Journal of Environmental Science and Management 22-1: 13-19 (June 2019) ISSN 0119-1144

Allelopathic Effects of Three Intertidal Marine Macrophytes on the Growth of *Nanochlorum* sp.

ABSTRACT

Benthic marine macrophytes produce secondary metabolites which when released to the environment could potentially influence the immediate surroundings to the detriment of their competitors (i.e., other species of algae or phytoplankton). The response of the phytoplankton, Nanochlorum sp. to crude extracts (1% and 4% concentration) of the intertidal macrophytes Gracilaria salicornia, Chaetomorpha linum and Sargassum polycystum was investigated. On the average, there was a significant decrease (40-61% decrease at 4% conc. and 11-40% decrease at 1% conc.) in cell densities three days after the addition of the extracts relative to the control. Crude extracts of some common intertidal macrophytes may contain allelochemicals that could inhibit the growth of phytoplankton. The potential applications of these allelopathic effects in controlling phytoplankton blooms in small ponds and tanks have been suggested and needs further investigation.

Dominic Franco C. Belleza[1*]
Ethel C. Wagas[1]
Jemma Lucitte J. Aaron[2]
Rex Samuel A. Abao Jr.[3]
Danilo T. Dy[1]

[1] Marine Biology Section, Biology Department, University of San Carlos, Cebu City 6000, Philippines and Graduate School of Fisheries and Environmental Science, Nagasaki University, Nagasaki, Japan
[2] Bohol Island State University, Clarin, Bohol, Philippines
[3] Xavier University, Cagayan de Oro, Philippines

*Corresponding author: bb53118027@ms.nagasaki-u.ac.jp

Key words: *Gracilaria salicornia, Chaetomorpha linum, Sargassum polycystum, reef flat, phytoplankton control, Mactan Island*

INTRODUCTION

Various strategies have been formulated to control the blooms of phytoplankton in closed systems. Large outdoor ponds typically use pumps connected to a series of filters to maintain water clarity. Some utilize ultraviolet light together with the filtration system to eliminate all organisms passing through the UV light. *Fitzgerald and Jackson* (*1979*) evaluated a number of commercially available algicides on *Chlorella pyrenoidosa* and *Phormidium inundatum* and found that copper-based products are the most effective. *Palmer and Maloney* (*1955*) did an extensive preliminary screening of various compounds for their potential as algicides and their results yielded many effective candidates. However, all were synthetic and one group in particular, the mercuric compounds, were not only highly lethal to algae but potentially toxic to other organisms as well. Biological control of nuisance phytoplankton is often done through the use of filter feeding bivalves such as mussels (*Petersen et al. 2008*).

More effective forms of phytoplankton control have previously been considered. Among them is the utilization of the intrinsic properties of macrophytes, specifically on their metabolites. Allelopathy is an effect of one plant or microorganism on the growth and survival of other species through the release of chemical compounds into the environment (*Singh and Chaudhary 2011*). The study of allelopathic interactions among algae and plankton has been problematic as highlighted by the review of *Gross et al.* (*2007*). These difficulties were observed under natural conditions when light competition, temperature and pH changes can mask allelopathic effects (*Keating 1977*). Alternatively, laboratory experiments can account for these effects and responses more appropriately.

The use of algal extracts to control microalgae is steadily gaining acceptance as an effective means of phytoplankton control. Extracts (i.e., fresh, dried, and aqueous) of marine algae were detrimental to dinoflagellates (*Wang et al. 2007*). Secondary metabolites from the extracts of the leaf-like blades of *Sargassum polyceratium* were known to inhibit growth and development of bacteria and planktonic larvae of invertebrates (*Thabard et al. 2011*). The effect of allelopathy in *Sargassum muticum* has also been proven

to be a viable substitute to toxic, heavy-metal based paints in preventing the attachment of fouling organisms (*Bazes et al. 2009*).

If closed systems employ energy intensive methods (i.e., pumps and filter systems) to maintain water quality and clarity while chemical algicides can indiscriminately eliminate non-target species of macrophytes (*Duvall et al. 2001*), then the allelopathic effect of some algal extracts can provide a better alternative over the conventional solutions to these problems. Some commercial algicides exhibit some form of environmental persistence (*Schrader 2005*), threatening organisms at the higher trophic levels (*Ylitalo et al. 2009*) long after the problem has subsided.

Ecologically, the allelopathic effect of macroalgae on the growth of phytoplankton is considered an important mechanism for regulating and stabilizing macrophyte - dominated waters (*Phillips et al. 1978*). Two types of equilibrium states were proposed by *Scheffer et al.* (*1993*), one which presents a clear-state dominated by aquatic vegetation and a turbid state characterized by high microalgal biomass. Shallow and enclosed bodies of water dominated by submerged plants can suppress phytoplankton biomass through the release of allelopathic, organic compounds (*Burnak and Beklioglu 2000*). If this is the case, then this clear-water state can be applied in smaller scales with the aid of natural algal extracts which could possibly produce the same effect. Utilizing the allelopathic effects of submerged macrophytes in mitigating phytoplankton blooms and subsequently improving water clarity is an efficient and cost-effective alternative to other potentially destructive methods. This is particularly relevant in stagnant/enclosed structures (i.e., aquaculture ponds, outdoor garden ponds) where constant nutrient supply and sunlight may produce conditions leading to phytoplankton blooms. Areas of interest for further research may be along the shallow coastal waters where the abundance of macrophytes on reef flats (*Costa et al. 2001*) and the diverse assemblage of macroalgae may lead to a discovery of various types of previously unknown allelopathic compounds. In this study, a laboratory experiment to show that small concentrations of crude algal extracts could significantly diminish the density of the phytoplankton, *Nanochlorum* sp., is hereby presented.

MATERIALS AND METHOD

The experiment followed a randomized complete block design experiment (RCBD) consisting of three treatments (i.e., crude extracts of red, brown and green algae) with one control (i.e., no algal extract), two concentrations of the extracts and six replications per concentration. The experiment was conducted in two batches (i.e., three replicates per batch).

Nanochlorum sp. was the microalgae used for this experiment's assay since it is a ubiquitous phytoplankton species in tropical marine waters from the family Chlorellaceae and is capable of producing blooms rapidly in controlled laboratory settings. For commercial applications, *Nanochlorum* sp. is encouraged to form blooms in aquaculture ponds as natural food for cultured species of fish and shellfish larvae. *Nanochlorum* sp. blooms are characterized by a light to deep green coloration of seawater. Furthermore, a decrease in *Nanochlorum* sp. cell density due to the effects of allelopathy would be immediately obvious due to the immediate color change in the water column. *Nanochlorum* sp. pure culture was obtained from Southeast Asian Fisheries Development Center (SEAFDEC). Upon arrival in the laboratory, the pure culture was transferred to a glass vessel and maintained at 15°C in a refrigerator. During the experiment, a modified f/2 nutrient medium (*Guillard and Ryther 1962*) was prepared using the utilization ratio, 1:2 (i.e., a total volume of 1 L of algae will require 2 mL of each component of f/2 medium). The modified f/2 medium had the following composition: $Na_2 \cdot EDTA$, $NaNO_3$, $NaH_2PO_4 \cdot H_2O$, NH_4Cl, $FeCl_3 \cdot 6H_2O$, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $CoCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H2O$, $Na_2MoO_4 \cdot 2H_2O$, Thiamine HCl, Biotin, and Cyanocobalamin.

For each replicate, a final scale-up volume of 125 mL in an Erlenmeyer flask was required. Hence, 3.25 mL of f/2 medium was first placed in the flask followed by the addition of 50 mL volume of *Nanochlorum* sp. pure culture, and finally, 71.75 mL sterilized seawater was added. The mouth of the flask was then sealed with an aluminium foil. Flasks were incubated in an environmental chamber set at 12 H:12 H, day:night cycle, and at 23°C ambient temperature. Photosynthetically active radiation (PAR) was 49.48 µmol s^{-1} m^{-2}, as measured by a Li-Cor (LI-250) light meter with a cosine corrected quantum sensor. Flasks were swirled daily for three days to facilitate even mixing of nutrients and microalgae. On the third day, initial counting of algal cells for 125 mL flasks were done using a light microscope and a haemocytometer. Average cell density (mL^{-1}) was obtained from three subsamples of each flask.

Algal extracts were prepared by collecting samples of *Gracilaria salicornia*, *Sargassum polycystum* and *Chaetomorpha linum* from the reef flat off eastern Mactan Island, central Philippines. Samples were brought to the

laboratory, segregated, and cleaned of their debris and encrusting epiphytes. Cleaned algal samples were blot dried and weighed according to the concentrations to be used. For the 4% concentration treatment, 10 g of the algal samples were homogenized with 10 mL sterilized seawater using mortar and pestle. The homogenized extract was filtered through cheesecloth to remove impurities and other unhomogenized solids prior to addition in the flasks containing the phytoplankton and nutrient medium while an additional volume of sterilized seawater was added to yield a final volume of 250 mL. The flasks were incubated in the environmental chamber for three days, after which, final counting of algal cells were done and the mean cell density was expressed as mL^{-1}. The 1% concentration (2.5 g macroalgae in 2.5 mL sterilized seawater) was prepared using the same procedure. It should be noted that phytoplankton cultures which failed to reach optimum cell counts (i.e., 1×10^6 cells mL^{-1}) were excluded.

The change in *Nanochlorum* sp. density (i.e., final – initial density) after three days incubation was used as the dependent variable. A model 1 nested ANOVA was used to check whether there was a significant difference between the two batches of experiments for both the 1% and 4% concentrations. Since there was no significant difference between the two batches, the replicates were pooled together and analyzed using one-way ANOVA to determine if there was a difference among the type of algal extract on the change in phytoplankton density. Homoscedasticity was tested using Levene's or Cochran C test. Tukey's HSD was used as a *post hoc* multiple comparison test should a significant result in ANOVA occurs. The significant level was set at $p = 0.05$. Both 1% and 4% algal extract concentrations were statistically treated separately due to logistic limitation in the implementation of the experiment.

RESULTS AND DISCUSSION

The type of crude extract was a significant factor in the suppression of phytoplankton growth in both test concentrations (**Table 1**). While all control flasks exhibited positive population growth (normal exponential growth phase in *Nanochlorum* sp.), treatments with the crude extracts showed decline in cell density. The decrease in growth of *Nanochlorum* sp. population was highest under *Sargassum polycystum* and *Chaetomorpha linum* extracts for 4% and 1% concentrations respectively, while *Gracilaria salicornia* had slightly decreased inhibition compared to the former two species (**Figure 1**). Concentrations of extracts also appeared to have an effect on the population growth of *Nanochlorum* sp., where higher concentrations (i.e., 4%) of the extracts resulted in more inhibition (ranging from 40-61%) on the population growth of *Nanochlorum* sp. after three days of culture. Visual observation shows settling of dead algal cells and an increased water clarity inside the culture flasks.

Table 1. Statistical results showing the effects of algal extracts on the chance in cell density of *Nanochlorum* sp. after three days of culture.

	Factors	SS	df	MS	F	p-level
1%	Type	2.15E+13	3	7.18E+12	3.7	0.03
	Error	3.87E+13	20	1.94E+12		
4%	Type	9.77E+13	3	3.26E+13	60.0	0.00
	Error	5.97E+12	11	5.43+11		

Marine macrophytes have been known to alter the structure of benthic ecosystems through chemical means (*Gross et al. 1996*). Despite this interesting phenomenon, the mechanism of their release from the actual plant and their effects on the target organism is still poorly understood (*Wium-Andersen 1987*). A study on algal secondary metabolites suggests that some species of macroalgae have inducible defense mechanisms which are triggered by direct herbivory or epibiosis (*Pereira et al. 2017*). This implies that allelopathic substances may be released into the immediate environment after algal tissues have been mechanically damaged by herbivore

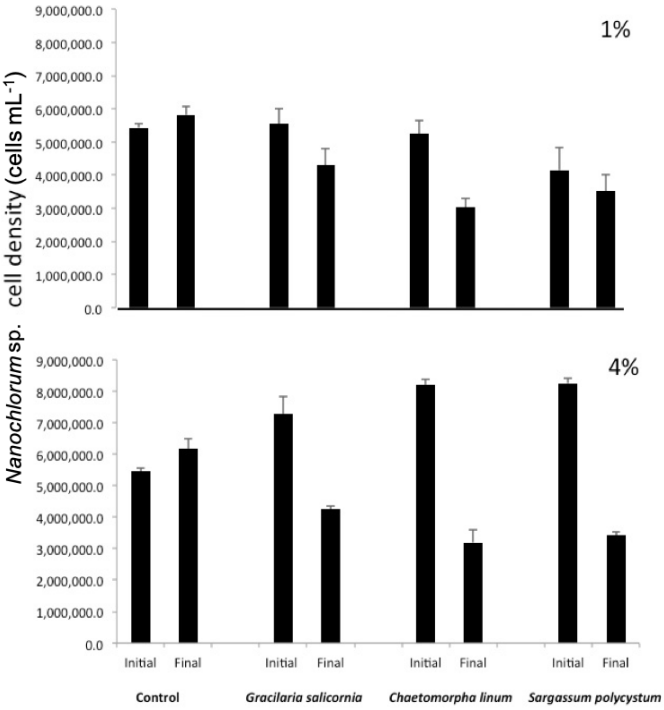

Figure 1. The effects of 1% and 4% concentrations of algal extracts of *Nanochlorum* sp. cell densities.

consumption or the attachment mechanisms used by fouling organisms and other epibionts. Similarly, the current experiment provided some indication of the detrimental effects of macroalgal extracts against a species of phytoplankton when *Nanochlorum* sp. was unable to reach cell densities similar to the control upon addition of the algal extracts.

The results corroborated with other studies. *Jasser* (*1995*) found out higher concentration of *Stratiotes aloides* extract led to increased allelopathic effects against natural phytoplankton. The algicidal effect could be due to the action of phenolic acids (*Usenko et al. 2002*). Other possible factors which may explain the mechanism of the allelopathic effects of macroalgae include the presence of methanolic extracts which has been demonstrated to exhibit algicidal activity (*Gross 1995*) by inhibiting the alkaline phosphatase activity (*Gross et al. 1996*) and the photosynthetic transport system (*Weston et al. 1999*). The inhibition of photosynthesis of competing primary producers is considered a very common mode of action among many photosynthetic organisms (*Gross 2003*). Ecologically, allelopathic compounds are released to prevent shading by epiphytic and planktonic primary producers (*Dondajewska and Budzyńska 2009; Mulderij et al. 2006*). Among planktonic organisms, inhibition of competitors have also been observed in the maintenance of harmful algal blooms, specifically with the dinoflagellate, *Karenia brevis*, by altering the physiology of the cell membrane and photosynthesis of other non-harmful phytoplankton species (*Poulin et al. 2018*). For macroalgae which are prone to the shading effects of phytoplankton blooms, allelopathy is one mechanism to maintain water clarity and reduce light attenuation. In this study, *C. linum* crude extracts showed significant decrease in *Nanochlorum* sp. cell density, suggesting a strong allelopathic effect on the phytoplankton population. This high degree of allelopathy shows resemblance to similar experiments utilizing macroalgae commonly found in shallow coastal waters like *Ulva pertusa* (*Nan et al. 2004*), *Ulva lactuca* (*Tang and Gobler 2011*) and *Gracilaria tenuistipitata* (*Ye and Zhang 2013*). Interestingly, the decomposition by-products of *Ulva prolifera* in green-tide events have been found to promote blooms of some red-tide forming species but not others (*Wang et al. 2012b*), highlighting the need for a controlled application of algal extracts to inhibit algal blooms and not just direct application of unprocessed algal biomass.

Furthermore, studies on crustose algae, which are also very prone to shading by large canopy-forming plants, show a very high degree of allelopathic activity as well. Crustose coralline algae from the genus *Lithophyllum* has been shown to produce low molecular weight, lipophilic allelopathic substances able to destroy the zoospores of the brown algae *Laminaria religiosa* (*Suzuki et al., 1998*) which form dense canopies as mature plants. For canopy-forming seaweeds such as members of the Sargassaceae, in this study 1% extracts from *Sargassum polycystum* seem to have a lesser effect on *Nanochlorum* sp. compared to *C. linum*. This is probably because phlorotannins present in brown algal species may have minimal allelopathic effect on phytoplankton. Epiphytes growing on the brown macroalga, *Ecklonia radiata*, for instance, showed no effect on the abundance and the distribution of these epiphytes despite phlorotannins exuded by the macroalgae (*Jennings and Steinberg 1994*). A study on *Sargassum thunbergii* demonstrates how its extracts of unsaturated fatty acids were able to inhibit the growth of red-tide dinoflagellates (*Wang et al. 2012a*). However, efficacy of the extracts depended on the type of extraction method and the concentration of pure extracts. This may explain the low performance of extracts at 1% concentration and an increased allelopathic effect at 4%. For *Gracilaria salicornia* a decrease in *Nanochlorum* sp. cell density of approximately 25.4% and 43% in 1% and 4% concentrations, respectively, still demonstrates an appreciable allelopathic effect. A study on extracts from *Gracilaria lemaneiformis* shows varying effects of different isolates on *Skeletonema costatum* (*Lu et al. 2011*) while *Ye and Zhang* (*2013*) shows *G. tenuistipitata* as an effective inhibitor for *Prorocentrum micans*. This may suggest the species-specific nature of allelopathic compounds which this study has not accounted for. However, the results in general suggests that efficacy may be greatly improved, hence, extract concentrations and extraction method should be a significant factor to be considered in future experiments.

The role of allelopathic effect by some macrophytes in reducing competition could be used as a tool to control unwanted algal growth in certain aquatic ecosystems (*Nakai et al. 1999*). The control of nuisance microalgal populations in tanks and ponds where the recirculation of water allows the sufficient interaction between allelopathic compounds and the target organism should be investigated further. More importantly, the allelopathic effect of macroalgal extract may be used as an alternative water clarifying agent over the use of industrial algicides. A review on the control of algal blooms using aquatic macrophytes underscores the increasing body of knowledge surrounding this application (*Hu and Hong 2008*). However, they also highlight the limitations towards its use on a commercial scale citing the effects of dilution and water currents may possibly not reflect the results in small-scale laboratory experiments. Hence, the

need for future studies focused on spatial and temporal effects to the allelopathic efficacy.

In retrospect, the relevance of using algal extracts for small-scale applications such as in the aquarium trade and in small aquaculture operations for maintaining water clarity holds potential. Utilizing the abundant, non-commercial species of tropical algae can be a cost effective substitute to the conventional methods without the consequence of pollution and toxic compounds persisting in the food chain. Concomitant to the use of algae, an extensive screening regime must be done to determine which species bear the highest potency of allelopathic compounds and the species of phytoplankton most vulnerable. Nevertheless, the effects on planktonic larvae of invertebrates and fish larvae must be taken into consideration as well, since it has been known that certain macroalgal species can inhibit settlement of recruits (*Kuffner et al. 2006*). The extent of their allelopathy to other species of phytoplankton must also be studied to determine threshold concentrations.

CONCLUSIONS AND RECOMMENDATION

The current study highlights the potential of common intertidal macroalgae as biological control agents for microalgal blooms. The addition of crude algal extracts led to the decline in microalgal cells leading to the conclusion that the crude extracts may contain some secondary metabolites which had an allelopathic effect on *Nanochlorum* sp. at both treatment concentrations. Secondary metabolites have an important ecological role for macroalgae as effective competitors for space in a highly dynamic environment. The production of metabolic by-products for this purpose can also be observed in other sessile taxa such as sponges (*Selvin and Lipton 2004*), corals (*Fenical and Pawlik 1991*), and ascidians (*Pisut and Pawlik 2002*), among many others. The critical consideration for the release of these allelochemicals was mechanical destruction of algal tissues, as was done here. However, other mechanisms which regulate the release of allelochemicals without tissue destruction would be worth exploring since clearwater states (*Burnak and Beklioglu 2000*) may also mean that macrophytes with high uptake rates of DIN and P were outcompeting phytoplankton and thus mask the true allelopathic effects. An interesting starting point for investigating this mechanism may be from the review by *Gopal and Goel* (*1993*) which attempts to gather evidence for allelopathy among aquatic plant communities and characterized allomones. Concomitant to this is expanding our knowledge of both algal taxonomy together with information on the mode of action of their allelochemicals (i.e. stimulatory/inhibitory action).

The effectiveness of allelopathic effects is highly dependent on the concentration of the extracted metabolites. The experiment in this study was able to provide some evidence of allelopathy through the use of a microcosm experiment. However, practical utility must also consider stability of extracts according to ambient environmental conditions such as temperature, pH, light and dilution effects. Furthermore, susceptibility of target and non-target organisms should be considered in assay studies as this may have broad applications (*sensu Cheng and Cheng 2015*) for managing plankton blooms in aquaculture enclosures, ameliorating nuisance algal bloom events in public beaches as well as mitigating plankton blooms in recreational aquaria and ponds.

REFERENCES

Bazes, A., Silkina, A., Douzenel, P., Fay, F., Kervarec, N., Morin, D., Berge, J.P., and Bourgougnon, N. 2009. "Investigation on the antifouling constituents from the brown alga *Sargassum muticum* (Yendo) Fensholt". *Journal of Applied Phycology* 21(4):395-403.

Burnak, S.L. and Bekliogl, M.. 2000. "Macrophyte-dominated clearwater state of Lake Mogan". *Turkish Journal of Zoology* 24:305-313.

Cheng, F. and Cheng, Z.H. 2015. "Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy". *Frontiers in Plant Science* 6: doi:10.3389/fpls.2015.01020.

Costa Jr, O.S., Attrill, M.J.,Pedrini, A.G., and De-Paula, J.C. 2001. "Benthic macroalgal distribution in coastal and offshore reefs at Porto Seguro Bay, Brazilian Discovery Coast". Proceedings of the 9th International Coral Reef Symposium, 9 pp. Bali, Indonesia

Dondajewska, R. and Budzynska, A. 2009. "The influence of green algae on Chlorococcal algae (*Desmodesmus* spp.)". *International Journal of Oceanography and Hydrobiology* 38:21-25.

Duvall, R.J., Anderson, L.W.J., and Goldman, C.R. 2001. "Pond enclosure evaluations of microbial products and chemical algicides used in lake management". *Journal of Aquatic Plant Management* 39:99-106.

Fenical, W. and Pawlik, J.R. 1991. Defensive properties of secondary metabolites from the Caribbean gorgonial coral *Erythropodium caribaeorum*. *Marine Ecology Progress Series* 75: 1-8.

Fitzgerald, G.P. and Jackson, D.F. 1979. "Comparative algicide evaluations using laboratory and field algae". *Journal of Aquatic Plant Management* 17:66-71.

Gopal, B. and Goel, U. 1993. "Competition and allelopathy in aquatic plant communities". *The Botanical Review* 59(3):155-210.

Gross, E. M. 1995. "Allelopathische Interaktionen zwischen Makrophytenund Epiphyten: Die Rollehydrolysierbarer Polyphenoleaus *Myriophyllum spicatum*". Ph.D. Dissertation. Christian Albrechts University, Kiel, Germany, 148 pp.

Gross, E. M. 1999. "Allelopathy in benthic and littoral areas: case studies on allelochemicals from benthic cyanobacteria and submersed macrophytes". In: Principles and Practices in Plant Ecology: Allelochemical Interactions (eds. Inderjit, K. M. M., Dakshini & Foy, C. L.) CRC Press, Boca Raton, pp. 179–99.

Gross, E.M., 2003. "Allelopathy of aquatic autotrophs". *Critical Reviews in Plant Science* 22:313-319.

Gross, E.M., Hilt-Körner, S.,Lombardo, P., Mulderij, G. 2007. "Searching for allelopathic effects of submerged macrophytes on phytoplankton– state of the art and open questions". *Hydrobiologia* 584:77-88.

Guillard, R.R.L and Ryther, J.H. 1962. "Studies of marine planktonic diatoms I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran". *Canadian Journal of Microbiology*, 8(2):229-239.

Hu, H.Y. and Hong, Y. 2008. "Algal-bloom control by allelopathy of aquatic macrophytes – A Review". *Frontiers of Environmental Science and Engineering in China* 2(4):421-438.

Jasser, I. 1995. "The influence of macrophytes on a phytoplankton community in experimental conditions". *Hydrobiologia* 306:21–32.

Jennings, J. G. and Steinberg, P.D.. 1994. "In situ exudation of phlorotannins by the sublittoral kelp *Ecklonia radiate*". *Marine Biology* 121:349-354.

.

Keating, K.I.. 1977. "Allelopathic influence on blue-green bloom sequence in a eutrophic lake". *Science* 196:885-887.

Kuffner, I.B., Walters, L.J., Becerro, M.A., Paul, V.J., Ritson-Williams, R. and Beach, K.S. 2006. "Inhibition of coral recruitment by macroalgae and cyanobacteria". *Marine Ecology Progress Series* 323:107-117.

Lu, H.M., Xie, H.H., Gong, Y.X., Wang, Q. and Yang, Y.F.. 2011. "Secondary metabolites fromthe seaweed *Gracilaria lemaneiformis* and their allelopathic effects on *Skeletonema costatum*". *Biochemical Systematics and Ecology* 39(4-6):397-400.

Mulderij, G., Smolders, A.J.P., and van Donk, E. 2006. "Allelopathic effect of the aquatic macrophyte, *Stratiotes aloides*, on natural phytoplankton". *Freshwater Biology* 51:554-561.

Nakai, S., Inoue, Y., Hosomi, M. and Murakami, A. 1999. "Growth inhibition of blue-green algae by allelopathic effects of macrophytes". *Water Science and Technology* 39:47–53.

Nan, C.R., Zhang, H.Z. and Zhao, G.Q.. 2004. "Allelopathic interactions between the macroalga *Ulva pertusa* and eight microalgal species". *Journal of Sea Research* 52:259-268.

Palmer, C.M. and Maloney, T.E. 1955. "Preliminary screening for potential algicides". *Ohio Journal of Science* 55 (1): 1-8.

Pereira, R.C., da Silva Costa, E., Sudatti, D.B. and da Gama, B.A.P. 2017. "Inducible defenses against herbivory and fouling in seaweeds". *Journal of Sea Research* 122:25-33.

Petersen, J.K., Nielsen, T.G., van Duren, L. and Maar, M. 2008. "Depletion of plankton in a raft culture of *Mytilus galloprovincialis* in Ria de Vigo, NW Spain. I. Phytplankton". *Aquatic Biology* 4:113-125.

Phillips, G. L., Eminson, D. and Moss, B. 1978. "A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters". *Aquatic Botany* 4:103-126.

Pisut, D.P. and Pawlik, J.R. 2002. "Anti-predatory chemical defenses of ascidians: secondary metabolites or organic acids?" *Journal of Experimental Biology and Ecology* 270(2): 203-214.

Poulin, R.X., Hogan, S., Poulson-Ellestad, K.L., Brown, E., Fernandez, F.M. and Kubanek, J.. 2018. "*Karenia brevis* allelopathy compromises the lipidome, membrane integrity, and photosynthesis of competitors". *Scientific Reports* 8(9572): DOI:10.1038/s41598-018-27845-9.

Scheffer, M., S.H. Hosper, M-L. Meijer, B. Moss and E. Jeppesen. 1993. "Alternative equilibria in shallow lakes". *TREE* 8(8):275-279.

Schrader, K.K. 2005. "Evaluation of several commercial algicides for control of odor-producing cyanobacteria". *Journal of Aquatic Plant Management* 43:100-102.

Singh, A.P. and Chaudhary, B.R. 2011. "Allelopathic potential of algal weed *Phithophora oedogonia* (Mont.) Ittrock on the germination and seedling growth of *Oryza sativa* L." *Botany Research International* 4(2):36-40.

Suzuki, Y., Takabayashi, T., Kawaguchi, T. and Matsunaga, K. 1998. "Isolation of an allelopathic substance from the crustose coraliine algae, *Lithophyllum* spp., and its effect on the brown alga, *Laminaria religiosa* Miyabe (Phaeophyta)". *Journal of Experimental Marine Biology and Ecology* 225: 69-77.

Tang, Y.Z. and Gobler, C.J. 2011. "The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy". *Harmful Algae* 10:480:488.

Thabard, M., Gros, O., Hellio, C. and Marechal, J.P. 2011. "*Sargassum polyceratium* (Phaeophyceae, Fucaceae) surface molecule activity towards fouling organisms and embryonic development of benthic species". *Botanica Marina* 54:147-157.

Usenko O.M., Sakevich, A.I., and Palamarchuk. V.D., 2002. "Influence of phenol acids of hydrophytes on the development of plankton algae". *International Journal of Algae* 4:20–28.

Wang, R.J., Xiao, H., Zhang, P.,Qu, L., Cai, H.J. and Tang, X.X. 2007. "Allelopathic effects of *Ulva pertusa*, *Corallina pilulifera* and *Sargassum thunbergii* on the growth of the dinoflagellates *Heterosigma akashiwo* and A*lexandrium tamarense*". *Journal of Applied Phycology* 19:109-121.

Wang, R.J., Wang, Y., and Tang, X.X. 2012a. "Identification of the toxic compounds produced by *Sargassum thunbergii* to red tide macroalgae". *Chinese Journal of Oceanology and Limnology* 30(5):778-785.

Wang, C., Yu, R.C. and Zhou, M.J. 2012b. "Effects of the decomposing green macroalga *Ulva* (*Enteromorpha*) *prolifera* on the growth of four red-tide species". *Harmful Algae* 16:12-19.

Weston, L., Nimbal, A., C.I. and Jeandet, P. 1999. "Allelopathic potential of grain sorghum (*Sorghum bicolor* (L.) Moench) and related species". In: Principles and Practices in Plant Ecology: Allelochemical Interactions. (eds. Inderjit, K. M. M., Dakshini and Foy, C. L.) CRC Press, *Boca Raton*, pp. 467–77.

Wium-Andersen. S. 1987. "Allelopathy among aquatic plants." Arch. Hydrobiol.–Beih. Ergebn. 27:167–172.

Ye, C.P. and Zhang, M.C. 2013. Allelopathic effect of macroalga *Gracilaria tenuistipitata* (Rhodophyta) on the photosynthetic apparatus of red-tide causing microalga *Prorocentrum micans*". *Information Engineering Research Institute Procedia* 5:209-215.

Ylitalo, G.M., Baird, R.W., Yanagida, G.K., Webster, D.L., Chivers, S.J., Bolton, J.L., Schorr, G.S. and McSweeney, D.J. 2009. "High levels of persistent organic pollutants measured in blubber of island-associated false killer-whales (*Pseudorca crassidens*) around the main Hawaiian Islands". *Marine Pollution Bulletin* 58:1922-1952.

ACKNOWLEDGMENT

We thank the Marine Biology Section, University of San Carlos for logistic support. The graduate scholarship grants extended by the Philippine Council for Aquatic and Marine Research and Development (PCAMRD) to DFCB, ECW, JLJA and RSAA Jr. are hereby acknowledged. We also thank Mr. Benjamin Pangatungan and Reynaldo Tobias, for their assistance. This is a research contribution of the Marine Biology Section, University of San Carlos.