Integrated Culture of *Haliotis asinina* and *Caulerpa racemosa* in Mariculture Park of Lianga Bay, Philippines

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Abstract: The two high-value extractive species, the *Haliotis asinina* and *Caulerpa racemosa* have been integrated into the existing commercial finfish culture of *Chanos chanos* in Lianga Bay, Surigao del Sur, Philippines. This study aimed to develop an efficient and economical means of aquaculture bioremediation through integrated multitrophic cultures. The growth rates were determined from *H. asinina* by monitoring shell lengths and weights in 180 days grow-out cultures, while growth rate and increments were measured for *C. racemosa*. The cultured species were subjected to proximate analysis to determine the nutrient composition such as crude fat, crude protein, and moisture. Monitoring of water nutrients such as ammonia and nitrate, and physico-chemical parameters were also conducted throughout the study. Results revealed that the the average growth rates of *H. asinina* showed significant difference in the individuals cultured in combination with *C. racemosa* when compared to those culture far from the seaweeds areas. On the other hand, the mean growth of *C. racemosa* showed a significant differences ($p < 0.05$), in favor of those cultured in the surface layers of the water column. Similarly, significant differences ($p < 0.05$) were likewise observed in the proximate analyses of the nutrient compositions, specifically for crude proteins between integrated cultures of *H. asinina* versus the monoculture; and; the integrated cultures versus wild populations of *C. racemosa*. In relation to water nutrients, the experimental site has minimal amount of ammonia and nitrate level compared to the site without integrated farming system and is densely occupied with fish cages. Therefore, the combination of these successful concepts, which have been proven effective in milkfish culture, should be advocated and expanded in the Region by integrating locally available extractive species.

Keywords: *Haliotis asinina, Caulerpa racemosa,* growth rate, proximate analysis.

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INTRODUCTION

The establishment of marine aquaculture (monoculture) has been recognized as a cause of ecological harm due to the ongoing development of farming practices and feeding methods (25). This form of intensive mariculture heavily relies on commercial feeds, which, when lost or released as effluent, result in the discharge of substantial amounts of inorganic nutrients. To address these concerns, the concept of integrated cultivation involving marine species such as *Eucheuma denticulatum, Perna viridis,* and *Crassostrea* sp., alongside valuable finfish species like *Chanos chanos* and *Tranchinotus blonchii,* has been proposed as a potential bioremediation approach. This approach offers the possibility of enhancing economic benefits through species diversification while mitigating feed contamination (28, 16, 19). The selected bioremediator species play a crucial role in removing inorganic nutrients such as nitrogen and phosphorus from the marine environment and seabed (22).

The donkey's ear abalone, *H. asinina* is one of the most commercially-important gastropods in the Philippines. However, overharvesting, poor management of the stock, and loss of habitat have led to dwindling of the resources, which may not only observed in the Philippines, but also worldwide (5). The culturing methods for this species can be land-based or sea-based, including land-based tanks, sea cages and the intertidal part ranching method (12). On the other hand, *C. racemosa* is widely cultured under modified conditions as an important functional food. This species is also one of the major source of livelihood in most areas in the Philippines. Considering these species as potential aquaculture produce in the country, integration of cultures as bioremediation is scarcely practiced among farmers.

It was believed that integrated cultivation of beneficial and functional species such as extractive species for the excess nutrients is currently sought as a potential solution. Some examples of the extractive species are the red macroalgae, such as *Eucheuma* and other *Gracilaria* species, can effectively absorb dissolved inorganic nitrogen (14). Development of integrated culture of tropical marine algae and shellfishes species with fish cultures can potentially reduce the negative impacts of aquaculture effluents in the marine environment. Moreover, this would provide diversified means of food production and facilitate resource sustainability for the needs of the community. It is the aimed of this paper investigate aquaculture bioremediation through integrated, multi-throphic cultures of *H. asinina* and *C. racemosa* in mariculture park of Lianga, Surigao del Sur.

MATERIALS AND METHOD

Study Area

The study was conducted in the mariculture zone of Lianga Bay, specifically in Barangay Liatemco, Lianga, Surigao del Sur (Figure 1). It is a semi-closed embayment where most fishpen are confined in the inner part of the bay.

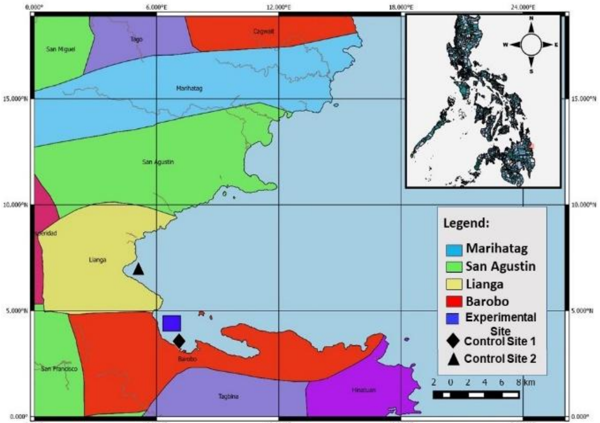

Figure 1. Map of Lianga Bay, Surigao del Sur showing the study sites.

Culture Scheme

The floating open-sea farm was set up in Lianga Mariculture zone. The cages were set-up, in the floating platforms of the milkfish cages. For the water nutrients, two (2) reference sites served as positive and negative controls. Positive control (control site 1) was situated adjacent (approximately 1 km) away from the experimental site with heavy dense fish cages. While the negative control (control site 2) was situated in Baucawe, Lianga, Surigao del Sur in which no fish farming activities were observed. Juvenile abalones were collected in the coastal area of Rizal, Barobo, Surigao del Sur. The samples were brought to Liatemco, Lianga, Surigao del Sur and acclimatized for 24 hours. After acclimatization, it was placed into the thick plastic containers (1/4x12x16 cm) as a culture media with bamboo as substrate in the stocking density of 30 individuals per container with nine replicates. Each sides of the fish cage was surrounded with abalone cages hanged about three (3) meters below the sea surface under a floating platform. Also, other set-up was installed away (approx. 50 meters) from the fish cage, the 6 containers were hanged below the NEMSU-LC Marine Cottage.

On the other hand, the fragments of *C. racemosa* were collected away from the fish cages and pens in Lianga Bay. The algal pieces were collected during low tide and placed in polyethylene bags and were then stored in a polystyrene icebox and transported to the NEMSU-LC Marine Cottage. The samples were then washed with seawater to remove the epiphytes before being placed in a 100-liter plastic tank with an aerator. After that, the algal fragments were transferred to the culture cages. A total of eighteen (18) cages deployed at three (3) depths: surface, middle, and bottom depth. The triple-layer cages were hanged and positioned alongside the floating fish cages using a rope. Each cage was planted with 50 grams of *C. racemosa,* utilizing a piece of tie box and allowed to grow for six (6) months.

Physio-chemical parameters

Physio-chemical parameters were conducted weekly to examine the water quality. Inorganic nutrients such as nitrite and ammonia were monitored. A contractor was hired to collect water samples at the two (2) control sites. After 180 days of culture, *H. asinina* and *C. racemosa* samples were subjected to proximate analyses. The samples were oven-dried at 180 °C for 72 h for H. asinine and 50 °C for 72 hours for C. racemosa . The dried tissues was then grounded into fine powder and used for proximate analysis. For abalone samples, the same method was employed from the outside cultured abalone for data comparison. The 250 grams of fine powder abalone and sea grapes were analyzed for the crude moisture, crude protein, and crude fat and were then sent to Department of Agriculture for the analysis of nutrient content.

Measurements of shell length (SL mm), shell width (SW mm), and body weight growth (BWG) were made twice a month from September 2021 to March 2022 using the vernier caliper and a digital balance. To minimize stress, exposure of the animals to the air was kept to a minimum by returning them immediately to the seawater as soon as measurements were made. Abalones in each cage was fed with about 25 grams of fresh seaweed *Gracillaria* sp. about every 2–3 days considering the excess feed coming from the fish station as the alternative. The abalones' total weight gain, shell length increment and specific growth rate was also computed to obtain the growth performance of the abalone. On the otherhand, *C. racemosa* fragments were measured for six months, with 15-day interval weight measurements for growth rate measurement. The growth and biomass yield of *C. racemosa* were determined using the following formula (2)

Growth Rate Analysis

$$\text{SGR } (\% \text{ g day}–1) = [\ln(Wt / W0) / t] \times 100$$

where Wt = final fresh weight at Day t (g), Wo = initial fresh weight at Day 0 (g), t = number of culture days

The T-test and One-Way Anova were used to test for differences on the growth performance of the *H. asinina* and *C. racemosa* as well as the proximate compositions of both species. The data was analyzed using microsoft excel to determine any significant difference among the types of culture method.

RESULTS AND DISCUSSION

Shell Length and Shell Growth Rate of *H. asinina*

The two treatments of *H. asinina* had no significant differences in terms of shell lengths after 180 days culture of 50.04 mm and 49. 30 mm (Fig. 2). In terms of average daily shell growth rate of *H. asinina*, cultured inside the cages with *C. racemosa* has obtained 0.012 mm day^{-1}, comparably higher to the abalone cultures outside the cages (0.006 mm day^{-}1) (Figure 3). The same species studied in Guimaras Island, Iloilo obtained highest growth rates after six months period (180 days) with 49.6 ± 0.11

mm average SL at stocking density of 50 individuals/m2 with initial shell length of 26–30 mm (10).

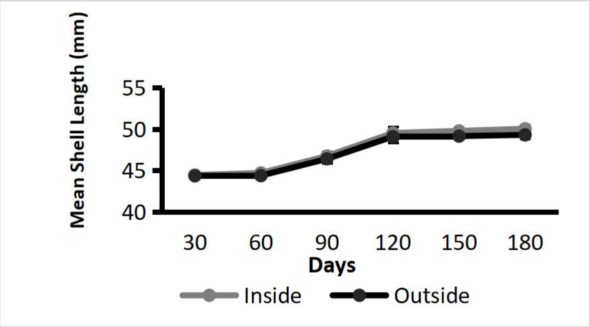

Figure 3. Monthly shell length of abalone cultured inside and outside in the fish cage for 180 days

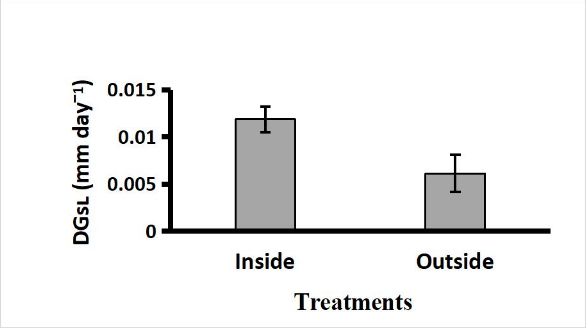

Figure 4. Average daily shell length growth rate of abalone cultured in the inside and outside fish cages.

In terms of daily weight growth rate, the samples inside the fish cage is 0.43 g day^{-1} higher compared to the samples outside the fish cages (0.3 g day^{-1}) (Figure 5). Also, this result obtained higher average rate compared to 0.11 g/day in the study of (16). On the other hand, no mortality rate was observed for both experimental sites for three (3) months (September to November, 2021). But around 30% mortality rate was observed starting from December 2021 to March 2022 (Figure 6). This abrupt mortality might be due to the hit of typhoon Ruby that greatly affect the sites.

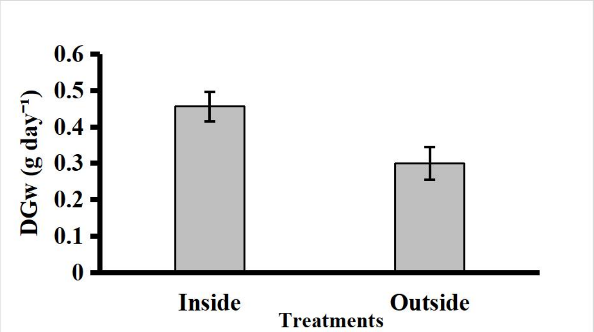

Figure 5. Average daily weight growth rate g day^{-1} of abalone.

Growth o*f C. racemosa*

The Table 1 highlights that the specific growth rate of *C. racemosa* decreased with increasing depth. The Surface depth exhibited the highest growth rate, followed by the Middle depth, and the bottom depth showed the slowest growth rate. At the surface depth, *C. racemosa* exhibited a high specific growth rate of 70.72 grams per day. This indicates that the seaweed experienced rapid growth, with an increment in weight of 6,456.17 grams, from an initial weight of 50 grams to a final weight of 6,506.17 grams.

Table 1. Th specific growth rate of *C. racemosa* in different depths

Depth	Initial Weight (g)	Final Weight (g)	Increment (g)	SGR (g) per day
Surface	50	6,506.17	6,456.17	70.72
Middle	50	2,816.50	2,766.50	30.61
Bottom	50	325.39	275.39	3.54

These findings suggest that the depth at which the seaweeds is located significantly influences its specific growth rate, with the surface area providing the most favorable conditions for rapid growth. The specific growth rate of *C. racemosa* cultivated using the sowing method was reported at 3.85 % g day^{l} after 30 days of culture (23). The increment of frond length was claimed to be one of the growth indicators for *Caulerpa* sp. as suggested by (22). The growth variations of *Caulerpa* were due to the differences in environmental factors and farming techniques such as off-bottom culture, tray, and sowing

Proximate compositions of *H. asinina* and *C. racemosa*

The Table 2 presents the proximate analysis comparing the composition of cultured abalone in two different farming systems the integrated culture and Monoculture. The crude protein content, a significant difference is observed between the two farming systems. Abalone cultivated within the integrated culture system demonstrates a notably higher protein content of 76.11%, whereas the monoculture system yields abalone with a lower protein content of 41.01%. This finding suggests that the integrating farming approach may be more effective in

Table 2. Proximate analysis of the *H. asinina* using T-test for independent samples

Composition (%)	Integrated Culture System	Monoculture	Related Article (Knauer et al. 1994)
Moisture	5.24 ± 0.02^{a}	3.94 ± 0.11^{a}	4.6
Crude Protein	76.11 ± 0.07^{a}	41.01 ± 0.42^{b}	42.1
Crude Fat	1.25 ± 0.18^{a}	1.37 ± 0.15^{a}	4.6

Notes:
i. Values are expressed as mean + SD, n= 9.
ii. Values in the same row with different superscripts letters are significantly different ($p < 0.05$)

promoting protein production in abalone compared to the monoculture system.

The proximate analysis comparing the composition of *C. racemosa* in two different conditions the cultured (integrating farming) and the wild was presented in Table 3. In terms of moisture content, the cultured seaweed has a significantly lower moisture level of 2.28% compared to the wild , which has a moisture content of 4.07%. This suggests that the cultured seaweeds has undergone some form of drying or processing, resulting in a reduced water content.

The crude protein of the cultured *C. racemosa* exhibits a higher protein content of 18.42% compared to the wild seaweeds, which has a lower protein content of 9.50%. This indicates that the cultivation process has contributed to an increase in protein production in the cultured seaweeds. This higher protein content can be beneficial for nutritional purposes. Regarding crude fat, both the cultured and wild seaweeds have relatively low fat contents. This suggest that *C. racemosa* is a low-fat food source regardless of the cultivation method.

Table 3. Proximate analysis of cultured and wild *C. racemosa*.

Composition	Cultured *C. racemosa*	Wild *C. racemosa*
Moisture (%)	2.28 ± 2.282^{a}	4.07± 0.08^{b}
Crude Protein (%)	18.42 ± 0.22^{a}	9.50 ± 0.15^{b}
Crude Fat (%)	1.29 ± 0.31^{a}	0.77 ± 0.34^{b}

Notes:
i. Values are expressed as mean + SD, n= 9.
ii. Values in the same row with different superscripts letters are significantly different ($p < 0.05$)

Protein regulates abalone and algal biological processes, and their activities can be characterized by enzymatic catalysis, transport, and storage (7). The high protein content of the abalone and seaweed culture could be a potential alternative source for animal protein replacements as food and as feed. The high protein and low-fat content in this study was possibly caused by increased nutrients and the high presence of commercial feeds from fish production in the area. As (30) reported that the higher the nitrogen and phosphorus content, the higher the protein. On the other hand, the fat content is inversely proportional to the protein content. This result is consistent with the findings of (17), where protein content is always inversely proportional to fat content. Whereas, the moisture content of seafoods strongly affects their microbiological and chemical stability and physical properties. They are also used to determine their nutritional composition (6).

Water Nutrients and Physico-chemical parameters

A similar pattern was observed in ammonia and nitrate from September 2021 to March 2022 (Figure 6). However, the control site area has higher inputs of water nutrients starting from December 2021 to March 2022. This result revealed that the water nutrients from control site 1 (Liatmeco) exhibits higher effluent contamination when compared to the experimental sites and control site 2 (Baucawe).

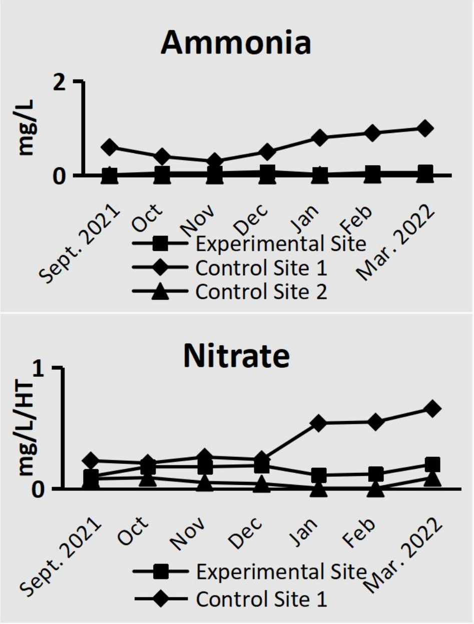

Figure 6. Water nutrients of the experimental and control sites.

The water nutrient increased be due to the dense fish cages in control site 1 and minimal to low water nutrients in other two sites due to the integration of extractive species and the absence of fish cages. The ammonia-nitrogen levels (0.00–0.045 mg L^{-1}) recorded during the study period were lower than that reported (8) (24) where they obtained results ranging from 0.05 to 6.20 and 0.01 to 1.35 mg L^{-1} , respectively. The levels of ammonia-nitrogen content found in the experimental site was far below the tolerance levels (15).

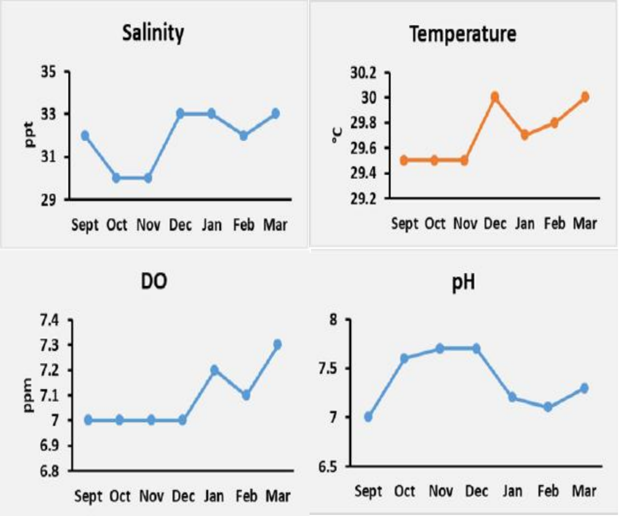

Figure 7. Water Quality Parameters.

Figure 7 shows the monthly water quality parameters in the experimental site from September 2021 to March 2022. The salinity in the experimental site ranges from 30 ppt to 33 ppt from September 2021 to March 2022 tend to have good salinity level for the *H. asinina* and *C. racemosa.* Since these species are generally sensitive to salinity changes, salinities lower than 30 ppt might result in poor growth and higher mortality rate for these species (23). (29) showed that maximum growth of *C. racemosa* occurred at a salinity of 36 ppt.

Meanwhile, the maximum specific growth rate for *C. racemosa* occurred at a salinity of 35 ppt (13). However, salinities in our cultured sites were slightly lower compared to their studies. The water temperature (28-32°C) is the appropriate range for fish culture (4). However, 25.9°C to 30.5°C, with an average of 27.9°C was recorded in the experimental site. These values were obtained because the water measurements were, in general, carried out from the morning to afternoon as recommended (1). During observations, relatively high DO (7.0-7.3 ppm) indicates good water quality conditions from the experimental site, whereas low DO reflects hypoxia (low oxygen), which may serve as a reduction for fish, abalone and macroalgae culture. On the other hand, the range of 7-8 pH level recorded in the study area was suitable for abalone and seaweeds culture along with fish culture, since the optimum range of pH in aquaculture is 7 to 8.5 (9).

The seaweeds was able to filter the amount of product in fish culture, also known as biofilters, to recycle the nutrients (or waste) that were present in and around the aquaculture farms. This helped growers improve the environmental performance of their aquaculture sites. Similar results were observed in *C. racemosa* from aquaculture effluent obtained from *P. latipinna* (sailfin molly) rearing tanks. Another possible factor is the unconsumed commercial floater feeds of the fish in the cage served as the fertilizer for the seaweeds (11). The use of macroalgae for effluent treatment is a prominent approach.

Previous studies have demonstrated that some Caulerpa species have the potential for bioremediation of intensive tank-based aquaculture due to the luxury uptake and fast growth rates (16). Another reason may be that the optimum growth of macroalgae requires specific chemical, biological, and environmental conditions (21). The commercial use of the fish and macroalgae-integrated system is essential to consider the factors affecting the growth of both fish and macroalgae, as well as the nutrient requirements (26). In particular, sea grape can absorb nutrients from aquaculture effluents, heavy metal wastewater, and toxic dye-contaminated wastewater methods (18; 27; 23).

The current data suggests culturing sea grapes *C. racemosa* in surface depths hanged in the fish cages is viable due to its fast-growing characteristics. Because of their effective absorption of effluent from the cultured milkfish, the existing nutrients coming from the floating feeds were filtered by the seaweed, particularly in the surface depths, which increases its growth rate on the surface compared to the middle and bottom depths with moderate growth rates. Their high growth rate in surface depths could provide farmers with interest in sea grape culture. Moreover, this species could clarify the environmental impacts of eutrophication (3).

CONCLUSION

This integration of *H. asinina* and *C. racemosa* with fish culture is the way to fasten the growth of these species. Moreover, the integration of these extractive species in the farming system can also be used as an effective culture method for the removal of inorganic nutrients, maintenance of water quality, and a reduction in water consumption for fish and macro-algal production.

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