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RESPONSES OF THE SEXUAL PROPAGULES OF *Enhalus acoroides* (L.f. Royle) TO MARICULTURE-DERIVED CHANGES IN WATER QUALITY

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ABSTRACT – Marine aquaculture (or mariculture) has induced varying levels of environmental change in coastal waters, including the decline of seagrass populations. A microcosm experiment was carried out for 115 days to examine the responses of *Enhalus acoroides* sexual propagules to changes in water quality, specifically nutrient enrichment and reduced light availability resulting from addition of artificial fish feed used in mariculture. Seed germination was delayed by two days in the enriched microcosms. Fish feed loading resulted in higher concentrations of dissolved nutrients (NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}) and suspended particulate inorganic (PIM) and organic matter (POM). The nutrient enrichment stimulated the growth of both planktonic and epiphytic algae as evidenced by increased suspended and epiphytic chlorophyll content. Consequently, the higher water column turbidity and denser epiphyte layer resulted in at least 7% increase in light attenuation. The lower light availability for the growing propagules may account for 13% and 40% reduction in seedling growth and survival, respectively. The length and biomass of the roots the seedlings in the enriched microcosms were reduced by 41% and 35%, respectively, hence the seedlings remained weakly attached to the substrate. As sexually derived propagules are essential for the persistence of genetically diverse seagrass populations as well as recovery and restoration of damaged seagrass areas, the inability of these propagules to establish and survive the deteriorating water quality associated with mariculture could ultimately lead to the decline of seagrasses along with its key ecosystem functions. Therefore, there is need to reevaluate mariculture practices and identify ways to maintain healthy and resilient seagrass meadows.

Keywords: Enhalus acoroides, mariculture, nutrient enrichment, seagrass, sexual propagules

INTRODUCTION

Seagrasses form one of the most important ecosystems in shallow coastal waters where they maintain high primary production (Duarte 1989) and provide shelter as well as feeding, breeding, and nursery grounds for a diverse array of marine invertebrate and vertebrate fauna (Howard et al 1989, Klumpp et al 1993). Their physical structure offers other important functions which include filtration and recycling of nutrients (Hemminga et al 1991), trapping and binding of sediments (Fonseca 1989) and shoreline protection through sediment stabilization and reduction of wave energy (Fonseca and Fisher 1986). However, the seagrass ecosystem is also one of the most threatened ecosystems due to its presence in shallow coastal areas (Orth et al 2006, Burkholder et al 2007), where it is often subjected to various

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anthropogenic disturbances.

Marine aquaculture or mariculture has been identified in the past few decades as one of the major disturbances causing significant loss of seagrasses (Orth et al 2006, Waycott et al 2009) because the fish farming structure consisting of fixed pens and floating cages are commonly placed adjacent to or directly on seagrass beds (Herbeck et al 2013, Tanaka et al 2014). Mariculture releases and leaves large amounts of uneaten fish feeds as well as feces of the cultured fish in the surrounding waters, and eventually accumulating on the seabed (Perez et al 2008, Holmer et al 2008, Ferreira et al 2016). The nutrients from these fish feeds stimulate the growth of both planktonic and epiphytic algae (Krause-Jensen et al 2008) that compete with seagrasses for light. In addition, turbidity from particulate organic and inorganic materials increases light attenuation, further affecting light available for the seagrass. Most studies on mariculture impacts on seagrasses focus on the health status of adult seagrass populations, including reductions in distribution area, cover and density, decrease in shoot size, appearance of necrosis in leaves (Dimech et al 2000, Cancemi et al 2003, Apostolaki et al 2009, Ruiz et al 2001), reductions in vertical rhizome growth (Marba et al 2006) and changes in epiphyte load (Frankovich and Fourqurean 1997). In contrast, studies on the response of seeds and seedlings to environmental changes are lacking, particularly for tropical species (Artika et al 2020). To date there is no study on the effects of water quality changes associated with mariculture on seedling development and growth. As seagrass restoration programs could be based on generative techniques (i.e. using seeds and seedlings) (Orth et al 2006b, Marion and Orth 2010, Statton et al 2013, Alagna et al 2015, Artika et al 2021), investigating the responses of these early-life phase of seagrasses to environmental stressors is important.

Enhalus acoroides is a common and dominant seagrass species in the Philippines. It exhibits both clonal and sexual reproduction from seeds. Its sexual propagules play a significant role in the maintenance and persistence of genetically diverse seagrass populations as well as recovery and restoration of damaged meadows, particularly when vegetative reproduction fails to withstand adverse conditions (Kendrick et al 2012). This genetic variability makes seagrasses more resilient to environmental change, offering greater adaptability and allowing seagrass ecosystems to recover more effectively from environmental stressors, including climate change (Unsworth et al. 2015). The ability of seeds to germinate and remain attached to sediments and subsequently develop into seedlings, grow, and thrive are critical steps in establishing new populations and the continued survival of existing genetically diverse populations in highly disturbed seagrass areas (Artika et al 221).

This study was carried out to evaluate the effects of fish feeds loading on selected response traits of the sexual propagules of *Enhalus acoroides*. It examined how fish feeds used in mariculture modifies the physical, chemical, and biological environment and how these changes affected the growth and survival of *E. acoroides* sexual propagules. A laboratory experiment was conducted to evaluate the responses of seagrass seeds and seedlings to changes in nutrient levels and light availability following fish feed loading. By investigating the responses of sexual propagules, this study aims to better understand the impact of these environmental changes and assess the potential for these propagules to grow and survive under mariculture-associated conditions. The findings could inform future management and restoration plans for damaged seagrass ecosystems.

MATERIALS AND METHODS

Enhalus acoroides seeds collection

Around 50 mature fruits of *E. acoroides* were collected from a seagrass meadow around Silaqui Is., Cape Bolinao, northwestern Philippines. In the laboratory, fruits were opened, and seeds were separated from the fruit capsule by pressing the capsules between the fingers. The seeds were thoroughly mixed in a plastic basin containing seawater. This was done to randomize the selection of seeds used in the experiment, although seeds of the same size were selected.

Figure 1. Location of the *Enhalus acoroides* seeds collection site.

The seeds were grown in 6 outdoor microcosms, consisting of 80-liter glass aquaria each containing six 4-liter pots filled with sediment to a depth of 9 cm. The sediment used as substrate was collected from the fruit collection site. The grain size profile of the substrate was as follows: medium to very coarse sand (60-63% g dry wt.), fine sand (17-19% g dry wt.), very fine sand (18% g dry wt.) and silt (1-4% g dry wt.). Experimental conditions were established to simulate the natural environment of *E. acoroides* within the spatial constraints of the microcosms. Stocking density was 6 seeds per pot or a total of 36 seeds per aquarium. This number approximates the average density of *E. acoroides* (i.e., 40 shoots.m^{-2}) in Cape Bolinao (Rollon 1998). The aquarium dimensions were 61 cm (L) x 35 cm (W) x 46 cm (H). The aquaria were covered on all sides with 1/2-inch styropore boards to eliminate shading by adjacent tanks (Rollon 1998) and were equipped with flow-through seawater supply to maintain ambient temperature and salinity. The seawater supply was pumped from a nearby strait where seawater salinity and temperature were 33.3±1.8 ppt and 28.9±1.2 ^{o}C, respectively. An aeration system bubbled air in the microcosms to provide mixing and to minimize CO_2 limitation and O_2 inhibition. The tanks were placed under shadeless conditions to provide equal ambient light availability to the systems. To simulate exchange rates in quiet embayments where seagrasses commonly occur, seawater exchange in the tanks was restricted to 10% of tank volume per day. During the experiment, the walls of the aquaria were maintained free of epiphytic growth by gentle brushing with a sponge at 4-day intervals. However, the loosened epiphytes from cleaning the aquaria were not removed to avoid uneven alterations of nutrient budgets in the systems.

Experimental design

The experiment followed a completely randomized design. The 6 microcosms were divided between Control (untreated) and Enriched (treated with fishfeeds), with three replicates each. The mariculture-derived nutrient enrichment was simulated by adding artificial fish feeds which are used in milkfish culture in Cape Bolinao. The feeds (milkfish finisher feeds) come in pellet form and are composed of 27% crude protein, 5% crude fat, 8% crude fiber, 12% crude ash and 13% moisture. Total nitrogen and phosphorus content of the fishfeeds were 3% and 0.04%, respectively. A daily dosage of 2 grams was added to each Enriched tank. This application scheme was derived from the actual amounts (calculated per volume of water) of fishfeeds applied in some of the fish pens in Cape Bolinao (approximately 3-16 sacks of feed added to a 5mx6m pen per day (Yap et al 2004)). The fish pellets were placed in small mesh bags (pore size = 0.5 mm) which were suspended midway down the tank to ensure that the nutrients would diffuse through the water column. At the end of each 24-hour period, the bags with the undissolved materials were removed and were replaced with bags of fresh pellets. The microcosm experiment ran from March to June 2016 (115 days), during which physico-chemical parameters and seedling responses were monitored.

Monitoring of physico-chemical variables

The levels of dissolved inorganic nutrients (NH_4^+, NO_3^- + NO_2^- and PO_4^{3-}), the vertical attenuation (K_d) of photosynthetically active radiation (PAR), pH, abundance of suspended particulate inorganic matter (PIM) and organic matter (POM) as well as suspended chlorophyll a (chl a) concentration were measured 6x per 30-day sampling period (i.e. T1: Mar 25-Apr 24; T2: Apr 25-May 25; T3: May 26-June 25). Concentrations of dissolved inorganic nutrients were measured spectrophotometrically as described in Parsons et al (1984). During each sampling period, three 250 ml of water samples from each microcosm were collected onto acid-washed Nalgene bottles. Water samples were obtained by drawing subsurface water through a Tygon tubing connected to a peristaltic pump. In the laboratory, the water samples were immediately filtered onto acid-washed and pre-combusted glass fiber filters (GFC) to remove all suspended solids and stored at -7°C for later analysis.

For pH measurements, subsurface water samples collected onto 50 ml screw-capped glass vials were used. The pH was measured immediately by using an Orion field pH meter (model SA-250).

The vertical attenuation of PAR through the water column was determined by taking simultaneous light readings at two fixed depths (distance between LICOR LI-1000 sensors was approx. 0.35 m). The light extinction coefficient, K_d, was calculated from the Lambert-Beer equation:

$$K_d = \frac{\ln \frac{I_o}{I_z}}{\Delta_z}$$

Suspended particulate organic matter (POM), particulate inorganic matter (PIM) and chl a concentrations were determined from water samples (n=3) collected as described for nutrients. Each water sample was filtered onto pre-combusted and pre-weighed glass fiber filters. For POM and PIM, the filters were oven-dried at 60°C to constant weights afterwards, followed by combustion at 550°C for 5 hours to get ash-free dry weight. The amount of POM (mg.liter^{-1}) was expressed as the difference between dry weight and ash weight (PIM, mg.liter^{-1}). For chl a, the filters were placed in screw-capped centrifuge tubes containing 15 ml of 90% acetone. After extraction in the dark at 5°C for 24 hours, the samples were centrifuged at 5000 rpm, and absorbance at 664 nm and 750 nm (absorbance due to turbidity) were measured. Chlorophyll a concentration was calculated following the equations given by Parsons et al (1984).

The amount of chl a was expressed in μg chl a. liter^{-1}.

Responses of the sexual propagules

Seed germination. The germination of *E. acoroides* seeds was monitored from the day these were planted until the first pair of true leaves appeared. The number of germinated seeds per treatment was recorded daily and was expressed as the percentage of the total number of seeds planted.

Seedling survival. At the end of each 30-day period, the number of live seedlings in each microcosm was counted. Seedling survival was calculated as follows:

$$Survival(\%) = \frac{number\ of\ live\ seedlings\ (N_t)}{total\ number\ of\ seeds\ planted\ (N_o)} * 100$$

Leaf growth rate and biomass. The modified blade-marking technique by Zieman and Wetzel (1980) was used to determine leaf growth rate (LGR), leaf surface area (LSA), and relative growth rate (RGR). When all the seeds had germinated, a hypodermic needle was used to carefully pierce 6 randomly selected shoots (i.e., 1 shoot per pot) just above the bundle sheath. Colored wires were used to tag each marked shoot for later reference. At the end of a 30-day period, the marked leaves were harvested by clipping them at the base. After removing all the epiphytic material, new leaf growth was measured as the distance that the holes travelled in every leaf. Total length and width of each leaf per shoot were also measured. Then, the leaves were oven-dried at 60°C to constant weight to get leaf biomass (mg dry wt.) per shoot. The following parameters were calculated (Rollon 1998):

(1) Leaf growth rate (LGR, cm^2.day^{-1})

$$LGR = \frac{\sum leaf\ length\ increments * width}{observation\ period(days)}$$

(2) Leaf surface area (LSA, cm^2.shoot^{-1})

$$LSA = total\ leaf\ lengths * width$$

(3) Relative growth rate (RGR, cm^2.cm^{-2}.sht^{-1}.day^{-1})

$$RGR = \frac{LGR}{LSA}$$

These procedures were repeated for the two succeeding sampling periods.

Seedling morphometrics and biomass. At the end of the experiment, all surviving seedlings that were not used in the growth rate measurements above were harvested including their below-ground plant parts. Twenty (20) seedlings per treatment were used for the measurements of shoot size and the number and biomass of leaves and roots. In the laboratory, the epiphytic material was scraped-off from each leaf with a spatula and was transferred into vials containing 5 ml of filtered seawater. The epiphyte suspensions were set aside for measurement of epiphytic chl a. Morphometric characteristics of each seagrass shoot (shoot height, number of leaves, mean leaf length and width, number of roots, mean root length) were measured. Then, the leaves and roots of each shoot were oven-dried at 60°C to constant weights. Above- and below-ground biomasses were expressed in g dry wt.shoot^{-1}.

The epiphyte suspensions obtained above were filtered onto pre-combusted and pre-weighed GFC filters. The filters (n=10) were placed in screw-capped centrifuge tubes containing 15 ml of 90%

acetone. After extraction in the dark at 5°C for 24 hours, the samples were centrifuged at 5000 rpm, and absorbance at 664 nm and 750 nm (absorbance due to turbidity) were measured. Chlorophyll concentrations were calculated following the equations given by Parsons et al (1984). The amount of chl a was expressed in $\mu g.cm^{-2}$. Epiphytic chl a content was standardized with the two-sided leaf area of the seagrass.

Data analyses

Significant differences in the concentrations of dissolved nutrients (NH_4^+, NO_3^- + NO_2^-, and PO_4^{3-}), suspended POM, PIM, and chl a, light extinction (K_d) as well as the leaf growth rate (LGR), leaf surface area (LSA), and relative growth rate (RGR) of the seagrass seedling across treatments and sampling periods were tested using two-way analysis of variance (ANOVA) ($p<0.05$). Residual analysis was used to diagnose departures from basic assumptions of analysis of variance, and transformations were applied if necessary to correct for non-normality and non-constancy for error variance. Tukey's honestly significant difference (HSD) procedure was used to test differences among individual means following ANOVA. On the other hand, t-test ($p<0.05$) was used to determine significant differences in epiphyte chl a and seedlings response variables (shoot height, number of leaves and roots, leaf chl a, length and biomass of leaves and roots) between treatments.

Principal components analysis (PCA) was used to determine the relationships between the physical, chemical, and biological variables. PCA reduces the dimensionality of the possibly correlated variables while retaining most of the information (Ringner 2008).

RESULTS AND DISCUSSION

The concentrations of dissolved inorganic nutrients varied significantly between control and enriched treatment (Fig. 2) but remained relatively constant with time ($p<0.05$ ANOVA). Ammonium concentration in the enriched microcosms was almost 10x higher than in the control. The concentrations of

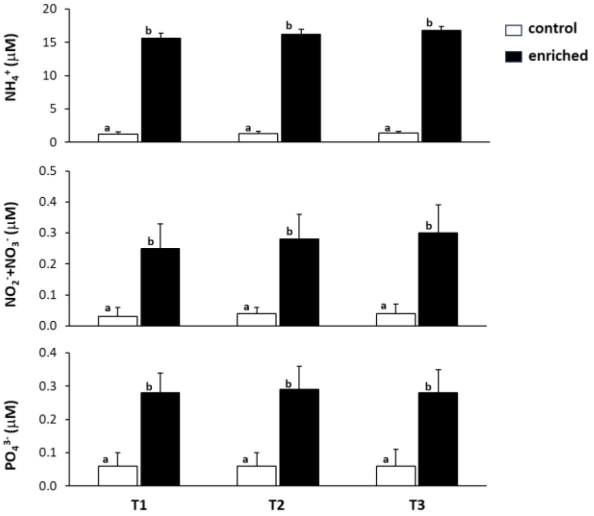


Figure 2. Variations in concentrations of inorganic nutrients between the control and enriched microcosms through time (T).

$NO_3^-+NO_2^-$ and PO_4^{3-} were about 5% higher than in the control microcosms. The elevated ammonium (~95% of total DIN) in the enriched condition may be due to the high organic matter content of the fish pellets. The lower relative concentration of nitrite and nitrate than ammonium suggests that nitrification could not match the high rate of organic matter loading and the subsequent ammonium production. On the other hand, no significant differences in pH (range: 8.0-8.1) were observed between the treatment and control as well as across time.

The amount of suspended POM, PIM, and chl a as well as the light attenuation coefficient were all significantly higher in the enriched microcosms than in the control microcosms ($p<0.05$ ANOVA) (Fig. 3). In the enriched microcosms, PIM constituted about 60% of the total suspended matter. The concentration of suspended chl a and vertical attenuation of PAR varied significantly between treatments ($p<0.05$) as well. In the enriched microcosms, K_d values were higher than in the control microcosms resulting in 19% light reduction.

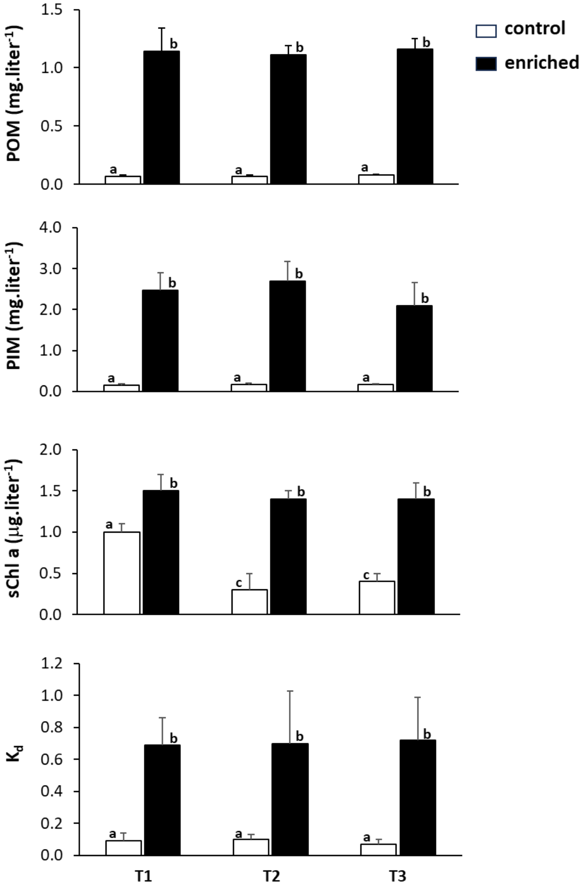


Figure 3. Average concentrations of suspended POM, PIM, and chlorophyll *a* and the extinction coefficient of light through the water column under control and enriched conditions through time (T).

Similarly, the chl a content of the epiphytic assemblage was also significantly higher under enriched condition than in the control (Fig. 4) ($p<0.05$ ANOVA). Epiphytic chl a concentration in the enriched condition was about 20x higher than in the control.

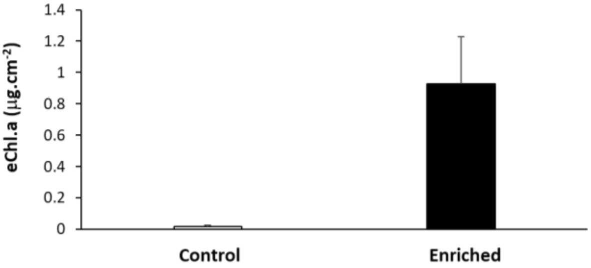


Figure 4. Average concentrations of chlorophyll a in the epiphyte layer on *E. acoroides* leaves measured at the end of the experiment.

The addition of fish feeds did not show any pronounced negative effect on the germination of *E. acoroides* seeds (Fig. 5). In both the control and enriched conditions, 100% germination was observed in all microcosms at about 14 days from the start of the experiment, although germination in the enriched microcosms was delayed by approximately two days. This suggests that the change in water quality from fish feeds addition may have minor effect on the germination process since 100% germination was achieved in both the control and enriched cultures.

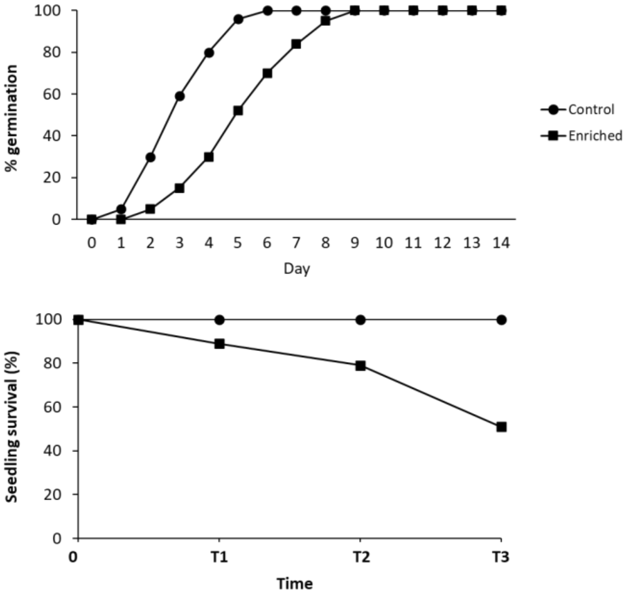


Figure 5. Percentage germination of *E. acoroides* seeds and seedling survival under control and enriched conditions.

On the other hand, seedling survival (Fig. 5), growth, biomass, and morphology were clearly affected by the abundance of suspended and attached biotic and abiotic materials (Fig. 6). Variations in leaf growth rate (LGR), leaf surface area (LSA), and relative leaf growth rate (RGR) were significant between treatments as well as across sampling periods ($p<0.05$). In terms of survival, all seedlings in the control microcosms survived until the end of the experiment while seedling loss of about 40% of the total population size was observed in the enriched microcosms. LGR, LSA, and RGR of the seedlings were lower by 48%, 4%, and 13% in the enriched microcosms, respectively. After three months of culture, seagrass seedlings in the control microcosms were taller than the seagrasses in the enriched conditions (Fig. 7). The addition of fish feeds reduced shoot size by about 48%. Seedlings in control microcosms also had more leaves and roots than those grown under enriched conditions. Moreover, the ratios of the above-ground (AG) and below-ground (BG) length and biomass of seedlings likewise showed significant difference between control and enriched conditions ($p<0.05$) (Fig. 8). Notably in the enriched microcosms, the root length and biomass were significantly reduced, hence higher AB:BG ratios. Most of the surviving seedlings in this condition were not firmly attached to the substrate due to poor root growth.

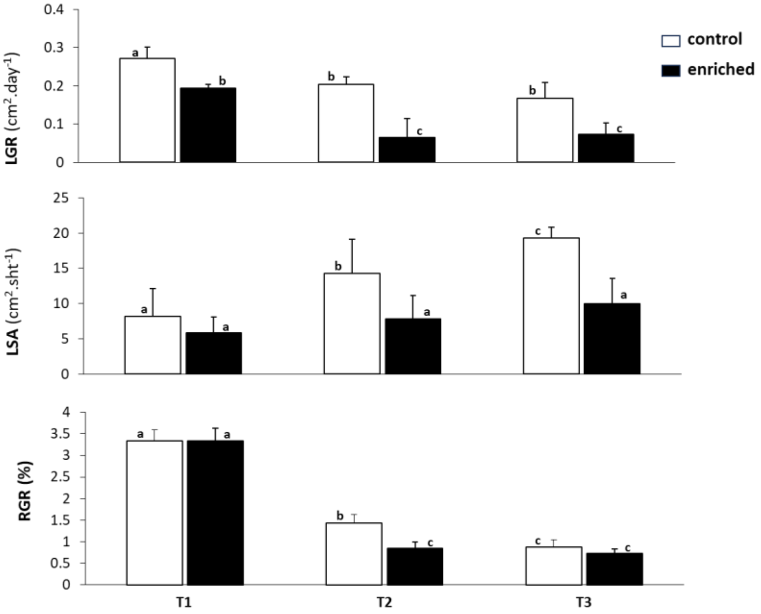


Figure 6. Variations in leaf surface area, leaf growth rate and relative growth rate of *E. acoroides* seedlings.

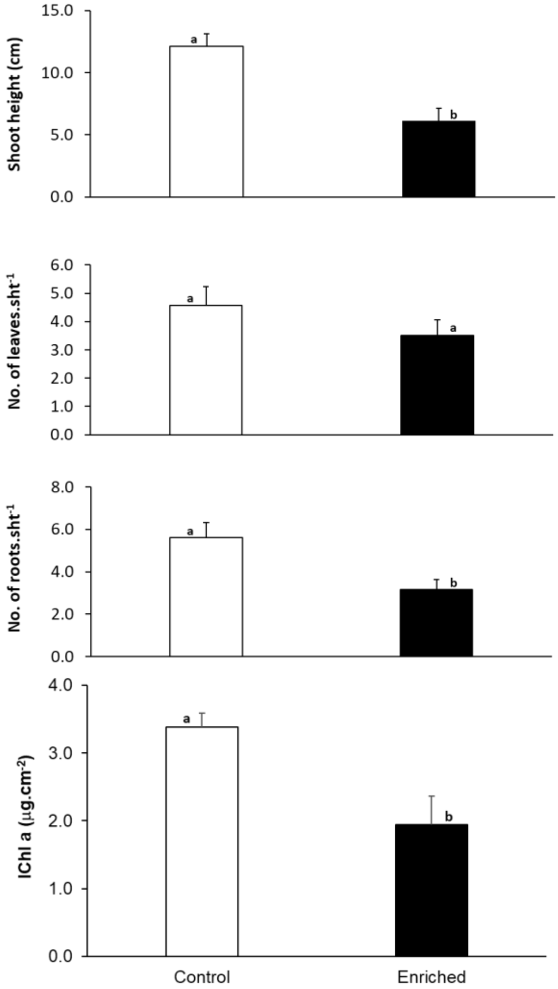


Figure 7. Comparison of *E. acoroides* shoot morphometrics and leaf chl a content under control and enriched conditions measured at the end of the experiment.

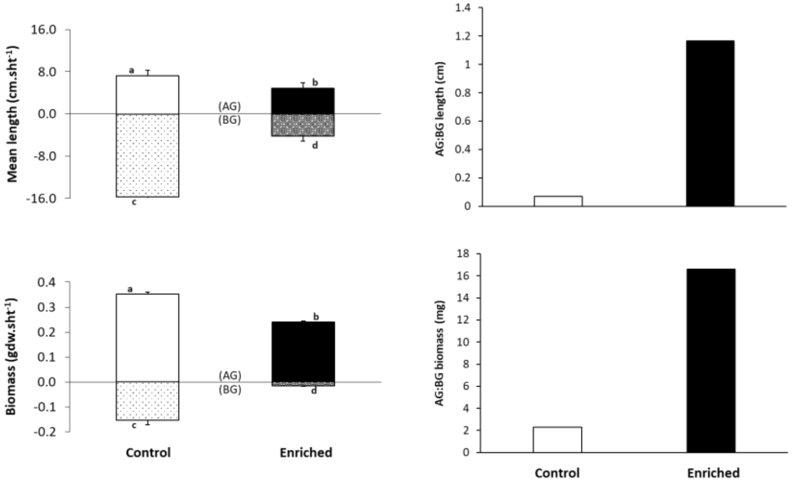
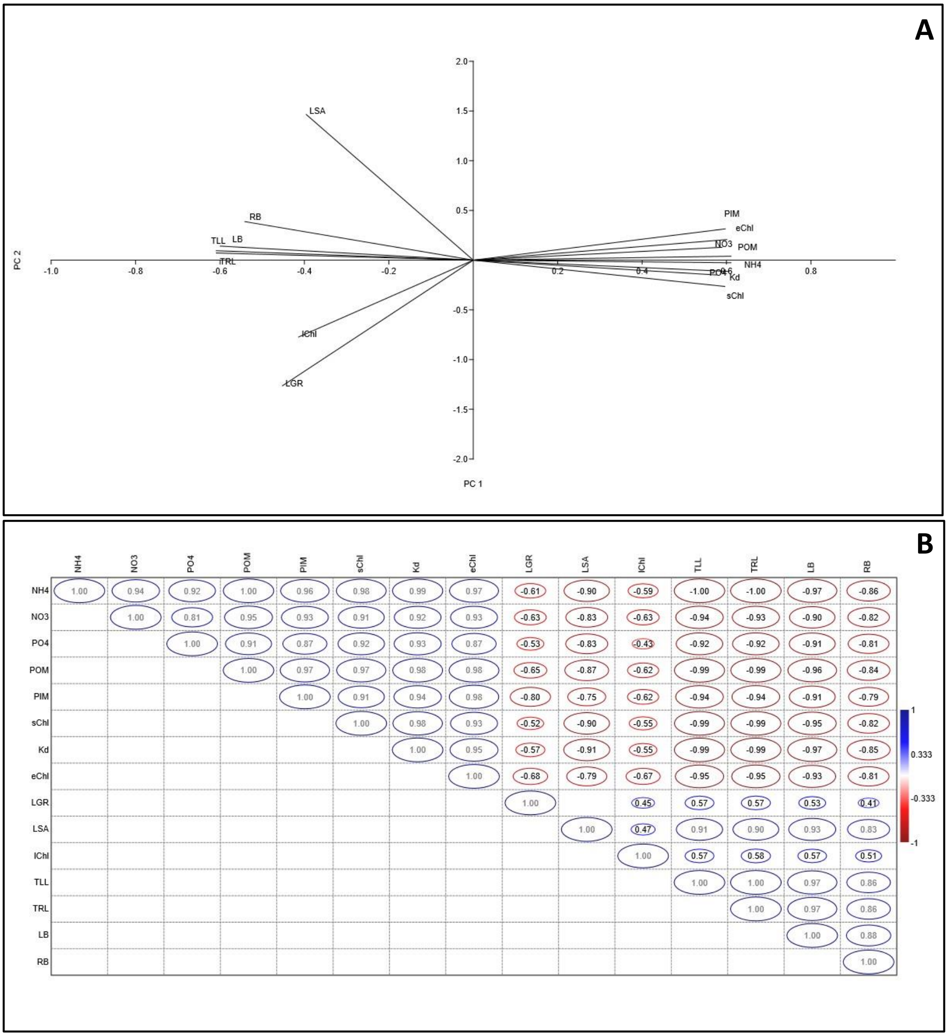


Figure 8. Mean length and biomass of the leaves and roots and ratios of above-ground (AG) and below-ground (BG) length and biomass of *E. acoroides* surviving seedlings in the control and enriched conditions measured at the end of the experiment.

PCA results showed that the first and second components (PC 1 and PC 2) represented 82.9% and 14.6% of the total variance, respectively (Fig. 9A). PCA loadings further revealed that nutrients, suspended particulate materials, light extinction as well as suspended and epiphytic chlorophyll contributed the most on PC 1 and indicated negative impact on the seagrass response variables. The correlation between the variables in the total dataset was confirmed by the Pearson correlation coefficient. As shown in Fig. 9B, water column light extinction was strongly positively correlated with NH_4^+, NO_3^- + NO_2^- and PO_4^{3-} as well as the suspended POM and PIM. Moreover, the strong positive correlation of nutrients with both the suspended and epiphytic chlorophyll a in the enriched microcosms indicates stimulated growth of planktonic and epiphytic algae, which require light for growth. These show that the nutrients and particulate matter from the fish feeds significantly reduced light availability for the seagrass propagules, which was evident in the negative correlations between these factors and the seagrass responses. Of the seagrass response variables, the above- and below-ground length and biomass of the seagrass seedlings were the most negatively affected by fish feeds loading.

Seagrass growth and productivity has been suggested to be largely a function of light (Wetzel and Penhale 1983, Dennison et al 1985) and nutrients (Hemminga et al 1991). Seagrasses act as natural biofilter for nutrients and (Sandoval-Gil et al 2016) and their growth and productivity are favored by increased nutrient concentrations (Burkholder et al 1994, Agawin et al 1996, Lee and Dunton 1999, Udy et al 1999). With respect to light, adult populations of *E. acoroides* have been shown to be the least sensitive to reduced light availability due to their lower light compensation point (Agawin et al 1996), larger size (Rollon 1998) and energy stored in their larger rhizomes. However, plasticity of plant growth has its limits (Philippart 1994), and any event that significantly reduces water clarity or alters nutrient levels, leading to substantial reductions in light availability, can result in the degradation or loss of seagrass populations. Thus, the survival and recovery of damaged seagrass populations may rely on the successful establishment, growth,



	NH4	NO3	PO4	POM	PIM	sChl	Kd	eChl	LGR	LSA	lChl	TLL	TRL	LB	RB
NH4	1.00	0.94	0.92	1.00	0.96	0.98	0.99	0.97	-0.61	-0.90	-0.59	-1.00	-1.00	-0.97	-0.86
NO3		1.00	0.81	0.95	0.93	0.91	0.92	0.93	-0.63	-0.83	-0.63	-0.94	-0.93	-0.90	-0.82
PO4			1.00	0.91	0.87	0.92	0.93	0.87	-0.53	-0.83	-0.43	-0.92	-0.92	-0.91	-0.81
POM				1.00	0.97	0.97	0.98	0.98	-0.65	-0.87	-0.62	-0.99	-0.99	-0.96	-0.84
PIM					1.00	0.91	0.94	0.98	-0.80	-0.75	-0.62	-0.94	-0.94	-0.91	-0.79
sChl						1.00	0.98	0.93	-0.52	-0.90	-0.55	-0.99	-0.99	-0.95	-0.82
Kd							1.00	0.95	-0.57	-0.91	-0.55	-0.99	-0.99	-0.97	-0.85
eChl								1.00	-0.68	-0.79	-0.67	-0.95	-0.95	-0.93	-0.81
LGR									1.00		0.45	0.57	0.57	0.53	0.41
LSA										1.00	0.47	0.91	0.90	0.93	0.83
lChl											1.00	0.57	0.58	0.57	0.51
TLL												1.00	1.00	0.97	0.86
TRL													1.00	0.97	0.86
LB														1.00	0.88
RB															1.00

Figure 9. Principal component analysis (PCA) of factors: (A) loadings of variables; and (B) correlation matrix of the physical, chemical, and biological factors and seagrass response variables.

and survival of the sexual propagules. The ability of seeds to germinate and the subsequent settlement, growth, and survival of seedlings are critical factors in establishing new populations and ensuring the continued survival of existing genetically diverse seagrass populations in the face of a constantly changing environment.

This study showed that the seeds successfully germinated following fish feed loading, although the process was slightly delayed. This suggests that light availability has minimal impact on germination, as successful germination was achieved across all microcosms. Germination of *E. acoroides* seeds was observed to occur even when still inside the fruit (Rollon 1998).

As the experiment progressed, the addition of fish feeds caused a significant shading effect on the seedlings, directly as a result of the increasing turbidity due to abundance of suspended particulate materials and indirectly by enhancing the algal growth in the water column and on seagrass leaves (Dimech et al 2000, Artika et al 2020, Wear et al 1999), despite the relatively controlled release of fish feeds in the experiment compared to typical mariculture activities. The downwelling photosynthetically active radiation (PAR) is attenuated due to both scattering and absorption processes by the optically active components including suspended and settled inorganic and organic solids, algae, and the water itself. Inorganic solids have the strongest scattering characteristic relative to other components while scattering of light by algae is less significant as compared to absorption (Kirk 1983). Algal cells can cause a substantial decrease in available light due to the various photosynthetic pigments which all contribute to absorption of PAR. Thus, the higher concentration of epiphytic algae in the enriched treatment may have more directly affected the seagrasses than the suspended particulate materials by actively increasing competition for available PAR.

Consequently, the lesser light availability resulted in the poor growth, development, establishment, and survival of *E. acoroides* propagules. Aside from low water clarity, the decrease in leaf surface area of the seagrass seedlings due to reduced leaf growth rate resulted in new epiphyte growth to be progressively concentrated on ever-smaller surface area causing even lesser amount of light received by the seagrass leaves. The reduction in relative leaf growth rate, number of leaves, and leaf chlorophyll content with decreasing PAR are strong indications of decreased net photosynthetic activity of the seedlings. The ratio of above-ground to below-ground biomass increased markedly because of decreased root production following fish feed loading. This was similar to *E. acoroides* seedling response to experimental nutrient treatment reported by Artika et al (2020). Under nutrient enriched condition, there was little need for root production and the substantial decrease in both length and biomass of the roots suggests that the plants may have allocated relatively more matter towards the leaves to maximize light harvesting (Goldsborough and Kemp 1988).

The progressive decline in growth and production of the seedlings due to sustained shading effect of suspended and settled materials may have led to the decreased structural integrity of the seagrass leaves and roots, eventually leading to death of some of the seedlings. As photosynthesis was not apparently sufficient to maintain the leaf material, the seagrass shoots became noticeably weakened and susceptible to sloughing under low light conditions. In addition, the substantial accumulation of epiphytes on seagrass leaves also hastens the deterioration of seagrass shoots by imposing greater physical drag so that the leaves are weighted down on the substrate or are easily torn off. On the other hand, the significant decrease in root tissue resulted in poor settling of the seedlings. The seedlings remained unattached to the substrate and became more vulnerable to physical disturbance. Root stabilization into the sediment is critical for the survival of the plant in the succeeding life stages (Artika et al 2020). It should be pointed out also that elevated nutrient levels and organic matter in sediment can create anoxic or hypoxic conditions, posing additional risk to seedlings by reducing oxygen availability and generating harmful compounds such as hydrogen sulfide (Brodersen et al 2017; Fraser et al 2016; MacLeod et al 2023).

CONCLUSION AND RECOMMENDATIONS

The overall poor performance of *E. acoroides* seedlings under experimental addition of fish feeds suggests that sexual propagules have a low potential to support maintenance and recovery as well as future restoration of *E. acoroides* populations in areas exposed to mariculture activities. Seagrass meadows can be effectively protected and restored by implementing management actions to control excessive inputs of nutrients as well as organic and inorganic materials associated with mariculture. Given that the conservation and management of seagrass ecosystems require an understanding of how they perform at both early and adult life stages, further studies, including field experiments, examining seedling responses to mariculture-associated environmental modifications will help better assess the future impact fish farming practices on the seagrass ecosystem.

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