Impacts of *Lab-lab* (Algal Mat) on Water Quality of *Chanos chanos* Aquacultures in Dagupan City, Pangasinan

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Abstract

Lab-lab is an algal mat that is commonly used as supplemental or full dietary feed for milkfish due to its rich concentration of essential nutrients. However, limited research surrounds the impact of *lab-lab* as this algal complex may differ in effects from previous literature since *lab-lab* is a mass of microorganisms that may differ in species, interactions, and effects. As such, this study aims to assess the impact of *lab-lab* on milkfish aquaculture and its potential effect on the milkfish itself. Data were collected in triplicates in four different aquaculture sites in Dagupan City, Pangasinan and surrounding areas. Data were collected using the GLOBE protocols for pH, water temperature, dissolved oxygen, and water conductivity. Furthermore, *lab-lab* samples were collected, extracted, and tested in a qualitative phytochemical screening. Obtained results suggested that dissolved oxygen, water temperature, and water conductivity were statistically different from sites with and without *lab-lab*. The results entail the positive impacts of *lab-lab* in milkfish growth using previous literature to corroborate our hypothesis. Furthermore, the presence of alkaloids, flavonoids, saponins, and terpenoids further reinforces the potential benefits of *lab-lab* as these phytochemicals promote fish growth.

Keywords: *Lab-lab*, algae mat, milkfish, water quality assessment

Research Questions

1. What is/are the effects of *lab-lab* on surrounding water areas, especially milkfish aquacultures, and the environment?
2. How does the effect of *lab-lab* on the water quality affect living organisms, specifically milkfish in aquacultures?
3. What is/are the phytochemical constituents of *lab-lab* that may affect living organisms, particularly milkfish?

Introduction & Review of Related Literature

Dagupan City, Pangasinan thrives on its local fishery industry, specializing in bangus, also known as milkfish or *Chanos chanos*. Renowned as the source of the "best tasting" bangus in the Philippines, Dagupan alone yielded 109,893.50 metric tons of milkfish in 2019, a quarter of the Philippines' total milkfish production (Sotelo, 2021). The production of bangus in Dagupan bears great economic importance, contributing a value of production of Php 44.2 Billion to the Philippines in 2018-2020 (DOST-PCAARD, n.d.). However, threats to its farming remain imminent.

Algal bloom remains a prevalent cause of fish kill, imposing numerous detriments to bangus aquacultures despite the resilience of bangus to diseases (Sotelo, 2021). In 2002, the nearby coastal town of Bolinao, Pangasinan experienced a *Prorocentrum minimum* bloom causing a six-million peso fish kill to which Azanza (2005) attributed the stark decline in oxygen levels in the area of below 5 mg/L. After a decade, a similar eutrophication event recurred due to the same algae; however, this second event induced a remarkably greater loss of five hundred million (500,000,000) Philippine pesos (Escobar et al., 2013). Notably, both mentioned events were highly associated with the decreasing water quality in the area brought on by excess nutrients from fish feeds (Azanza, 2005; Escobar et al., 2013).

A combination of fish cage practices and excessive synthetic feed use has made it common for nutrient waste to accumulate (White, 2011). These methods disrupt the natural flow of water in rivers and lakes and increase nutrient levels in the water. A stretch of three or four days with little to no sunlight will cause algae to start to consume oxygen and deplete fishes of their supply (Florida Lakewatch, 2000). In summary, synthetic feed has posed multiple challenges to the production of bangus as well as the livelihood of the people in Dagupan. However, using natural feed such as *lab-lab* will hopefully reduce the negative environmental impacts of bangus aquaculture.

Lab-lab is a common Filipino term for algae mats on the bottom of milkfish ponds (Fortes & Anne, 2010; Sotelo, 2011). This mass of microorganisms and algae is being used as supplemental or full dietary feed for milkfish due to its rich concentration of essential fatty acids and its potential to increase the "survivability of farmed species" (Nagappan et al., 2021). However, limited research evaluates the impact of *lab-lab* as this algal complex may differ in effects from previous literature since *lab-lab* is a mass of microorganisms that may differ in species, interactions, and effects.

This study aims to determine whether *lab-lab* is beneficial to bangus aquaculture and the environment in the long term using the Globe Hydrosphere protocol. Additionally, *lab-lab* samples will be subjected to qualitative phytochemical testing to determine natural compounds that may be beneficial to the bangus diet.

Research Methods

Water Quality Assessment

The water qualities of bangus aquacultures were assessed following the hydrosphere protocols of the GLOBE program. Specifically, the methods employed were the water temperature, transparency, pH, electrical conductivity, and dissolved oxygen procedures. Four different sample sites were assessed with three replicates of data collected from each site, *viz.*, Bonuan bangus farm (No *lab-lab*), Dawel River Cruise (Bangus Site; No *lab-lab*), Bangus farm with *lab-lab*, and *lab-lab* growth site.

Water Temperature. A calibrated wireless thermometer probe (PASCO, PS-3210) was lowered into the sampling sites to a depth of 10 cm for three minutes; after which, the data was recorded. However, an additional minute was added when the temperature still fluctuate before recording the data.

pH Level. A wireless pH probe (PASCO, PS-3204) was first rinsed with distilled water and dipped into a beaker containing sample water for one minute. The probe was left for an additional minute when the pH is still changing before recording the data.

Electrical Conductivity. A wireless conductivity sensor (PASCO, PS-3210) was used to measure the electrical conductivity of the sample water in µS/cm. The probe was first rinsed with distilled water and dipped into a beaker containing sample water for one minute. The probe was left for an additional minute when the pH is still changing before recording the data.

Dissolved Oxygen. A wireless optical dissolved oxygen probe (PASCO, PS-3224) was used to measure the dissolved oxygen concentration in the sample sites. The probe was first washed with distilled water before the data collection. The probe was submerged in the sample site for 1 minute. An additional minute was added whenever the data still fluctuated before the data was recorded.

Crude Extraction of *Lab-lab*

Samples of *lab-lab* were collected and phytochemically tested for the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols. The samples were first rinsed in tap water and then distilled water to remove the dirt ingrained in the samples. The samples were then sun-dried and ground to a coarse powder. 5g of the resulting powder was macerated in methanol for 24 hours and filtered twice using a Whatman no. 1 filter paper. Lastly, the filtrate, or the crude methanol extract (CME), was subjected to various qualitative phytochemical assessments.

Phytochemical Analysis of Lab-lab Extract

Alkaloid. The test for alkaloids utilized Wagner's test. Briefly, Wagner's reagent was first prepared by dissolving 2g of iodine and 6g of KI in 100mL of water. Then, a few drops of Wagner's reagent was added to a test tube containing 1mL of CME. A reddish-brown precipitate indicated the presence of alkaloids.

Flavonoid. The presence of flavonoids in the extract was determined using the alkaline reagent test. 2mL of 2% NaOH solution was first prepared and added to a test tube containing 1mL of CME. A few drops of diluted acid were then added to the working solution. The development of an intense yellow color after the addition of sodium hydroxide, which turns colorless after the addition of a few drops of dilute acid, indicated the presence of flavonoids.

Phenol. A small amount of CME was taken (1mL) in a test tube and 1 to 2 drops of 5% Iron III chloride ($FeCl_3$) were introduced. A blue, green, red, or purple color is a positive test.

Saponin. A drop of $NaHCO_3$ solution was added to 5mL of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. A stable honeycomb-like froth indicated the presence of saponins.

Tannin. Braymer's test was used to detect the presence of tannins. 1mL of CME was stirred with 3mL distilled water where a few drops of 10% ferric chloride were added. Black or blue-green coloration or precipitate was taken as positive result for the presence of tannins

Terpenoid. Based on Salkowski's test, 1mL of the crude extract was separately shaken with 2 mL of chloroform followed by the addition of 2 mL concentrated H_2SO_4 along the side of the test tube. A reddish-brown coloration of the interface indicates the presence of terpenoids.

Statistical Analysis

One-way Analysis of Variance (ANOVA) was performed to determine the presence of significantly different means in at least two sampling sites. The water quality data with significant differences were then subjected to the Tukey's HSD test for multiple comparisons to determine the significantly different pairs of sampling sites. Furthermore, all numerical data were presented as mean ± standard error (SE) while binary notation assessment (present or not present) was utilized in evaluating the presence of phytochemicals in the *lab-lab* crude extract.

Results

Water Quality

The water quality assessments yielded results showing that there is statistically significant differences in conductivity ($F_{3,8}$ = 5.05, P=0.030), dissolved oxygen ($F_{3,8}$ = 342.63, P=0.000), and temperature ($F_{3,8}$ = 99.48, P=0.000) levels across at least two of the sampling sites. In contrast, there are no statistically significant differences ($F_{3,8}$ = 3.86, P=0.056) in the pH level across all four sampling sites. The average of each of the four sites and their SEs are listed in the table below and pairs the significantly different sites:

Table 1. Water quality data on pH, conductivity, dissolved oxygen, and temperature on four different milkfish aquaculture in Dagupan City, Pangasinan and surrounding areas.

Sites	pH*	Conductivity (µS/cm)*	Dissolved Oxygen (mg/L)*	Temperature (ºC)*
Site 1[1]	4.68 ± 0.015	20220.3 ± 13.32[a]	5.71 ± 0.116[bcd]	29.3 ± 0.36[hi]
Site 2[2]	4.70 ± 0.003	20200.8 ± 11.25	4.44 ± 0.116[bef]	29.1 ± 0.17[jk]
Site 3[3]	4.66 ± 0.007	20136.5 ± 26.48[a]	7.62 ± 0.234[ceg]	30 ± 0.26[hjl]
Site 4[4]	4.66 ± 0.007	20185.5 ± 3.12	11.04 ± 0.123[dfg]	32.3 ± 0.17[ikl]

* Values are shown as mean ± SE for each of the parameters with data collected in triplicate. Means of different superscripts in a column are significantly different at 5% level using Tukey's test.

[1] Bonuan bangus farm (No *lab-lab*)

[2] Dawel River Cruise (Bangus Site) [No *lab-lab*]

[3] Bangus farm with *lab-lab*

[4] *Lab-lab* growth site

Sites 1 and 4 (95% C.I. = [4.63, 6.04]); and, 2 and 4 (95% C.I. = [5.90, 7.30]) reported the highest statistically different results for dissolved oxygen levels with both pairs having P=0.000. Similarly, sites 1 and 4 (95% C.I. = [2.33, 3.67]); and, 2 and 4 (95% C.I. = [2.53, 3.87]) also reported the most significant differences in temperature levels with both having $P=2\text{x}10^{-6}$. On the other hand, only sites 1 and 3 (95% C.I. = [-155.90, -11.64]) showed significant differences in electrical conductivity with P=0.024.

Phytochemical Analysis

Briefly, the qualitative phytochemical analysis tests showed the presence of alkaloids, flavonoids, saponins, and terpenoids as constituents of the crude *lab-lab* extract. Other phytochemicals, such as phenols and tannins, were absent in the crude extract.

Table 2. Phytochemical screening results of *lab-lab* crude extract.

Phytochemical Constituent	Results*
Alkaloids	+

Flavonoids	+
Phenols	-
Saponins	+
Tannins	-
Terpenoids	+

*Results are indicated by either the presence (+) or absence (-) of the phytochemical

Discussion

Water Quality Assessment

Dissolved oxygen (DO) refers to oxygen gas that has been dissolved in water that is crucial for aerobic aquatic organisms for survival and reproduction (Ali et al., 2022). In this study, the most significant observation is the increase in the levels of dissolved oxygen whenever there is more *lab-lab* present; for instance, sites 1 and 4, and 2 and 4 yielded significantly different results for dissolved oxygen levels with both pairs having P=0.000. In the cited example, site 4 recorded the highest average concentration of dissolved oxygen with 11.04 ± 0.123 mg/L, whereas sites 1 and 2 only reported roughly half of that of site 4 with 5.71 ± 0.116 and 4.44 ± 0.116 mg/L, respectively. This may be due to the high photosynthetic activity of *lab-lab* in the area (PHILMINAQ, n.d.). Consequently, this increase in dissolved oxygen may support milkfish development as fish growth, physiology, biochemistry, reproduction, and behavior can all be affected by low DO levels (Ali et al., 2022).

Even so, the area must not be saturated with *lab-lab* as too much may cause decomposing *lab-lab* to acquire more oxygen than producing it (Okwor et al., 2019). According to Freshwater Aquaculture (2019), levels of DO go down during the night, and low DO levels during the day may indicate fish kill for the next day; it is also important to note that *lab-lab* is best kept in shallow ponds as an increase in darker, deeper areas may lead to a stark decrease in dissolved oxygen. In addition to this, growing and keeping *lab-lab* during the drier seasons have been reported to be more beneficial than harmful (Fortes & Anne, 2010). In summary, too much or too little *lab-lab* in an area will be detrimental to the growth, development, and reproduction of Bangus (Fortes & Anne, 2010; Francis-Floyd, n.d.; Freshwater Aquaculture, 2019)

On the other hand, conductivity measures the amount of ions dissolved in it, specifically its degree of mineralization, causing its ability to conduct electricity. As such, conductivity is also often used to estimate the salinity of the sample water as both have a directly proportional relationship (United States Environmental Protection Agency, 2022). Conductivity is greatly important in monitoring osmotic pressure. A study on common carp has shown that excess (higher) salinity resulted in less growth of the freshwater fish as "the fish spend increasingly more energy for maintaining osmoregulatory balance as salinity increases" (Boyd, 2017). Based on the results in Table 1, average electrical conductivity was recorded to be lower in sites with *lab-lab* compared to sites without; for example, conductivity is measured lowest in Site 2 and highest in Site 3, making them the most and least saline sites, respectively. The results may entail the potential effect of *lab-lab* in regulating water conductivity.

Furthermore, all bangus sites recorded average temperatures within the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) standards. According to NSW Government (n.d.), temperature has a direct influence on vital factors such as growth, oxygen demand, food requirements and food conversion efficiency; higher temperatures accelerate metabolic rate leading to starvation while lower temperatures lead to a significant delay in stomach evacuation (Handeland et al., 2008). As such, the data shows that despite differences in the recorded temperature between sampling sites, milkfish on the aquacultures are growing under optimal conditions. Additionally, the recorded data of the study corroborates with the findings of USGS (2019) that DO is higher in warm water than it is in cold water. Accordingly, the data show that Site 2 displays the lowest recorded DO and temperature while Site 4 displays the highest recorded DO and temperature.

As for the pH level of the aquaculture sites, all four sample areas recorded similar results with no significant differences between any of the sample sites ($P>0.05$); however, all sites have also significantly lower pH than that of the SEAFDEC/AQD standards which is between pH level of 6 to 8 (Ledesma, 2023). Sites 1, 2, 3, and 4 have recorded an average pH level of 4.68, 4.70, 4.66, 4.66, respectively. According to Mustapha (2019), pH levels between 4 and 5 inhibits reproduction among warm-water pond fishes. This is contradictory to the observations of this study as the aquaculture sites have been reported to have numerous juvenile and adult milkfish. One potential explanation for this inconsistency is human error during data collection. The pH probe was only rinsed with distilled water due to the unavailability of the buffer solution recommended to recalibrate the pH probe before use. Another potential explanation for these low pH levels may be due to mine wastes, industrial effluents, and recent floodplain draining (*pH | US EPA*, 2023; Tomasso, 1994).

Phytochemical Analysis

Out of the six qualitative phytochemical tests, four of them were detected in the *lab-lab* sample. Namely, these are alkaloids, flavonoids, saponins, and terpenoids. Phenols and tannins were not detected in the sample.

Alkaloids. In plants, alkaloids are compounds that act as a defense against pests and predators (Rosa et al, 2007). Likewise, alkaloids have been observed to act as immunostimulators in fish such as the Blunt snout bream and the Red tilapia (Chakraborty and Hancz, 2013; Tastan and Salem, 2021; Ye et al, 2019), a type of freshwater fish.

Immunostimulants aid the immune system to protect a host from disease (NIH National Cancer Institute, n.d.). The presence of alkaloids in *lab-lab* may be a possible explanation for why locals deem bangus as healthy and "resistant to disease" (Asanza, 2005), although further studies need to be conducted for this association to be verified. The presence of this phytochemical has also been associated with weight gain in the same Blunt snout bream (Ye et al, 2019) and may be a possible reason why *lab-lab* is used as an alternative to fish feed.

Flavonoids. In humans, the consumption of certain flavonoids can promote the growth of a fish, which may also be attributed to the growth of bangus; however, these effects are subject to the sex of individual fish as well as different life stages (Chakraborty and Hancz, 2013).

Saponins. Saponins are also possibly linked to an increase in weight gain in fish (Francis et al, 2002), and this may also be a factor in why bangus is fed *lab-lab.* Even if saponins are harmful to other cold-blooded organisms, previous studies have proven that plants that are rich in saponin are beneficial for fish to feed on, especially with regard to promoting growth (Makkar et al, 2007).

Terpenoids. Terpenoids were detected in the *lab-lab* sample. Although terpenoids have been found to have pharmacological benefits, their effects on bangus and other fish remain uncertain (Ludwiczuk et al., 2017).

Results from this study indicate that the use of *lab-lab* as an alternative to traditional synthetic feed is beneficial to both the fish and the environment, albeit with some limitations.

Conclusion

Lab-lab has the potential to become an environmentally sustainable alternative to traditional fish feed if its growth is properly monitored and regulated to reduce the risk of having a low level of dissolved oxygen. The use of a natural fish feed such as *lab-lab* will be able to reduce nitrogenous waste in fish ponds and cages and may prevent further degradation of water bodies and their surrounding areas. However, factors such as weather, climate, temperature, and time of day must be taken into account to be able to further ascertain how much *lab-lab* should be grown in an area to prevent algal bloom. Moreover, more studies should be conducted regarding the effects of alkaloids, flavonoids, saponins, and terpenoids on bangus populations. This is to ensure the association between the presence of these chemicals and the possible positive effects this has on bangus aquaculture. Overall, *lab-lab* may be able to become a more environmentally sustainable alternative to synthetic fish feed due to its effects on water quality and the phytochemicals it provides to bangus.

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I make an impact

Fishkill and algal blooms are worldwide contributing factors to losses in aquaculture and fish farming businesses. As these issues are also experienced locally, specifically in Dagupan, this spurred the research to investigate the impacts of algae in aquacultures in Dagupan City, Pangasinan.

I am a data scientist

Water quality data on pH, electrical conductivity, dissolved oxygen, and temperature were collected in aquaculture sites in Dagupan City, Pangasinan and surrounding areas such as the nearby town of Binmaley. This data was then subjected to comparative analysis, related to each factor, and correlated to existing data and studies to solidify and confirm the data collected.