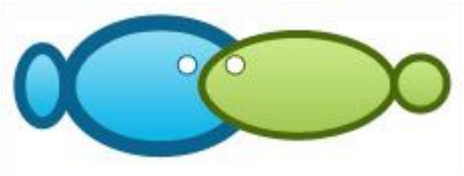

Fish hydrolysate derived from fish waste increased the growth of *Kappaphycus alvarezii*

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Abstract. The study was conducted to compare the growth performance of *Kappaphycus alvarezii* fertilized with two sources of fish waste liquid fertilizers at various concentrations using hanging long-line method for 45 days at Pilaper Island, Masinloc, Zambales. Growth was measured in terms of weight gain (WG) and daily growth rate (DGR). Water parameters were also monitored throughout the experimental period. The fish waste consists of internal organs, gills, scales and fins of milkfish (*Chanos chanos*) and tilapia (*Sarotherodon melanotheron*) was obtained from the local market in Pangasinan. Processing of samples into fermented products was done at Chemistry Laboratory of Pangasinan State University-Binmaley Campus. The milkfish and tilapia waste were placed in a separate fermented vat and added with brown sugar and Effective Microorganisms (EMO) at ratio of 1:1. Fish waste was then allowed to ferment for 10 to 15 days. The liquid fertilizers were harvested by sieving it with the aid of fine meshed net. There was significant increase on growth in terms of WG in *K. alvarezii* within 4-weeks culture period. Increased in WG and DGR was significantly high ($p < 0.05$) when *K. alvarezii* was fertilized with tilapia hydrolysate compared to that of the milkfish waste. The highest WG of 169.89±3.202g was obtained for 10 mL L^{-1} concentration and a DGR of 11.41±0.044 using tilapia fish waste. This study shows that the two fish hydrolysate could be used as liquid fertilizers to improve the production of *K. alvarezii* in hanging long-line.

Key Words: fish hydrolysate, *Kappaphycus alvarezii*, growth, nitrogen.

Introduction. *Kappaphycus alvarezii* (Doty) Doty ex Silva is an economically important red tropical seaweed highly demanded for its cell wall polysaccharide, being the most important source of K-carrageenan in the world (Bixler 1996). The market of carrageenan continues to grow and current sources of cultivated eucheumatoids seem incapable of meeting demand, at least in quality, price and volume for the requirements of the processing industry (Ask et al 2003). Commercial cultivation of *K. alvarezii* was developed in the Philippines during the latter half of the 1960s using local varieties selected from the wild (Parker 1974). It is one of the larger tropical red algae in the world species (Trainor 1978) which yields carrageenan, a commercially important polysaccharide. Carrageenans are used in a variety of commercial applications as gelling, thickening, and stabilizing agents, especially in food products such as frozen desserts, chocolate milk, cottage cheese, whipped cream, instant products, jellies, pet foods, sauces, feed ingredients and also being processed into a fertilizer (Trono 1992). Recently, culture of *K. alvarezii* shift interest on the cheaper and environmental friendly source of fertilizer that can be fully applied and replace the artificial media which are chemically produced (Hurtado et al 2009).

According to the report of Philippine Statistics Authority (PSA), the fishery production increased by 1.16% in year 2017. But the production of milkfish (*Chanos chanos*) in the Philippines continuosly decline from 75 thousand metric tons in 2015 to only 71 thousand metric tons in 2017. Milkfish and tilapia (*Sarotherodon melanotheron*) are one of the main fisheries commodity in Philippines in termns of aquaculture

production. In past years, Philippines milkfish and tilapia producers experinced vast mortality caused by sudden change of water temperature, high water salinity, euthrophication, and other problems related to water parameters. Recently, some aquaculture farms and cages practices lesser stocking density and number of cages per operators were narrowed down to address these problesms.

Those above mentioned problems caused an increased in the amount of discard milkfish and tilapia. To minimize and effciciently utilize the discard and by products of milkfish and tilapia in the Philippines, we looked into the potential of those byproducts from fish to be used as fertlizer. We processed the internal organs, gills, scales and fins to make a fish hydrolysate and used it as liquid fertilizer for culturing *K. alvarezii*.

Fish hydrolysate could be a valuable resource for culture of *K. alvarezii.* Afonso & Borquez (2002) stated that wastewater coming from seafood processing plants do not contain known toxic and carcinogenic substances. In addition, there were no significant differences between corn steep liquor and fish waste digestion blend (2.5N–1.1P–1.2K); and a fish soluble and molasses blend (4N–0P–1.7K) in growing blackberry cultivars under organic production system (Fernandez-Salvador et al 2015). Further, in the study of Yong et al (2014), the natural seaweed extract (NSE) showed a significant high growth when used to enrich the propagule of *K. alvarezii*. Since there are limited studies on the application and use of fish hydrolysate on culture of *K. alvarezii,* this study aims to investigate the effect of fish hydrolysate from milkfish and tilapia on growth of *K. alvarezii* when used as liquid fertilizer.

Material and Method

Processing of liquid fertilizer. The milkfish and tilapia waste includes the internal organs, gills, scales and fins which was procured in the local market, and it was brought to the Chemistry Laboratory of Pangasinan State University-Binmaley campus for the production of fish waste liquid fertilizer. To prevent the reduction in N-P-K content of the collected fish waste, it was processed without washing it with water.

The milkfish and tilapia waste were placed in a separate vat for fermentation process. The fish waste was weighed first to know the amount of brown sugar to be added. The sample to sugar ratio used in this experiment was 1:1 (sample:sugar). Addition of Effective Microorganisms (EMO) containing lactic acid bacteria was conducted there after which act as a deodorizing bacterium that reduced the foul odor of the fermented fertilizer. After adding sugar and EMO, the vat was then sealed. Fermentation process of fish waste took place for 10 to 15 days. The liquid fertilizers were then filtered and harvested using a fine mesh net to remove particles in the liquid. The filtered fish wastes were further processed by placing it in a refrigerator overnigt to allow coagulation of fats present in the samples. Coagulated fats were removed manually using spoon and the liquid fertilizers were packed in a cleaned and sanitized plastic bottles.

The N-P-K content of processed liquid fertilizer was analyzed and tilapia hydrolysate contained nitrogen 2.28% phosphorus 1.78% and potasium 0.23% while milkfish contained nitrogen 1.95%, phosphorus 0.69% and potasium 0.23%.

Culture of Kappaphycus alvarezii. Two liquid fertilizers made from tilapia and milkfish waste having five different concentrations (4 mL L^{-1}, 6 mL L^{-1}, 8 mL L^{-1}, 10 mL L^{-1} and 12 mL L^{-1}) each were used as the treatments in this study. Each treatment was replicated ten times. The cultures were situated at the seaweed farm of Bureau of Fisheries and Aquatic Resources 3 - Technology Outreach Station for Marine Water Development (BFAR3- TOSMWD) at Pilaper Island, Masinloc, Zambales and culture period lasted from December 3, 2015 to January 18, 2016 (45 days) (Figure 1).

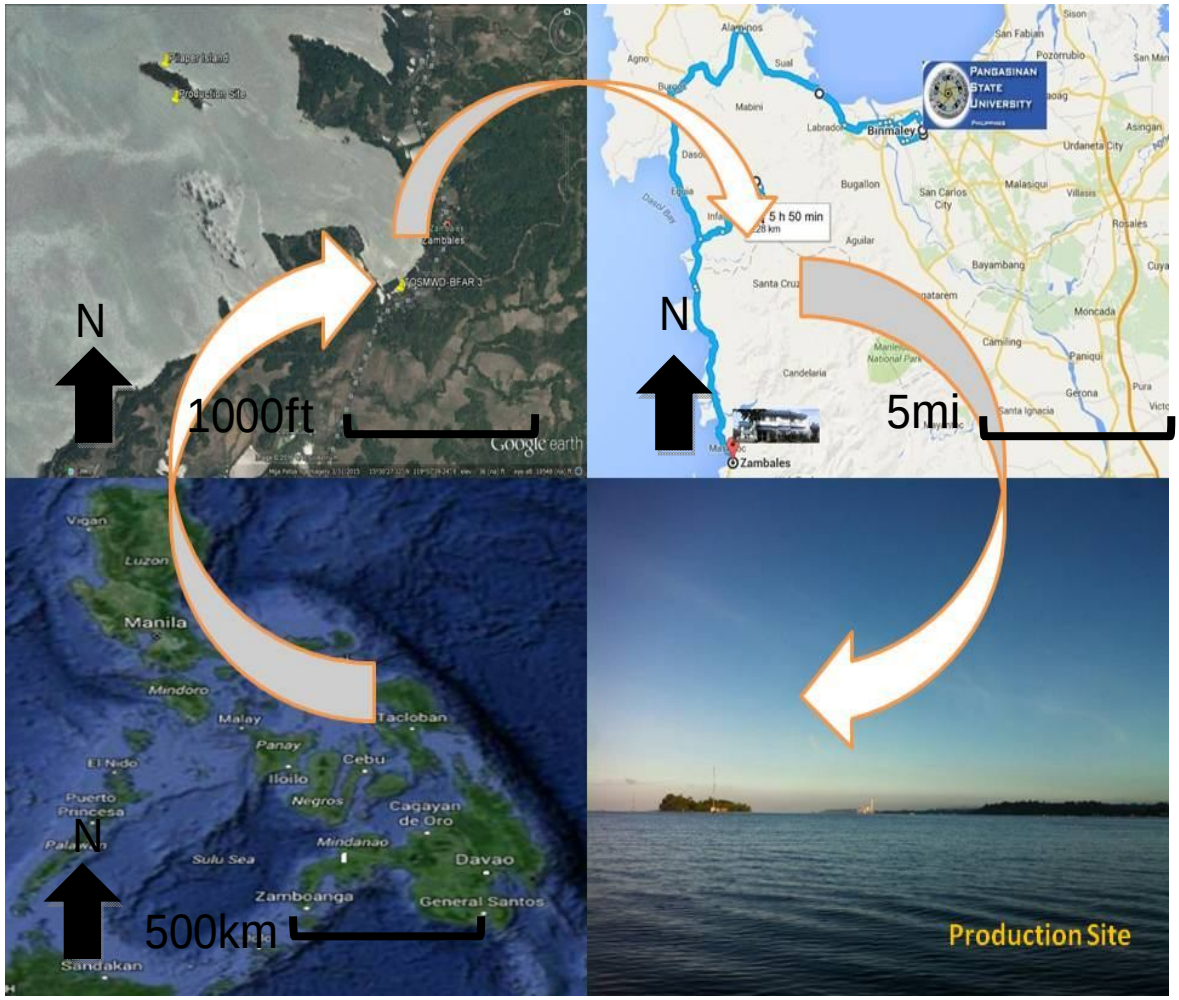

Figure 1. Culture site of *K. alvarezii* at Pilaper Island, Masinloc, Zambales.
Map and image source: Google earth V 9.1.39.1. Pilaper Island, Masinloc, Zambales. 15°30'23"N, 119°57'38"E, Eye alt 703 m. DigitalGlobe 2015. http://www.earth.google.com .

Matured *K. alvarezii* were harvested from the nursery farm of TOSMWD Station as the source of propagules for planting. Propagules were cut into sizes of 10 g per replicate. Prior to planting, seedlings were fertilized with fish waste liquid fertilizer using different concentrations. Amount of liquid fertilizer used in fertilization was measured using a beaker and the concentration was diluted into 1 liter using seawater. After preparing the fish waste liquid fertilizer, the propagules were placed on a plastic tub and soaked in 8 liters diluted fish waste liquid fertilizer for 12 hours with aerators. After soaking, fertilized propagules were brough into the culture site and were tied on to the cultivation rope using a plastic straw.

A hanging long-line method was utilized as culture method for this experiment. A 15 mm in diameter monolines polyethylene rope with a length of 12 meter provided with floaters and sinkers were used as culture structure. In setting, both ends of the rope that served as the frame of the monoline were tied to floaters with sinkers. The distance of each monoline was 1 meter apart and it was installed parallel to the water current.

The tying of seaweeds were done in the afternoon and was conducted off-shore. Each cultivation rope had an interval of 30 cm each seedlings. Cultivation ropes with seaweed seedlings were brought and set to the production site after tying. The arrangement of *K. alvarezii* propagules were rotated weekly to maximize the opportunity of *K. alvarezii* to be exposed to similar environmental conditions.

The 2x5x10 factorial in Complete Randomized Design (CRD) was used as set-up the layout in this study.

Sampling and monitoring of Kappaphycus alvarezii. Cultures were monitored daily at 9:00 am, using a motorized boat. The water parameters such as salinity and temperature were recorded and also the growth of *K. alvarezii*. Salinity was measured using the *Atago* refractometer and temperature was measured using mercury thermometer. Any impaired culture structures were repaired and epiphytes were removed from the *K. alvarezii*. Additional floaters (plastic bottles) were also tied in between of seaweeds to support the monoline, this was done when the monoline cannot maintain the distance of seaweeds from the surface due to the increase of their weight.

Growth was computed using the formula:

$$\text{Weight gain} = W_2 - W_1$$

where: W_2 = final weight and W_1 = initial weight.

The average daily growth rate (DGR = % day^{-1}) was expressed as the percentage increase in wet weight per day for each replicate which was calculated according to the formula:

$$DGR = [ln \text{ (final weight/initial weight) /duration of culture}] \times 100$$

Data analysis. Data on weight was subjected to two-way analysis of variance followed by Duncan's Multiple Range Test to test for significant differences among treatment means at 0.05 level (Gomez & Gomez 1984).

Results and Discussion. The result of growth of *K. alvarezii* fertilized with various concentrations of milkfish and tilapia waste liquid fertilizer in terms of gain in weight after 4-week of culture is shown in Table 1. Both the main effect and sub-effect showed interaction and significantly affect ($p < 0.05$) the weight gain (WG) and DGR of *K. alvarezii*. The result of two-way ANOVA indicates that the growth in terms of weight gain was significantly high ($p < 0.05$) in tilapia hydrolysate compared to that of milkfih. Increasing concentration of tilapia hydrolysate up to 10 mL L^{-1} increased significantly the WG and DGR of *K. alvarezii.*

Table 1

Growth performance of *Kappaphycus alvarezii* after 45 days of culture (wet weight in (g) and ±SEM)

Main treatment	*Sub-treatment (mL L^{-1})*	*Mean WG (g)*	*DGR*	*Main effect*	*Sub-effect*	*Interaction*
Milkfish	4	130.70 ± 0.86^{d}	10.83 ± 0.015^{f}	< 0.0001	< 0.0001	< 0.0001
	6	119.21 ± 1.32^{e}	10.62 ± 0.025^{g}		< 0.0001	< 0.0001
	8	129.03 ± 1.004^{d}	10.80 ± 0.017^{g}		< 0.0001	< 0.0001
	10	111.03 ± 2.007^{g}	10.47 ± 0.039^{g}		< 0.0001	< 0.0001
	12	114.06 ± 2.217^{f}	10.53 ± 0.042^{g}		< 0.0001	< 0.0001
Tilapia	4	166.49 ± 2.002^{b}	11.37 ± 0.027^{b}	< 0.0001	< 0.0001	< 0.0001
	6	141.51 ± 2.092^{c}	11.01 ± 0.033^{c}		< 0.0001	< 0.0001
	8	142.71 ± 1.223^{c}	11.02 ± 0.019^{d}		< 0.0001	< 0.0001
	10	169.89 ± 3.202^{a}	11.41 ± 0.044^{a}		< 0.0001	< 0.0001
	12	140.27 ± 2.779^{c}	10.99 ± 0.047^{e}		< 0.0001	< 0.0001

*mean with the same superscript are not significantly different at $p < 0.05$.

Loureiro et al (2010), Borlongan et al (2011) and Li et al (1990) reported the DGR of *in vitro* growth rate of variants of *K. alvarezii* treated with extract from *Ascophyllum nodosum*, Acadian Marine Plant Extract Powder (AMPEP) and an intermitent application of ammonium. The DGR for *A. nodosum* treated had 5.9% day^{-1}, AMPEP had 1.3-4.1% day^{-1} while 4.6% day^{-1} for intermitent fertilization which was lower compared to the DGR in present study (11.41±0.044).

The difference in growth performance was due to the NPK content in tilapia hydrolysate. According to Hanisak (1983) the ability of macroalgae including *K. alvarezii* to utilize various source of inorganic (nitrate, nitrite, and ammonium) and organic (urea)

nitrogen differ from each other. Lluisma (1992) reported that *K. alvarezii* has a low nitrogen requirement and it also exhibits surge ammonium uptake similar to that observed in some macroalgae (Dy & Yap 2001; den Haan et al 2016).

Aside from nitrogen, another factor that affected the WG and DGR of *K. alvarezii* was the amount of phosphorus present in tilapia hydrolysate. In nutrient-enriched estuaries, the increased in both nitrogen and phosphorus concentrations on water resulted to large scale macrolagal bloom especially of species belonging to the genera *Enteromorpha*, *Ulva*, *Cladophora*, and *Chaetomorpha* (Raven & Taylor 2003; Hurd et al 2014). Phosphorus is said to limit the production for macroalgae (Littler et al 1991). Phosphorus surge uptake has said to be observed in P-deficient macroalgae like the *Palmaria palmata* that belongs to phylum Rhodophyta (Martínez & Rico 2004). Once exposed in high P-concentration in water diffusion of PO_4^{3-} into the organism's extracellular space take place resulting to surge uptake of phosphorus (Hurd & Dring 1990). In this study, we were not able to measure the amount of nitrogen and phosphorus content of *K. alvarezii* after 45 days of culturing it using fish hydrolysate. But, it is possible that the amount of phosphorus in tilapia hydrolysate up to 10 mL L^{-1} is sufficient to provide the required phosphorus for *K. alvarezii* and significantly increase the WG and DGR.

The factors that affect the rate of nutrient uptake are a high surface:volume ratio (Fong et al 2004), and nutrient storage capacity of macroalgae (Fujita 1985). The increased in concentration of tilapia hydrolysate up to 12 mL L^{-1} showed decreased in WG and DGR. This might be due to the limited ability of *K. alvarezii* to store high content of nitrogen and phosphorus in their system.

Water parameters in terms of salinity and temperature limit the growth of *K. alvarezii*. The water parameters in this study were monitored throughout the whole experimental period. Based on the monitored level of salinity and temperature, the range for the salinity was 30-31 ppt and for the temperature was 29-31°C. This fluctuation is within the optimum level of salinity (30-34 ppt) and temperature (29-32°C) needed for the growth of *K. alvarezii* (Neish 2008). In the current experiment, water parameters did not affect the growth performance of *K. alvarezii*.

Conclusions and recommendations. We conclude that tilapia hydrolysate at level of 10 mL $^{-1}$L gives optimum growth to *K. alvarezii* cultured in an open water. The nitrogen and phosphorus content (N-2.28, P-1.78) of tilapia hydrolysate was sufficent and significantly enhance the growth of *K. alvarezii*. Further increased in concentration of tilapia hydrolysate upto 12 mL L^{-1} had significant deacreased in WG and DGR indicating that *K. alvarezii* has limited ability to store high amount of nitrogen and phosphorus in its system.

The detailed study on the ability of *K. alvarezii* to utilize nitrogen and phosphorus is recommended with the focus on its ability to synthesize different nutrients available in the water and within its system. In addition, growth of macroalgae could be used as an indicator of the quality of water in an area. Development of water monitoring system by studying the behavior of nutrient uptake of different species of macrolagae including commercial ones should be developed.

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