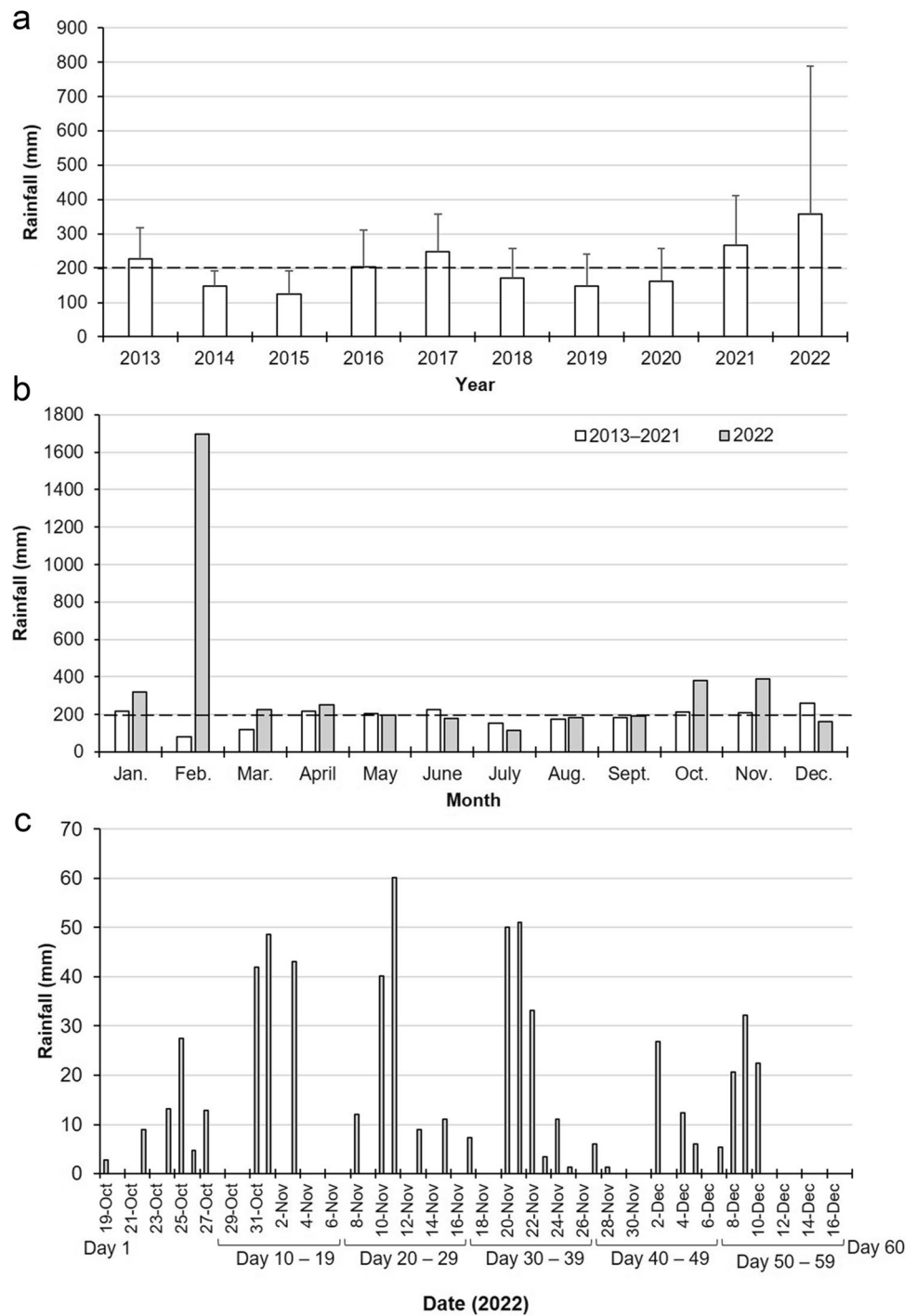


Fig. 6 Current circulation (5 m depth) at the farm in Silungun. Data obtained from Copernicus Marine Environment Monitoring Service (CMEMS; <https://data.marine.copernicus.eu/product>). The length of the arrow indicates the velocity and direction of the current circulation

The mean SGR of the wild-collected eucheumatoids grown on tie-tie and basket net method, showed a decreasing trend against time. The decreasing SGRs can be attributed to: i) the biofilms, ice-ice disease and pests, which occurred due to the slow (less than 0.10 m s^{-1}) water current at the farm site during the study period, ii) an adaptation period required by the wild eucheumatoids to a different growing mechanism (i.e., attached upright to the substrate in the natural habitat as opposed to hanging upside down from the cultivation lines when domesticated in the farm), and iii) a different environment in the farm, which included a change

Fig. 7 Rainfall (mm) at Semporna **a.** mean rainfall from 2013 – 2022 ($n = 12$, mean $\pm$ SD), **b.** mean rainfall recorded in 2013 – 2021 and total rainfall in 2022 by month, **c.** daily rainfall during the study period



in light intensity, spectral light quality and the intensity of ultraviolet radiation, temperature and type of substratum. These justifications are supported by the robust growth observed for commercial cultivars grown by the farmer at the same farm. For the basket net method, the occurrence of biofilms, ice-ice disease and pests may have been caused by a reduction in water flow through the nets, due to the smaller mesh size (6 mm) compared with the 10 – 40 mm mesh used in previous studies. This smaller mesh size

was successful in preventing grazing by herbivores (i.e., fish, including rabbit fish, sea turtles), but may have been too restrictive in terms of current circulation, which promoted pest establishment and ice-ice disease. For the tie-tie method, the prevalence of ice-ice on individuals led to the deterioration and loss of sections of the thallus into the water column, which subsequently caused weight loss and hence, a reduction in SGR between sampling periods. More importantly, the various cultivation methods reported in the

existing literature (Hurtado et al. 2001; Rao et al. 2008; de Góes and Reis 2011; Kasim and Mustafa 2017; Hendri et al. 2020; Munawan et al. 2021; Rasnijal et al. 2021) have been trialled using established cultivars as opposed to the wild eucheumatoids newly introduced into a farming system as in the current study. Thus, it is not unexpected for the SGR to be lower compared to values reported from other studies using commercial cultivars. Despite the loss of some of the wild eucheumatoids, the surviving individuals have the potential though to be developed as cultivars subject to further phenotyping characterization.

Although the factors of cultivation method and genera did not exert any significant influence on the mean SGR of the eucheumatoids, the mean SGR was notably higher on Day 10 – 19 and Day 20 – 29 when the tie-tie method was used. However, as the study period progressed, the mean SGR of eucheumatoids grown using the basket net method was higher than the tie-tie method, particularly for *E. denticulatum*. Negative SGRs were observed when the tie-tie method was used because of the greater loss of biomass due to ice-ice disease and grazing by herbivores compared to when the basket net was used. Kasim and Mustafa (2017) and Rasnijal et al. (2021) reported similar observations in the growth of *K. alvarezii* cultivar in Indonesia, whereby the mean SGR was higher when using net system compared to tie-tie, because the cage structure deterred grazing by herbivorous organisms. Kasim and Mustafa (2017) recorded SGR of $3.52 - 3.69\% \text{ day}^{-1}$ for the floating cage and $2.41 - 2.90\% \text{ day}^{-1}$ for tie-tie, whereas Rasnijal et al. (2021) recorded $2.45 - 6.58\% \text{ day}^{-1}$ for net bag and $1.59 - 6.71\% \text{ day}^{-1}$ for tie-tie. The mean SGR recorded for wild-collected *Kappaphycus* spp. in this study is lower than those reported by Kasim and Mustafa (2017) and Rasnijal et al. (2021) for commercial cultivars that are well adapted to the cultivation system. Moreover, the net system of each study was different: Kasim and Mustafa (2017) used a strung confinement of PVC pipe and a box with dimension of $100 \text{ cm} \times 440 \text{ cm} \times 50 \text{ cm}$ with 1 cm diameter of multifilament nets, whereas Rasnijal et al. (2021) used monofilament gillnet number 40 with mesh size 3.17 cm. In contrast, de Góes and Reis (2011) recorded no significant difference in the SGR of farmed *K. alvarezii* in Brazil grown using tie-tie in 60 mm mesh of nylon net ($3.4 \pm 0.8\% \text{ day}^{-1}$) and 5 m tubular net with 20 mm mesh ($3.2 \pm 0.5\% \text{ day}^{-1}$). Similarly, the mean SGR of wild-collected *E. denticulatum* in this study ($0.37 - 2.62\% \text{ day}^{-1}$) is lower than previous reports for commercial cultivars. Munawan et al. (2021), when studying the growth rate of *E. denticulatum* using different net methods (Vertinet and Horinet with 10 to 40 mm mesh) reported the SGR as 7.69 and $9.16\% \text{ day}^{-1}$, respectively before the value decreased over time. On Day 42 of their study, the value of SGR for Vertinet and Horinet was 3.54 and $4.56\% \text{ day}^{-1}$, respectively.

Hendri et al. (2020) on the other hand reported the SGR for *E. denticulatum* as $3.95 - 7.77\% \text{ day}^{-1}$ when using the net bag method. Previous reports of eucheumatoid cultivation using the tie-tie method generally reported positive SGR, such as in the Philippines, whereby a SGR of $2.3 - 4.2\% \text{ day}^{-1}$ was reported for *K. alvarezii* (Hurtado et al. 2001), and in Northwest India, whereby a SGR of $3.64 - 13.98\% \text{ day}^{-1}$ was reported for *K. alvarezii* (Rao et al. 2008). The lower mean SGR observed in our study compared to $0.44 - 3.74\% \text{ day}^{-1}$ reported by Narvarte et al. (2022) for ten wild *K. alvarezii* cultivated for 42 days in an outdoor land-based hatchery is likely to be attributed to the uncontrolled factors in the open sea, such as water temperature and depth, light intensity, pests and biofilms. However, the growth rates of all the wild *K. alvarezii* strains were observed to decline across sampling days (Narvarte et al. 2022), similar to what was observed in the current study. The authors attribute this observation to the presence of a higher proportion of young and meristematic tissues that contributed to new growth at a faster rate during the first sampling time point, but as the seaweed thallus ages, the higher proportion of mature tissues contribute to slower growth rates at the later sampling time point.

Results of this study highlight the importance of farm site selection for the development of new cultivars from wild populations, where optimal environmental conditions, which include current velocity and substratum with minimal pests, are necessary given the vulnerability of wild eucheumatoids compared to established cultivars. Site selection for seaweed farms is challenging in Semporna waters due to the presence of sea turtles which are protected wildlife. The basket net method developed in this study is now being trialled at a different farming area in Sebangkat with stronger water currents, where significant losses of seaweed have occurred in the past due to turtle grazing. Preliminary results are promising with keen interest from the Semporna Fishermen Association to adopt this method for farming of commercial cultivars.

Environmental parameters are known to be important factors in determining the growth rate and health condition of eucheumatoids on commercial farms, especially water temperature, salinity, light intensity, current velocity, bottom type, depth and water pollution (Foscarini and Prakash 1990; Ward et al. 2021; Bohari and Musbir 2022). From this study, the environmental parameters (salinity, temperature, pH and rainfall) were significantly different ($p < 0.05$) between study period. Temperature was found to be inversely correlated with the rainfall, with temperature decreasing when the study site received higher rainfall on Day 10 – 19. Out of a total of 60 days of the study, no rain was recorded for 30 days of the study period at the study site. Heavy (more than 31 mm) rain was only recorded for 9 days at the study site. In October and November 2022, the rainfall was more

than 380 mm in the study area, which was 50% higher compared to the southwest monsoon (May to September 2022), with the monthly rainfall being less than 195 mm (based on the rainfall data from METMalaysia). The survival rate of the wild-collected eucheumatoids was high when the seawater temperature was below 30 °C. Kasim and Mustafa (2017) reported that the SGR of *K. alvarezii* increases with a decrease in temperature and increase in salinity and current velocity. A later study by Oedjoe et al. (2022) on the effect of temperature on *K. alvarezii* reported higher SGR and carrageenan yield at 29 to 31 °C compared to 32 to 35 °C. The temperature recorded during this study ranged from 28.7 to 30.5 °C was, therefore, within the optimum temperature (27 to 30 °C) for growth of the eucheumatoids (Kumar et al. 2020).

Biofilms, pests and ice-ice disease were reported on the wild-collected *Kappaphycus* spp. and *E. denticulatum* by Day 10 – 19 and this negatively impacted the mean SGR and survival rate of the seaweeds. The occurrence of the biofilms, pests and ice-ice disease was recorded for both wild-collected *Kappaphycus* spp. and *E. denticulatum* throughout the study period. However, the type of pests or species composition of the biofilms were not recorded in this study. The biofilms, pests and ice-ice outbreaks were likely caused by the low current velocity of 0.03 – 0.10 m s⁻¹ at the farm during the study period. Wakibia et al. (2006) and Riyaz et al. (2019) reported that low water current velocity is one of the attributing factors for the development of ice-ice disease in *K. alvarezii* and *E. denticulatum*. A current velocity below 0.1 m s⁻¹ typically elevates biofilm coverage and attachment of pathogenic microorganisms to the thallus of eucheumatoids (Kambey et al. 2021). This recent study suggests that wild-collected eucheumatoids are more prone to biofilms, pests and ice-ice disease outbreaks in periods of low current velocity.

Ice-ice disease is a persistent problem in the seaweed farming industry (Tahiluddin and Terzi 2021). The ice-ice disease is characterised by the depigmentation, whitening or bleaching of the infected thalli, which is then weakened before breaking and/or disintegrating (Trono and Valdestamon 1994; Faisan et al. 2021; Ward et al. 2021). In this study, when badly infected, the thalli typically broke off and fell from the cultivation line when using the tie-tie method compared with the basket method, which retained the infected thallus within the basket. Abdullah et al. (2020) similarly reported the loss of *K. alvarezii* farmed using tie-tie from the cultivation lines into the water due to pests and ice-ice disease infection. Usually, when ice-ice occurs in commercial farming, the farmers will remove the entire infected individuals from the farm to prevent the spread of the disease. For this study, the infected section of the individual was removed before either tying the remaining part back to the cultivation line or placing it back into the basket net,

which may also have led to the reduced SGR observed. Ice-ice disease, however, is reported to occur seasonally and can be affected by location. For example, Alibon et al. (2019) reported that ice-ice disease occurred from July to September in the Philippines and Badraeni et al. (2020) reported that ice-ice disease occurred from September to October in Indonesia. High ice-ice incidence and pest outbreaks have been previously reported in seaweed farms in the Semporna area from May to August (DOFM 2022). Even though the current study was not conducted from May to August, a proportion of the wild-collected *Kappaphycus* spp. and *E. denticulatum* were still badly affected by ice-ice disease and pests. At the end of this study, however, the survival of the wild-collected *Kappaphycus* spp. was 46% and *E. denticulatum* was 51%. In particular, those cultured using the basket net method had a higher survival indicating that these individuals were not only able to adapt to their new environment, but were able to survive the additional stressors imposed by heavy rainfall, periods where seawater temperatures were in excess of 30 °C, and both ice-ice and pest outbreaks. Further investigations for desirable traits, such as the high carrageenan yield and quality are now underway using these individuals to determine their longer-term potential for new cultivar development.

Conclusion

In this study, we compared the growth and survival of wild eucheumatoids at a commercial farm site using two cultivation methods. The tie-tie method recorded better growth rate, but basket net method showed better survival rate of the wild-collected eucheumatoids in Silungun, Sabah, Malaysia. Farming of wild eucheumatoids using the tie-tie method allowed more space for them to grow but at the same time, the relatively smaller-sized wild eucheumatoids with few branching were more likely to become detached from the cultivation lines and were more vulnerable to grazing. In contrast, growth was more restricted due to less space within the basket net, but the loss of biomass from grazing and complete loss from detachment were prevented. The results suggest that the established tie-tie cultivation method is not suitable for wild-collected eucheumatoid during the early process of domestication due to the low survival rate (i.e., high lost or detachment of the seaweeds from the cultivation lines probably due to their small sizes ranging from 20 to 81 g and with sparse branching), compared with commercial cultivars, where the initial seedlings typically weigh 100 g and have more robust branching. The wild-collected eucheumatoids also require time to adapt to the high light intensity, high temperature, shifts in spectral quality and ultraviolet intensity experienced in the new growing environment (i.e., tied to lines near the surface) compared with their natural

subtidal benthic environment in the wild. The development of new cultivars from wild populations, therefore, is a process which will take time and patience, often with low success rate, but it is inevitable that this process is essential to safeguard the future of the seaweed aquaculture industry. Although the domestication of wild eucheumatoids by farmers is largely undocumented, it has been undertaken by a small number of farmers despite the amount of time and effort required to develop a robust growing cultivar. Farm site selection is also crucial for the development of new cultivars from wild populations, where optimal environmental conditions which include current velocity and substratum with minimal pests are necessary given the higher vulnerability of wild eucheumatoids to pests and disease outbreaks compared with established cultivars. The results suggest that to maximise survival and growth, a combination of the two cultivation methods maybe required, with the basket net method used for the early adaptation stage followed by the tie-tie, when the individuals have grown to approximately 100 g. Using the basket net method will increase the initial costs of domesticating new cultivars, but ultimately it will protect the new wild-collected eucheumatoids from herbivores, prior to their transfer to commercial cultivation methods (tie-tie) applied by most of the farmers. Results to date imply that the wild-collected *E. denticulatum* individuals were less vulnerable to environmental changes, pests and ice-ice disease compared to *Kappaphycus* spp. Nevertheless, further study is necessary to determine the impact of climate change on the distribution and changes in population size of the important wild stocks to inform conservation policies and risk mitigation plans, such as setting up biobanks, by the various stakeholders to build resilience in the seaweed industry.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10811-024-03325-8>.

Acknowledgements The authors would like to give special thanks to the officers of the Department of Fisheries Sabah (Mr. Nor Sri Shahri-zan, Mr. Melvin Kinsuat), Mr. Sazali Binsali, Mr. Ali Maulana Mohd Yusah, Muhammad Azam Asri and the local Suluk farmer Mr. Fadzri Abd. Jauhari and his family for their kind assistance during fieldwork in Semporna, Sabah.

Authors' contributions Conceptualization: Phaik-Eem Lim; methodology: Nurulafifah Yahya, Ku Nor Afiza Asnida Ku Mansor, Azhar Kassim; formal analysis and investigation: Nurulafifah Yahya, Sze-Wan Poong; writing—original draft preparation: Nurulafifah Yahya, Sze-Wan Poong; writing—review and editing: Juliet Brodie, Elizabeth J. Cottier-Cook, Joanna Wilbraham, Saul Mallinson; funding acquisition: Phaik-Eem Lim, Juliet Brodie, Elizabeth J. Cottier-Cook.

Funding The research was funded by the UK Department for Environment, Food and Rural Affairs (DEFRA) for the Global Centre on Biodiversity for Climate (GCBC) project (SCG22001; IF041-2022)—Innovative Seaweed Aquaculture: A Nature Based Solution for Biodiversity Restoration & Poverty Alleviation in a Time of Accelerating

Global Climate Change. This study is partially supported by the Higher Institution Center of Excellence (HICoE) grant (HICoE IOES-2023F).

Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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