TRANSACTIONS ON SCIENCE AND TECHNOLOGY

Impacts of Associated Fauna on Seagrass During The Conditioning Period In Husbandry Tanks: Gaya Island, Sabah, Malaysia Case Study

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ABSTRACT Sustainability of seagrass restoration raised concern especially limitation and condition of donor seagrass meadows. To counter this, "gardening" approach can be applied by growing seagrass shoots asexually and sexually in a nursery facility. This study was carried out to identify the fauna species associated with seagrass in the husbandry tanks at Marine Ecology Research Centre (MERC), Gaya Island, Kota Kinabalu, Sabah, Malaysia. Associated fauna was identified to the lowest taxa, while their behaviour and potential impacts on seagrass growth were recorded weekly for 9 months (April 2016 to December 2016). Bite marks on the seagrass leaves were reconfirmed through isolation of fauna with seagrass leaves. Total of 18 species of fauna identified, mostly were mesograzers foraging on seagrass or epiphytic algae. Those are polychaeta, grammarid amphipod, sphaeromatid isopod, sea hares, nerites snails and greenspine sea urchin which left specific bite marks on the seagrass leaves. Also, there is discovery of boring bivalve residing inside the rhizome of the seagrass. Quarantine protocol should implement in the future nursery facility, by removing harmful organisms and introduce beneficial organisms as biological control, to ensure higher survival and growth of seagrass.

KEYWORDS: Seagrass, nursery, fauna, herbivory grazing, propagation
Received 13 April 2020 **Revised** 6 July 2020 **Accepted** 7 July 2020 **Online** 12 July 2020

Original Article

INTRODUCTION

Seagrass restoration through transplantation had resulted various degree of success, depending on local environment, methodologies and limitation of donor shoots (van Katwijk *et al*., 2016; Thorhaug *et al*., 2020). Although it is recommended that seagrass shoots should not collect more than 25% of the total donor coverage but there is limited study on the condition and recovery of seagrass at donor site, which is raised a concern about sustainability of large seagrass scale transplantation. Thus, for seagrass restoration success would require large scale transplantation (van Katwijk *et al*., 2016), which is involved huge number of planting units that directly increase cost of labour, time and money to collect and replant. This may yield little success and may also resulting in degradation of the existing donor seagrass meadows. To counter this, new approach can be applied through "gardening", similar in coral restoration (Baria-Rodrignez *et al*., 2018). Both underwater and land-based facilities can produce planting units, through sexual and asexual seagrass propagation. This method would encourage large scale seagrass restoration with low degree of damage to the existing donor seagrass meadows.

Landed based nursery can promote sustainability of long term and large-scale restoration (Mohamad-Saupi *et al*., 2016; Ishida-Castaneda *et al*., 2019), through cost-effectiveness and allocate more resources into transplantation and restoration. For seagrass, survival of planting units in the underwater nursery vary depending on local environment scenario such as current movement, biofouling, predatory or herbivory impacts (Frias-Torres, 2015) and water parameters fluctuation. Thus, it is required much more resources to manage the nursery and maintaining the health of planting units compared to land based nursery such as time, labour (SCUBA diving to clean biofouling organisms, monitoring etc) and financial support. Land based nursery can modify the environment parameters to ideal growing condition (Quimpo *et al*., 2019), with less temperature and salinity fluctuation. This method can also introduce biological control to counter biofouling organisms and algae (Nithyanandan *et al*., 2018), minimize parasitism and diseases impacts (Pratt, 2017). Toh *et al.*, (2014) reported that land-based nursery can promote higher survivorship and growth through nutritional enhancement and sustain genetic biodiversity. There were various attempts to cultivate seagrass seedlings in laboratory facility (Kirkman 1998; Thangaradjou & Kannan, 2008; Infantes & Moksnes, 2018). The results indicate that the laboratory raised seedlings has better survival rate compared to in vitro and propagules collected directly from donor sites. However, the seagrass health and growth at the land-based nursery may affected by its associated marine fauna.

Borneo Marine Research Institute (University Malaysia Sabah), together with Marine Ecology Research Centre (MERC) has taken an initiative to "gardening" seagrass in a nursery facility at Gaya Island, Kota Kinabalu, Sabah. This aim to reduce the damage of the seagrass donor site and preparing for an asexually and sexually propagation. The objectives of this study were to identify species and observe the behaviour of seagrass associated fauna in the husbandry tanks. The potential impacts of the fauna on the seagrass health were also recorded. This would assist future establishment of landbased seagrass nursery with biological control, enhance cost effectiveness and encourage large scale of seagrass restoration.

METHODOLOGY

A total of five seagrass species (*Halophila ovalis, Enhalus acoroides*, *Cymodocea serrulata, Cymodocea rotundata* and *Halodule uninervis)* were collected for this study. The clod of seagrasses shoots (approximately 20 cm x 20cm) were collected from a donor site at Gaya Bay, Gaya Island (Figure 1). Sediment stick on the seagrass shoots were removed and shoots were placed inside the plastic container kept submerged with seawater. Sandy sediment at adjacent non-vegetated sea floor was collected to fill in the plastic bags for seagrass seedling. Seagrass was transferred to nursery facility at Marine Ecology Research Centre (MERC) located nearby the Malohom Bay (Figure 1). Seagrass shoots were separated according to species, then prepared into planting units (2-4 shoots each) before planted into plastic bags. All planting units were placed randomly in the two husbandry tanks (dimension 1.6m x 1.3m x 0.5m), which were equipped with overflow system, sand filter 100 µm size and received ambient sunlight filtered by transparent roofs. This is part of seagrass conditioning period before transplanting activities at adjunction coastal areas.

Presence of any seagrass associated fauna and physical change of the seagrass (e.g., bite marks on the seagrass leaves and leaves colour change) in the seagrass husbandry tanks were recorded weekly from April to December 2016 (9 months). Photo of fauna was taken with digital camera (Olympus Tough TG5) or observed under compound microscope Carl Zeiss (serial no. 48599) to aid in species identification. Behavioural observations based on fauna grazing activities were also recorded to identify their impacts on the seagrass. For examples, bite marks on the seagrass leaves were identified based on its morphologic characteristics and shape. Mesograzers found nearby the grazed leaves were isolated into a small container with three pieces of seagrass leaves. The bite marks from isolated fauna species were then reconfirmed with those found in the husbandry tank. In addition, physical post-mortems were carried out on any dead seagrass shoots and dissecting any necrotic tissue to identify any obvious underlying causes. All organisms were identified based on morphological characteristics from various sources (Hutchlings & Reid, 1991; Shipway, *et al*., 2016; Holzer, & Rueda, 2011).

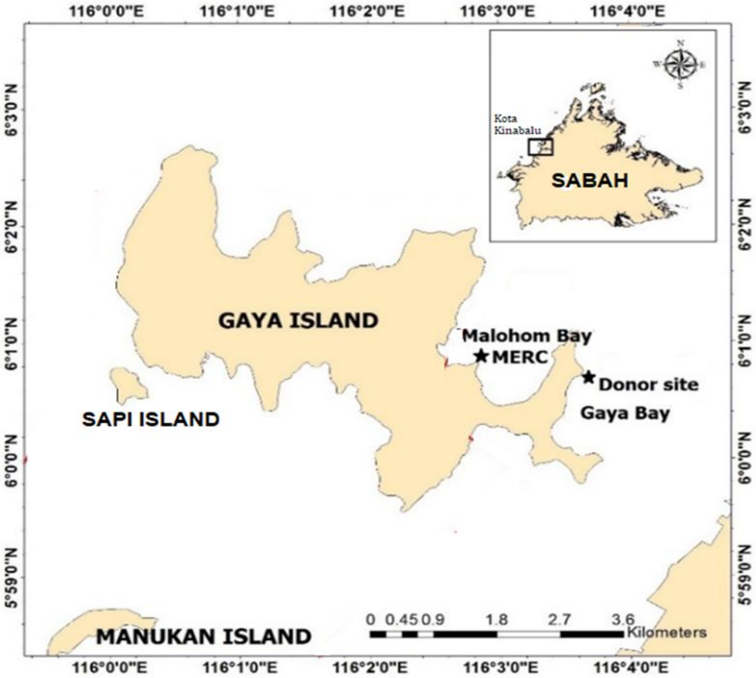

Figure 1. Location of the seagrass donor site (Gaya Bay) and husbandry tanks (Malohom Bay) at Gaya Island, Sabah, Malaysia

RESULTS AND DISCUSSION

Inventory and Behaviour of Associated Marine Fauna

Behaviour observations of associated fauna in the seagrass husbandry tanks are explained in Table 1, while inventory of associated fauna was listed in Appendix 1**.** Among 18 species of fauna identified in the husbandry tanks, seven species were mesograzers. Those species were identified as polychaetes, grammarids amphipods, sphaeromatid isopods, nerites snails and greenspine sea urchin. Of the mesograzer assemblage, the polychaetes and amphipods species are commonly found associated with natural seagrass meadows (Guidelti, 2000; Gambi *et al*., 2003), grazing on the seagrass or epiphytes growing on the leaves surface. Two species of fauna, polychaete and amphipod were making nest on the seagrass leaves by secreted silky substance attracted leaves together (Table 1). While, juveniles greenspine sea urchin were attaching the seagrass leave fragments on its body, together with rubble fragments as camouflage.

Three sea hares species from the family of Aplysiidae was detected three weeks after the seagrass planting units placed in the husbandry tanks. The translucent green sea hare spends most its life cycle on seagrass leaves while, the other two sea hare's species (lined sea hare and blunt end sea hare) were observed feeding on thick layer of epiphytes, leaving behind feeding trails. All three sea hare species were observed, mated and laid eggs masses in the husbandry tanks which is similar finding reported by Wong *et al*. (2016).

Table 1. Identified marine fauna and observation of their behaviour in the seagrass husbandry tanks.

No	Name /species	No of species	Observation / behaviour
1	Sea hare (Line sea hare, blunt end sea hare, green sea hare)	3	All three species grazing on a thick epiphytic mat with feeding trails observed. Line and blunt end sea hares laid strings of egg masses on seagrass tank's wall and seagrass. Green sea hare observed on the seagrass and feed on encrusting fauna. It laid transparent egg mass on seagrass leaves.
2	Polychaete	1	Formed long tube nest by attaching two seagrass leaves together with silky threads. It feeds on seagrass leaves and left irregular bite marks. Faeces were observed green in colour.
3	Grammarid amphipod	1	Formed a nest by attaching leaves fragments on living seagrass leaves. Several of the nests occupied by two individuals with different sizes. It grazes on seagrass leaves and left serrated circular pattern bite marks on the leaves.
4	Sphaeromatid isopod	1	Camouflage or hide on the surface of the sediment or seagrass sheath. This species devour almost all part of the leaves expect vein structure.
5	Nerite snails	3	Sucking sap out from the seagrass leaves and leaving empty tissues structure with cell wall intact. The leaves were transparent to naked eyes.
6	Olive snail	1	Hiding in the sediment most of the time and only come out to feed on other snails.
7	Seagrass boring bivalve	1	Bore and feed on seagrass rhizome (*Enhalus acoroides* and *Cymodocea rotundata*). Calcareous lining tube inside rhizome and emerging calcareous tube from meristem of the plant can be observed.
8	Sea urchin	1	Juvenile greenspine sea urchin attached itself with seagrass leave fragments and rubble fragments.

There are two types of gastropod species with special adaptation observed in the husbandry tanks. The predatory snail, olive snail hides in the sandy sediment most of the time, hunting for other snails' species, like a terrestrial assassin snail (*Clea helena*). While rare boring bivalve was identified inside death seagrass shoots of *Enhalus acoroides* and *Cymodocea rotundata* (Yap *et al*., 2018). This bivalve was bored and ingested in the seagrasses' rhizome materials and creating a calcareous lining hollow that allowing it to move and live within rhizome.

Impacts of associated Marine Fauna to seagrass health

A total of 495 planting units were placed into the husbandry tank (5 species seagrass) and survival rate was approximately 70% in two months. Mortality was caused by natural causes and partially were uprooted by overgrown thick algae and grazing pressure by mesograzers. Some of the mesograzer assemblages were grazed partially on the seagrass leaves. For example, an isopod that has devoured whole leaves except the petiole and rhizome of *H. ovalis*. This is directly contributed to the *H. ovalis* mortality as this species is sole genus with mono-meristematic non-leaf replacing (Calumpong & Fonseca, 2001).

Bite marks on the seagrass leaves serve as important identification key to identify mesograzer and its grazing pressure on the natural seagrass meadows. Although there were no direct observations of the grazing activities but isolation of these associated marine fauna would help to determine the bite marks. Four types of bite marks were identified which are irregular serrated by polychaete (Figure 2a), random circular by amphipod (Figure 2b), leaf almost devoured by isopod (Figure 2c) and plant sap sucked by nerites snails that created transparent cell wall structure on seagrass leaves (Figure 4d). Nevertheless, these findings will help in identifying the grazing impacts on natural seagrass meadows.

Figure 2. Species-specific bite marks by mesograzers in husbandry tanks. (a) Irregular serrated by polychaete, (b) random circular bite marks by amphipod, (3) leaves almost devoured by isopod and (d) transparent leaves by nerite snail.

Post-mortem of seagrass shoots found a boring bivalve species from the family of Teredinidae (Haga, 2006; Shipway *et al*., 2016) living within the rhizome of *E. acoroides* and *C. rotundata*. This new boring bivalve species was associated with lower growth rates ((0.220±0.038 cm day^{-1} in infested shoots, 0.738±0.036 cm day^{-}1 in un-infested shoots, (Yap, *et al*., 2018)). Furthermore, it is appeared to have selective infestation nature towards the thicker rhizome seagrass species which have a higher nutrient content such as in *E. acoroides* and *C. rotudata* species (Terrados *et al*., 1999). This speculation requires further study.

The snail's species (Table 1: Family: Cerithidae, Marginelidae and Neritidae) found in the husbandry tank are commonly found in the tropical seagrass meadows (Fong *et al*., 2018). Snails such as creeper snail (*Rhinoclaris longicaudata)* were observed feeding on detritus and may not give any direct negative impact to seagrass. Only snail species from family Neritidae was observed in sucking plant sap and leaving empty plant tissue, transparent to the naked eye (Figure 2d) and harmful to the plant (Holzer & Rueda, 2011). In contrast, there were three species of sea hares observed as beneficial to seagrass by removing epiphytic algae (Hamatani, 1962). Epiphytes compete for space, sunlight and nutrient in the water column. This would reduce productivity and photosynthesis of the seagrass in land-based nursery. In the future, sea hare species can be introduced as biological control for the epiphytes population on seagrass. It is also possible that faeces from the grazers beneficial to seagrass growth (van Montfrans *et al*., 1984; Gagnon *et al*., 2020).

According to Wong *et al*., (2016), part of sea hare's life cycle is planktonic larval stage and easily introduced into the husbandry tanks from the donor site. Snail species was transferred to the husbandry tanks via sandy sediment or seagrass shoots. Other associated fauna species can be passed through the seawater filter if their larval and eggs are smaller than 100 μm and then settle in the husbandry tank. The conditioning process such as husbandry tanks can be applied as a necessary quarantine protocol for future seagrass nursery establishment. Those associated fauna that do not benefit to seagrass should be removed physically before putting into husbandry tanks.

CONCLUSION

A total of 18 associated fauna species identified in the husbandry tanks and were basically dependent on seagrass as a source of food and protection. High number of mesograzers in the husbandry tank affect the survival rate of seagrass species before the transplant activities at natural environment. Thus introduce beneficial organisms such as sea hare as biological control or epifaunal bivalves to enhance seagrass growth and increase survival rate of seagrass planting units at land based nursery. This method can be applied to ensure better seagrass quality as part of diet to green turtle and dugong in aquaria, or rescue centre. It is suggested that a quarantine protocol should be implemented during husbandry by removing associated organisms that would affect the seagrass health and increase survival and growth rate of the seagrass.

ACKNOWLEDGEMENTS

We would like to thank the Ministry of Higher Education (MOHE) through Project FRG0424-SG-1/2015, Universiti Malaysia Sabah and Marine Ecology Research Centre (Dr. Robert Tan and Mr. Alvin Wong) in providing facility and support in this study. We acknowledge and extend appreciation to the staffs of both institutions, in providing assistant in this study.

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Appendix 1: List of marine fauna identified in the husbandry tanks.

Family	Genus	Species	Common name	Remarks
Aplysiidae	*Stylocheilus*	*striatus*	Lined sea hare	Epiphytes grazer
Aplysiidae	*Dolabella*	*scapula*	Blunt end sea hare	Epiphytes grazer
Aplysiidae	*Petalifera*	*punctulata*	Green sea hare	Epiphytes grazer
Plakobranchidae	*Elysia*	*ornata*	Ornate leaf slug	Sap-sucking
Haminoeoidea	Unable to ID		Headshield slug	-
Nereididae	*Platynereis*	*dumerilii*	Polychaete	Seagrass grazer
Lysianassidae	Unable to ID		Grammarid amphipod	Seagrass grazer
Sphaeromatidae	Unable to ID		Sphaeromatid isopod	Seagrass grazer
Temnopleuridae	*Salmacis*	*spharoides*	Greenspine sea urchin	Seagrass grazer
Aiptasiidae	*Aiptasia*	sp	Aiptasia anemone	-
Cerithiidae	*Rhinoclaris*	*longicaudata*	Creeper snails	Detritivore
Cerithidae	Cerithium	rostratum	Snail	Detritivore
Marginellidae	Unable to ID		Snail	-
Neritidae	*Smaragdia*	*viridis*	Nerite snails	Sap sucking
Neritidae	*Smaragdia*	*sauverbiana*	Nerite snails	Sap sucking
Neritidae	*Vitta*	*virginea*	Nerite snails	Sap sucking
Olivadae	*Oliva*	*australis*	Olive snail	Snail predator
Teridinidae	*Zachsia*	*New species*	Boring bivalve	Bore into rhizome of seagrass