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Growth Performance, Survival, and Filtration Efficiency of Green Mussel (Perna viridis) Spats Fed with Different Algae

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ABSTRACT

A feeding trial was conducted to assess the potential of four species of marine microalgae as diets for green mussel *Perna viridis* spats. Species of algae tested were: *Isochrysis galbana*, *Tetraselmis tetrahele*, *Chaetoceros calcitrans*, and *Skeletonema tropicum*. Growth, survival and filtration rates were assessed. Highest growth rate was observed in spats fed with *C. calcitrans* followed by *I. galbana* and *T. tetrahele*, and *S. tropicum*, respectively. No significant differences in the survival of spats was observed in all feeding treatments. Further, filtration rate as factor affecting bivalve filter-feeding behavior was also measured. Highest filtration rate was observed in groups fed with *C. calcitrans* and *I. galbana*. The present study provides valuable information on the suitable diets for the nursery culture of green mussel spats. Furthermore, our results provide a basis for developing a better feeding strategy for the production of mussel spats in captivity.

Key words: green mussel, spats, microalgae, filtration rate, *Perna viridis*

INTRODUCTION

Green mussel (*Perna viridis*) is considered as one of the traditional aquaculture commodities in the Philippines. Over the years, it became one of the most cultured bivalves which contributed to a volume of 18,761.76 metric tons to the total aquaculture production of the country (Laxmilatha et al., 2011). However, this past decade data shows a significant reduction of mussel production (FAO, 2016; PSA, 2016). One of the pertinent reasons for the decline of its production is linked to the decrease of the available seeds (spats) supply in the wild. Moreover, the primary cause of the decline of wild spat population is the deterioration of the coastal environmental brought about by pollution and climate change (Marshall et al., 2010; Laxmilatha et al., 2011).

Sustainability of mussel seed supply is an important component in the success of the shellfish industry. Thus, the mussel industry throughout the world is slowly focusing on producing their seed supply from the hatchery. At present, several countries in Europe (Spain, France, Wales, the Netherland and Ireland) had shifted to hatchery-produced spats or 'seeds' for commercial purposes. Just recently the first mussel hatchery was established in the country (Mero et al., 2019). This is to answer the needs of the grow-out farmers to mitigate their reliance on spats from the wild. Therefore, there is a need to focus on how to produce mussel seeds for sustainable green mussel farming. While there is ongoing development in hatchery production, nursery rearing is also a critical stage before spats are transferred for grow-out culture (Helm and Bourne, 2004). The purpose of nurseries is to rapidly grow small spats at low cost to a size suitable for grow-out culture (FAO, 2004). One of the factors that affect the growth and survival of mussel spats in the nursery is the availability of appropriate algal diet. Some of the algal species used for bivalve aquaculture belongs to the genera *Chaetoceros, Thalassiosira and Skeletonema* (diatoms), *Isochrysis, Pavlova* (prymnesiophytes), *Tetraselmis and Pyramimonas* (prasinophytes) and *Dunaliella* (chlorophytes) (Gosling, 2003; Helm and Bourne, 2004; Brown and Blackburn, 2013; Maquirang et al., 2019). Higher percentage of the bivalve production cost is expended on the production of algal food (Coutteau, 1996; Helm and Bourne, 2004).

Hence, there is a need to utilize optimum algal species to support growth and development of the early stages of green mussel so that grow-out farmers will be provided with the good quality spats to maximize production (Fidalgo et al., 1994). Previous studies have already assessed appropriate diets for the early larval stages of green mussel at hatchery phase. The algal species *Isochrysis galbana*, *Chaetoceros calcitrans*, or their combination promotes better growth and survival of the D-stage to pediveliger larval stages of green mussel (Apines at al., 2020). The same trend was also observed in the study of Maquirang et al. (2019) during the pediveliger to early spat (newly settled larvae). It was also observed that *Isochrysis galbana*, and *Chaetoceros calcitrans* are more digestible and stimulates maximum growth. Here, the different species of microalgae that are appropriate for the nursery culture of green mussel *Perna viridis* spats were assessed.

MATERIALS AND METHODS

Experimental Treatments

Spats (3-4mm) used in the study were acclimatize in 12L capacity plastic rectangular tank filled with 8L filtered seawater with partial water change daily, fed experimental algae. Feeding experiment started after 7 days of acclimation. Thirty spats were randomly distributed to each experimental tank provided with oyster shells as substrates. Algae used as feeding treatments were: *Isochrysis galbana* (size: 5 - 6µm), *Tetraselmis tetrahele* (size length 10 -16µm; width 8-11µm), *Chaetoceros calcitrans* (size: length 8µm; width 5µm) and *Skeletonema tropicum* (length 10 µm; width 9µm). All treatments were replicated three times. Algal diets were given twice daily at 8-9am in the morning and 4-5pm in the afternoon. Food ration was calculated based on the biomass of the spats held per tank. The food ration computed based on the formula of Helm and Bourne (2004):

$$V = (Sx0.4)/(WxC)$$

where, V is the volume of the algae (L) required to provide the daily ration, S is the live weight of the spat (mg) at the start of the week, W is the weight of algal cells required and C is the algal cell density (cells per µl). The values 0.4 refers to the ration as dry weight of the algae (mg) per mg live weight of spat. Spats are weighed weekly to adjust its food ration.

The duration of feeding experiment is 30 days or until the spats reach the ideal size for grow-out culture. At the end of the experiment growth and survival of spats were assessed. Guillard's (1973) formula for calculating the specific growth rate (SGR) of the mussel spats was used:

SGR (length) = [(ln final weight – ln initial weight) / experimental days] x 100

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Survival was also computed:

% Survival = (number of live spats / number of initial stocks) x 100

Algal Culture

Algal stock cultures were sourced out from SEAFDEC-AQD, Tigbauan, Iloilo and sub-cultured in the hatchery. Methods of algal culture were based from that of de la Peña and Franco (2013). Briefly, algae were batch cultured in 800mL glass bottles and 10L plastic carboys using UV filtered seawater. Cultures of both *Isochrysis galbana*, *Tetraselmis tetrahele* were enriched with Conwy (Walne, 1970) medium while diatoms, *Chaetoceros calcitrans and Skeletonema tropicum* cultures were added with F-medium fertilizer (Guillard and Ryther 1962). All cultures were exposed to 24-hour constant illumination and provided with continuous aeration.

Filtration Rate

The filtration rate (FR) of spats given with different algal species was also measured. The same algal species used in the feeding experiment was used in this analysis. Using the indirect method of calculation used by Pestana et al. (2009), mussel spat filtration rate (FR), was determined. Briefly, mussel spats (0.9-1.1cm in length) were acclimated, unfed for 24 hours and two spats were placed in 500mL plastic container filled with sand-filtered seawater without aeration. FR was computed 1 hour after the algal feeds were given. A subsample (20mL) was taken from each container after 1 hour and algae were counted under the compound microscope (Motic, Kowloon, HK) using a haemacytometer (Neubauer Improved Brightline, Marienfield, Germany). FR was calculated using the formula of Sprung 1984:

$$\mathrm{FR} = \frac{v}{n}\left\{\left(\frac{\ln C_{\mathrm{o}} - \ln C_{\mathrm{t}}}{t}\right) - a\right\}$$

where v is the volume of water (mL), n the number of larvae stocked , t the duration of experiment (h), C_o the initial cell density (cells mL^{-1}), C_t the final cell density (cells mL^{-1}) and a the correction factor using the control.

$$a = \frac{\ln C_{\mathrm{o}} - \ln C'_{\mathrm{t}}}{t}$$

where C'_t is the final cell density in control (cells mL^{-1}).

Statistical Analysis

Statistical analysis of the actual specific growth rate, percentage survival and filtration rate were all analyzed using a One-Way Analysis of Variance (ANOVA) using SPSS version 16. A Tukey HSD post-hoc test was used to identify differences among specific treatment groups. In all cases, level of confidence was assigned at $p<0.05$ comparison of treatment means.

RESULTS AND DISCUSSION

This study determines the ideal algal diet for the nursery culture of green mussel spats. There was no significant difference observed in the survival of spats among treatments (Table 1). However, significantly higher growth rates were observed in treatments fed with *C. calcitrans* and *I. galbana* compared with treatments fed with *T. tetrahele and S. tropicum.* The same observation was seen in the study of Apines-Amar et al. (2020) and Maquirang et al. (2019) when they conducted feeding trials in the early larval stages of the green mussel at hatchery phase. In both studies significant increase in the growth were observed when the larvae were fed with *C. calcitrans* and *I. galbana*. Thus, both species of algae are considered to be ideal diets for the hatchery culture of green mussel. The result of this study also conforms to the result of the feeding trial conducted in another mussel species *Mytilus galloprovincialis*, wherein spats that were fed *Chaetoceros sp.* have better growth performance (Esquivel and Lobina, 1994). Furthermore, the same results was also observed in *Argopecten ventricosus* (Lora-Vilchis and Doktor, 2001), *Pinctada imbricata* (Lodeiros et al., 2016), and *Ostrea edulis* (Laing and Millican, 1986.) Although in other species of mollusk, *I. galbana* showed better result in inducing growth of *M. mercenaria* (Wikfors et al., 1992) and *C. virginica* (Epifanio, 1979). As described by Ukeles (1970), *I. galbana* and *C. calcitrans* were both considered as ideal algal diet for early stages of marine invertebrates. On the other hand, *Tetraselmis* was recommended by Helm and Bourne (2004) for bivalve spats. However, based on the result of the present study, *T. tetrahele* failed to induce optimum growth in green mussel spats. The same observation was also reported in *O. edulis (*Laing and Millican, 1986*)* as well as in *C. gigas* and *T. philippinarum* (Laing and Verdugo, 1991) when they used *Tetraselmis sp*. as one of their feeding treatments. In this study, significantly lowest growth was observed in spats fed with *Skeletonema tropicum.* In contrast, the temperate species *S. costatum* performed well in inducing growth of other mollusks. It ranked second to *Chaetoceros spp*. for improving the growth performance of *O. edulis* juveniles (Laing and Millican, 1986). Furthermore, O'Connor et al. (1992) considered *Skeletonema costatum* as best diet for *Saccostrea commercialis* without significant difference to *Chaetoceros spp*. This indicates that different species of mollusk may require different types of algae to satisfy their nutritional requirements for growth.

Table 1. Length, weight specific growth rate and survival of green mussel spats fed experimental diets for 30 days.

Algal Treatments	Length SGR % day^{-1}	Weight SGR % day^{-1}	Survival (%)
I. galbana	2.81±0.042^{a}	10.731±0.047^{ab}	98±1.0
C. calcitrans	3.23±0.062^{a}	11.333±0.167^{a}	93±2.0
Skeletonema sp.	2.25±0.365^{b}	7.707±0.648^{c}	89±5.0
Tetraselmis sp.	2.30±0.218^{b}	8.994±0.453^{bc}	94±2.0

Values are shown as mean ± standard error. Values with different superscripts are significantly different.

Although not measured in the present study, nutritional composition of different species of algae used in the feeding experiment may influence the growth performance of green mussel spats. Protein, considered as the most important factor that determines the nutritive value of the species, functions primarily in supplying nitrogen and essential amino acids (EAAs) needed for tissue biosynthesis (Brown et al., 1997). Carbohydrates and lipids on the other hand act as major source of energy source and energy reserves. FAO (2004) presented a data that includes protein, carbohydrate, and lipid composition of the different algal species in terms of percent dry weight. Based on the data, the percent dry weight for protein, carbohydrates, and lipid of *C. calcitrans*, *I. galbana*, *Tetraselmis sp.* and *Skeletonema sp*. are as follows: protein 34, 29, 31, 28; carbohydrate 6.0, 12.9, 12.0, 4.0; lipid 16, 23, 10, and 13. Furthermore, highly unsaturated essential fatty acids (HUFAs) composition of these algae may also play a very important factor in the growth and development of mussel spats. *Chaetoceros calcitrans* is rich in EPA (icosapentaenoic acid), which ranges from 21- 26% (Napolito et al., 1990, Rivero-Rodriguez et al., 2007) of total fatty acids (TFA). Whereas, *I. galbana* contains 0.9-6.89% (Volkman et al., 1989; Rivero-Rodriguez et al., 2007), *Tetraselmis* have 3.9-5.88% (Fernandez-Reiriz et al., 1989; Volkman et al., 1989; Renaud et al., 1999) and *Skeletonema* contains 13.0% EPA of TFA (Renaud et al., 1999). Aside from EPA, DHA (docosahexaenoic acid) plays a key role in bivalve nutrition. *Isochrysis galbana* is known to contain high levels of DHA, which is 20.47-26.8% (Napolito et al. 1990, Rivero-Rodriguez et al., 2007), while the DHA level of *C. calcitrans* ranges from 1.16-2.3%, *Skeletonema* is 1.8% (Renaud et al., 1991) and zero or trace for *Tetraselmis* (Volkman et al., 1989; Fernandez-Reiriz et al., 1989;

Renaud et al., 1999; Patil et al., 2007; Rivero-Rodriguez et al., 2007). Thus, based on the data gathered from various literature both *C. calcitrans* and *I. galbana* have better nutritional profile compared to *Skeletonema* and *Tetraselmis*.

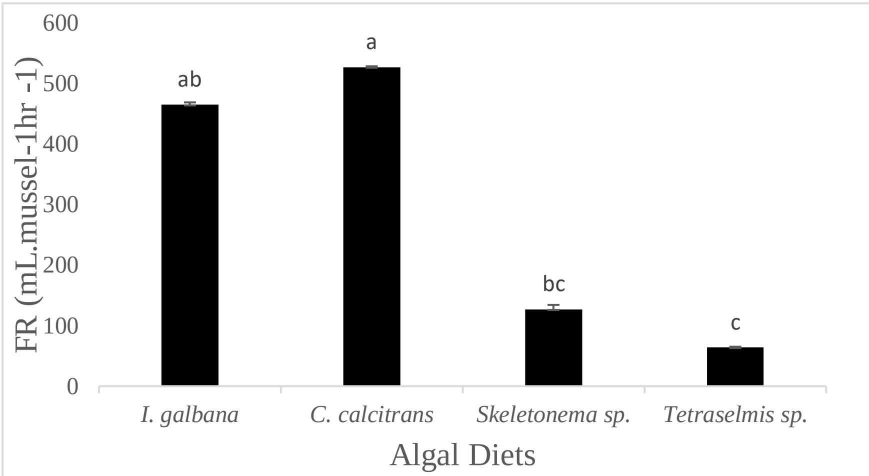

Figure 1. Filtration rate (mL.mussel^{-1} hr^{-1}) of spats fed experimental diets. Values with different superscripts are significantly different.

Other factors such as cell size, cell wall composition and digestibility of algal species may also influence their assimilation by the mussel spats. Furthermore, these factors may affect the feeding behavior which could influence filtration mechanism of the organism and then eventually contributes to their growth difference. This study also examines the filtration rate of spats given with different algal species. Highest filtration rate was observed in spats fed *C. calcitrans*, followed by the groups fed with *I. galbana* (Figure 1). Both treatments were not statistically different since their cell sizes are nearly similar, *C. calcitrans* measures 5-8µm while *I. galbana* is 5-6µm (de la Peña and Franco, 2013). Lower filtration rate was observed in both *S. tropicum* and *T. tetrahele*, both species have bigger cell sizes which ranges from 9-16µm (de la Peña and Franco, 2013). This observation conforms to the work of Bougrier et al. (1997) and Laing (2004) wherein filtration rate of *Tetraselmis sp.* is significantly lower than *C. calcitrans* for *C. gigas* and *P. maximus* juveniles. Morphological structure of the algae may also affect the efficiency of the organism to filter them. Poor filtering efficiency by mussel spats for *T. tetrahele* could be due to its thick theca or cell wall (Epifanio, 1979). *Tetraselmis* was poorly assimilated by *C. virginica* and *M. mercenaria* which results to their inferior growth. While the cells of *Skeletonema* are made up of silica rods and occur in chains (de la Peña and Franco, 2013) which could hinder and affect proper filtration and ingestion of the algae by the mussel spats. In contrast, the cell wall composition of *I. galbana* and *C. calcitrans* were easily digestible (Lora-Vilchis and Maeda-Martinez, 1997; Martinez-Fernandez et al., 2004) which supports the higher growth rate in treatments fed *with C. calcitrans*, followed by groups fed with *I. galbana*. This was further supported by the study of Maquirang et al. (2019) that *C. calcitrans* and *I. galbana* can be ingested and digested more efficiently by the green mussel pediveliger larvae based on the epifluorescence microscopy analysis. It was furthered explained in the study that morphological structure can be a factor in the digestibility of the algae. *I. galbana* have no distinct cell wall, thus it is considered as naked flagellate (Martínez-Fernández et al., 2004). This characteristic makes the algae more efficient to digest and assimilated. Whereas, the distinguishing characteristic of *C. calcitrans* is the presence of long setae or spines. However, the presence of spines did not hamper the filtering and digestion efficiency of the larvae when fed with the algae. The setae or frustule fragments of *C.calcitrans* cells may have facilitated the breakdown of other algal cells which results in better digestion efficiency of the larvae (Ragg et al., 2010) which eventually results in better growth.

CONCLUSION

The determination of the optimum algal diet for the nursery culture of green mussel provides a very important information in developing strategies to enhance their production. The result of present study showed that the ideal algal diet for mussel nursery were *C. calcitrans* and *I. galbana*. However the use of mixture or combination of different algal species can be explored. Combination of different algal species can enhance nutritive profile of the diets that can improve growth and survival of spats in the nursery.

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LITERATURE CITED

Apines-Amar, M.J.S., D.C. Rendaje, L.A.G. Piñosa, L.V.Jr. Laureta, A.N.D. Masculino, F.L. Pedroso, F.F.C. Mero, T.P.V. Capaque and J.F. Cadangin. 2020. Effects of water quality, stocking density, water exchange frequency, and food, on growth and survival of the green mussel, Perna viridis larvae. Isr. J. Aquacult. Bamid., 72: 1-13.

Brown, M.R., S.W. Jeffrey, J.K. Volkman and G.A. Dunstan. 1997. Nutritional properties of microalgae for mariculture. Aquaculture, 151: 315–331.

Brown, M.R. and S.I. Blackburn. 2013. Live microalgae as feeds in aquaculture hatcheries. In Advances in Aquaculture Hatchery Technology (eds G. Allan and G. Burnell). Woodhead Publishing Limited. Cambridge, UK.

Bougrier, S., A.J.S. Hawkins and M. Heral. 1997. Preingestive selection of different microalgal mixtures in *Crassostrea gigas* and *Mytilus edulis*, analyzed by flow cytometry. Aquaculture, 150: 123-134.

Coutteau, P. 1996. Micro-algae. In Manual on the Production and Use of Live Food for Aquaculture (eds P. Lavens and P. Sorgeloos). FAO Fisheries Technical Paper No. 361. FAO, Rome.305 pp.

De la Peña, M.R. and A.V. Franco. 2013. Culture of marine phytoplankton for aquaculture seed production. Aquaculture Extension Manual no. 55. Southeast Asian Fisheries Development Center-Aquaculture Department, Tigbauan, Iloilo, Philippines.

Epifanio C. E. 1979. Growth in bivalve molluscs: nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* (Gmelin) and the hard clam Mercenaria mercenaria (L). Aquaculture 18:1-12.

Esquivel, B.C., and D.V. Lobina. 1994. Growth of *Mytilus galloprovincialis* fed with four microalgae and two feeding regimes. Journal of the World Aquaculture Society 25(3): 471-476.

Fernandez-Reiriz, M.J., A. Perez-Camacho, M.J. Ferreiro, J. Blanco, M. Planas, M.J. Campos and U. Labarta. 1989. Biomass Production and Variation in the Biochemical Profile (Total Protein, Carbohydrates, RNA, Lipids and Fatty Acids) of Seven Species of Marine Microalgae. Aquaculture 83: 17-37.

Fidalgo, J.P. A. Cid, I. Lopez-Munoz, J. Abalde and C. Herrero. 1994. Growth and biochemical profile of juvenile mussels *Mytilus galloprovincialis* fed on different algal diets. J. Shellfish Res., 13: 67-75.

Food and Agriculture Organization of the United Nations (FAO). 2004. Hatchery culture of bivalves a practical manual. FAO, Rome, 175 pp.

Food and Agriculture Organization of the United Nations (FAO). 2016. The state of world fisheries and aquaculture. FAO, Rome, 204 pp.

Gosling, E. M. 2003. Bivalve Molluscs Biology Ecology and Culture. Fishing News Books. Oxford, UK: Blackwell Publishing. pp. 443.

Guillard, R.R.L. 1973. Methods for microflagellates and nannoplankton. In Handbook of Phycological Methods. Culture Methods and Growth Measurements (Stein, J., eds.), 69-85. Cambridge University Press, New York.

Guillard, R.R. and J.H. Ryther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol., 8: 229-239.

Handbook of Phycological Methods. Culture Methods and Growth Measurements. 1973. Cambridge University Press, New York. Handbook of Phycological Methods. Culture Methods and Growth Measurements. J. Stein (ed.), 69-85. Cambridge University Press, New York.

Helm M.M. and N. Bourne. 2004. Hatchery Culture of Bivalves: A Practical Manual. A. Lovatelli (ed.), FAO Fisheries Technical Paper 471. Rome, Italy. 203 pp.

Laing, I. 2004. Filtration of king scallops (*Pecten maximus*). Aquaculture, 240: 369-384.

Laing, I. and C.G. Verdugo. 1991. Nutritional value of spray-dried *Tetraselmis suecica* for juvenile bivalves. Aquaculture, 92: 207-218.

Laing, I. and P.F. Millican. 1986. Relative growth and growth efficiency of *Ostrea edulis* L. spat fed various algal diets. Aquaculture, 54: 245-262.

Laxmilatha, P., G.S. Rao, P. Patnaik, T. Nageshwara Rao, M. Prasad Rao, and B. Dash. 2011. Potential for the hatchery production of spat of the green mussel *Perna viridis* Linnaeus (1758). Aquaculture, 312: 88-94.

Lodeiros, C.J., L. Freites, A. Marquez, M.E. Glem, M. Guevara and P.E. Saucedo. 2016. Comparative growth and survival of spat of the Caribbean pearl oyster, *Pinctada imbricata* cultivated indoor with microalgae diets and outdoor with natural diet. Aquacult. Nutr., 23(3): 511-522.

Lora-Vilchis, M.C. and A.N. Maeda-Martinez. 1997. Ingestion and digestion of Catarina scallop *Argopecten ventricosus-circularis*, Sowerby II, 1842, veliger larvae with ten microalgae species. Aquac. Res., 28: 905-910.

Lora-Vilchis, M.C. and N. Doktor. 2001. Evaluation of seven algal diets for spat of the Pacific Scallop *Argopecten ventricosus*. J. World Aquacult. Soc., 32(2): 228-235.

Maquirang, J.R.H. , F.L. Pedroso, M.J. Apines-Amar, L.A.G. Piñosa, D.C. Rendaje, J.F. Cadangin, F.F.C. and C.C. Baylon. 2019. Ingestion, digestion, growth and survival of green mussel *Perna viridis* pediveliger larvae fed different microalgae. Fish. Sci. doi:10.1007/s12562-019-01367-3.

Marshall, R., S. McKinley and C. Pearce.2010. Effects of nutrition on larval growth and survival in bivalves. Rev. Aquacult. 2: 33-55.

Martinez-Fernandez, E., H. Acosta-Salmon and C. Rangel-Davalos. 2004. Ingestion and digestion of 10 species of microalgae by winged pearl oyster *Pteria sterna* (Gould, 1851) larvae. Aquaculture, 230: 417-423.

Mero, F. F. C., F.L. Pedroso, M.J.S. Apines-Amar, J.F. Cadangin, D.C. Rendaje, C.S. Verde, J.R.H. Maquirang, and L.A.G. Piňosa. 2019. Influence of water management, photoperiod and aeration on growth, survival, and early spat settlement of the hatchery-reared green mussel, *Perna viridis*. Int. Aquat. Res., doi: 10.1007/s40071-019-0226-9..

Napolito, G.E., R.G. Ackman, W.M.N. Ratnayake. 1990. Fatty acid composition of three cultured algal species (*Isochrysis galbana*, *Chetoceros gracilis* and *Chaetoceros calcitrans*) used as food for bivalve larvae. J. World Aquacult. Soc., 21: 122-130.

O'Connor, W.A., J.A. Nell and J.A. Diemar. 1992. The evaluation of twelve algal species as food for juvenile Sydney rock oysters *Saccostrea commercialis* (Iredale & Roughley). Aquaculture, 108: 277-283.

Patil V., T. Kallqvist, E. Olsen, G. Vogt, H. Gislerød. 2007. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquacult. Int., 26: 1–9.

Pestana, D., A. Ostrensky, W.A. Pereira Boeger and M.R. Pie. 2009. The Effect of Temperature and Body Size on Filtration Rates of *Limnoperna fortunei* (Bivalvia, Mytilidae) under Laboratory Conditions. Braz. Arch. Biol. Technol., 52: 135-144.

Philippine Statistics Authority. 2016. Selected Statistics on Agriculture. Philippine Statistics Authority, Quezon City, Philippines. 69 pp.

Ragg, N.L., N. King, E. Watts and J. Morrish. 2010. Optimising the delivery of the key dietary diatom *Chaetoceros calcitrans* to intensively cultured Greenshell™ mussel larvae, *Perna canaliculus*. Aquaculture, 306: 270-280.

Renaud, S.M., L.V. Thinh and D.L. Parry. 1991. The gross composition and fatty acid composition of 18 species of tropical Australia microalgae for possible use in mariculture. Aquaculture, 170: 147-159.

Rivero-Rodriguez, S., A.R. Beaumont and M.C. Lora-Vilchis. 2007. The effect of microalgal diets on growth, biochemical composition, and fatty acid profile of *Crassostrea corteziensis* (Hertlein) juveniles. Aquaculture, 263: 199-210.

Sprung, M. 1984. Physiological energetics of mussel larvae (*Mytilus edulis*). I. Shell growth and biomass. Mar. Ecol. Prog. Ser., 17: 283-293.

Ukeles, R. 1970. Nutritional requirements in shellfish culture. In K. S. Price and D. L. Maurer (ed.). Proceedings of the Conference on Artificial Propagation of Commercially Valuable Shellfish - Oysters. Univ. Delaware, Newark, Del. p. 43-64.

Volkman, K., S.W. Jeffrey, P.D. Nichols, G.I. Rogers and C.D. Garland. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol., 128: 219-240.

Walne, P.R. 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea, Mercenaria and Mytilus*. Fish. Invest. London Ser. II, 5: 1-62.

Wikfors, G.H., G.E. Ferris and B.C. Smith. 1992. The relationship between gross biochemical composition of cultured algal foods and growth of the hard calm, *Mercenaria mercenaria* (L.). Aquaculture, 108: 135-154.