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The Study of Pholas Orientalis's Life Cycle and Its Relationship with the Ecological Parameters in States of Kedah, Perak and Selangor, Malaysia

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Abstract: *The angelwing clams or known locally as 'siput mentarang'; Pholas orientalis, can be one of the potential species for aquaculture industries in Malaysia and hopefully in near future it can be an option for farmers to culture the clams. As most bivalves culture system, the seeds are obtained naturally and collected by farmers on certain time of the year. Thus, knowledge of the clam's life-cycle will be advantageous in aquaculture especially in farm's planning and scheduling. Three locations in Kedah, Perak and Selangor have been selected for this study. The samples were collected periodically using a systematic sampling method starting from March 2015 to April 2016 at each sampling sites. Plankton net was used to collect the eggs, larvae and umbo stage of P. orientalis, while soil samples were collected at depth of about 0.5m using a scoop for juvenile and adult stage analysis, and soil quality. The study showed that the three locations had different seasonal timing of the life-cycle developmental stages of the clams. A combination of abiotic factors such as temperature, salinity, dissolved oxygen and nutrient may contribute to the differences of the developmental stages of P. orientalis within the study areas. It was found that the abundance of food (planktons) contributed greatly to the life cycle timing of the clams.*
Keywords: *angelwing clams, aquaculture, life-cycle, Pholas orientalis*

I. Introduction

Pholas orientalis (Gmelin, 1791) or known locally as 'Siput Mentarang' (Malay) is a marine bivalve characterized by two thin elongated shells hinged on a flexible ligament; which located in the sagittal plane. It is edible and marketed either fresh or dried in Hongkong, Malaysia, Thailand and Philiphines [1] [2] [3] [4] [5] [6]. According to [7], it becomes one of the highly sought bivalves in Central Philippines due to its juicy, sweetly and tenderly meat. In Malaysia, it is commercially important and highly valued food especially among the Malays communities yet its biology is poorly understood [8].

The natural habitat of the clams was closed to the river mouth where the polluted effluents from the industries and houses were discharged directly into the sea [9] The clams were found to burrow in compact muddy sand in intertidal and sublittoral areas to a depth of about 0.3m. Previous study reported that environmental conditions influence the reproductive activities of marine invertebrates, especially bivalves; their gonads may have considerable variations between places and times in terms of years [10] [11] [12] [13] [14]. Another important environmental parameter that could affect the reproduction of the bivalve species is the breeding pattern. It appears to have variation that linked with the differences of the temperature and food supply [15] [16] [17] [18].

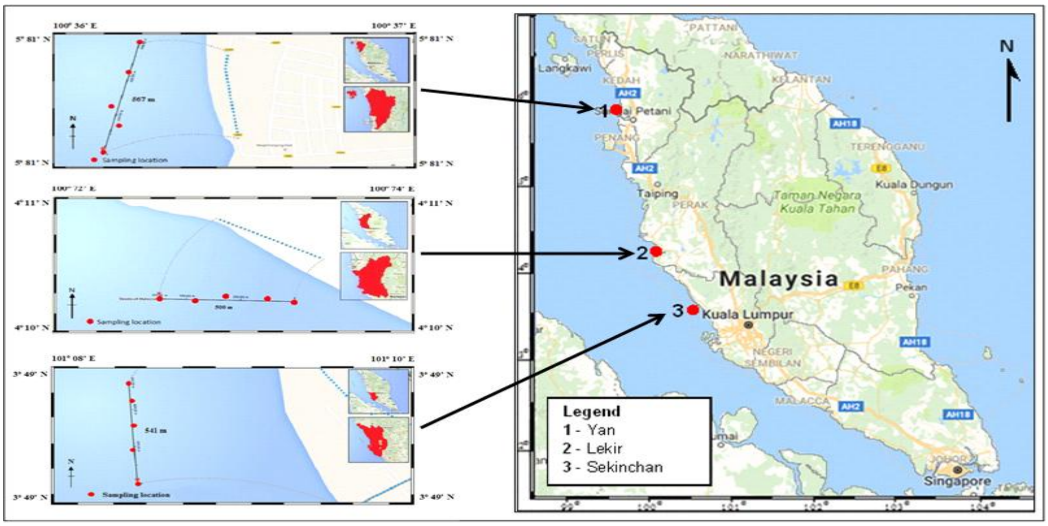
Over fishing of the clams may lead to extinction and currently in Malaysia, it has been hard to find and considered as seasonal species [8]. The only feasible way to overcome this problem is by initiating mass production through aquaculture. Since seeds could be obtained naturally, knowledge on a life cycle of a particular bivalve is important to farmer especially during seeds collection. Farmers need to know when and where to collect the seeds so that they will be able to plan and organize their farming schedule. Other biological information essential in aquaculture will be the size and time of maturity, spawning, growth rate and its distribution. Hence, the study on the life cycle of this species is necessary to obtain in-depth knowledge that may lead to sustainable production of the bivalves. Study of the life cycle of the clams has been conducted in the coastal zone of bays in Perak, Kedah and Selangor which were located at the West Coast of Peninsular Malaysia. The bays area are characterized by muddy and sandy sediments with abundant natural invertebrates.

P. orientalis, can be one of the potential culture species for aquaculture industries in Malaysia and in near future it can be an option for for farmers to cultivate them. Thus, knowledge of the clam's life-cycle will be advantageous in aquaculture especially in planning and scheduling of the farm. The aims of this study are therefore, to determine the life cycle of *P. orientalis* at three locations in Perak, Kedah and Selangor and determining the ecological parameters that may affect their existence.

II. Materials And Methods

2.1 Sampling locations

The study was conducted in three coastal states of Malaysia, namely, Kedah, Perak and Selangor (see Map 1.0). Location in each state such as Yan, Kedah (Lat: 5.81°, Long: 100.36°) and Sekinchan, Selangor (Lat: 3.49°, Long: 101.09°) were selected based on information given by the locals about the existence of the clams whereas in Lekir, Perak (Lat: 4.11°, Long: 100.73°), the clams were newly discovered and published survey was so far absent.



Map 1: Map of study and sampling location in (1) Yan, (2) Lekir and (3) Sekinchan (Adapted from [9])

2.2 Collection and analysis of the sample

Data collection of the clams was done once a month for duration of fourteen months starting from March 2015 to April 2016 at each sampling site. A systematic sampling method was used where it was done at five points in each sampling sites. Soil samples were collected at 0.5 m depth using a scoop and kept in plastic bags. The scooping was done gently to prevent the shell from breaking. The physico-chemical parameters of water such as temperature, salinity, pH, DO and TDS were analyzed *in situ* using multisensory probes YSI Pro Plus. Soil property such as total N (%) and total C (%) were analysed using Leco CNS-2000 automated dry combustion instrument [19], extractable P (ug/g) and K (ug/g) were analysed using Bray-2 and ammonium acetate extraction method respectively [20]. Meanwhile, planktonic animals were sampled using plankton net with the mesh size of 100 µm. Although the purpose is to sample the egg with the sizes of 50 ± 0.24µm, larvae and umbo, the greater mesh size of plankton net was used due to the water condition which was eutrophic. Consecutively, the larger mesh size also had prevented clogging inside the plankton net. The samples collected through plankton net were transferred into the sampling bottle by dipping the net into the bottle containing seawater which was collected at the sampling point. The bottle was then labeled and kept in a storage box before transferred to laboratory for observation and identification of the eggs and microorganism present in water samples.

III. Results And Discussions

The life cycle of the clams generally can be divided into five stages which are egg, larvae, umbo, juvenile and adults [21]. The fertilization of the eggs occurs externally [22] and in the water surrounding the adults [23]. According to [21], about 1 to 2 million of eggs released by a spawner at a time depending on its size. As pointed out by [24], the diameter of the mature eggs is 50 ± 0.24µm and the shape is spherical.

The study showed that the three locations had different seasonal timing the of the life-cycle of *P.orientalis*. During sampling, only three stages were easily identified, which were; eggs, juvenile and adult stage whereas the researchers were unable to identify the larvae and umbo stages. To complete the cycle, studies by [22] and [24] done in captivity were referred and presented in table 1, table 2 and table 3. In Yan and Lekir, eggs released between July 2015 to December 2015 and between March 2015 to August 2015 respectively. In both locations, the eggs developed into juvenile after a month being released. Three months are required for the juvenile to develop into adult stage in these two locations. Meanwhile, in Sekinchan, eggs released between December 2015 to January 2016 and juvenile was found in January 2016. Adult stage was observed after two months of juvenile stage. The complete cycle of the developmental stage of *P. orientalis* in Sekinchan was found to be faster compared to other sites. Data collected showed that, in Sekinchan, it took only four months for the eggs to develop into adult stage. Meanwhile, in Yan and Lekir, it took about five months for the egg to develop into adult stage.

Table 1: The developmental stages of *Pholas orientalis* in Yan, Kedah.

Stages					Month	Year
Adult	Juvenile	Umbo	Larvae	Egg		
					March	2015
					April	
					May	
					June	
		✓	✓	✓	July	
	✓	✓	✓	✓	August	
	✓	✓	✓	✓	September	
	✓	✓	✓	✓	October	
✓	✓	✓	✓	✓	November	
✓	✓	✓	✓	✓	December	

✓	✓				January	2016
✓					February	
✓					March	
					April	
					May	

Table 2: The developmental stages of *Pholas orientalis* in Lekir, Perak.

Stages					**Month**	**Year**
Adult	**Juvenile**	**Umbo**	**Larvae**	**Egg**		
		✓	✓	✓	March	2015
	✓	✓	✓	✓	April	
	✓	✓	✓	✓	May	
	✓	✓	✓	✓	June	
✓	✓	✓	✓	✓	July	
✓	✓				August	
✓					September	
✓					October	
✓					November	
					December	
					January	2016
					February	
					March	
					April	
					May	

Table 3: The developmental stages of *Pholas orientalis* in Sekinchan, Selangor.

Stages					**Month**	**Year**
Adult	**Juvenile**	**Umbo**	**Larvae**	**Egg**		
					March	2015
					April	
					May	
					June	
					July	
					August	
					September	
					October	
					November	
		✓	✓	✓	December	
	✓	✓	✓	✓	January	2016
	✓				February	
✓					March	
✓					April	
✓					May	

Three groups of microorganism consists of zooplankton, phytoplankton and protozoa were observed in water samples from Kedah, Perak and Selangor. Phytoplankton (microalgae) was the largest group being observed with two phylum; dinoflagellate and diatoms (see Table 4) A total number of 38 species of microalgae from 27 genus were identified from water samples. According to [21] the growth of *P. orientalis* in natural habitat was found to accelerate with the present of high primary productivity. In this study, high protein content microalgae; *Chaetoceros* sp., *Skeletonema* sp. and *Isochrysis* sp. were found to be present in all water samples. Previous studies by [25] [26] [27] [28] reported that the growth of juvenile clam was better when fed with centric diatom such as *Skeletonema costatum* and *Chaetoceros sp.*

Figure 1 showed the cell density of microalgae in the study locations. As suggested by [29], *P. orientalis* needs about 15 x 10^4 cells/ml to 29.4 x 10^8 cells/ml of microalgae when cultivated in the laboratory in order to survive. Apparently, in all study locations, the amount of cell density meets the clam's nutritional requirement where Yan recorded 1.9 x 10^6 cells/ml to 2.6 x 10^6 cells/ml with the average of 2.28 x 10^6 cells/ml, Lekir recorded 1.88 x 10^6 cells/ml to 2.38 x 10^6 cells/ml with the average of 2.18 x 10^6 cells/ml and Sekinchan recorded 1.5 x 10^6 cells/ml to 2.13 x 10^6 cells/ml with the average of 1.83 x 10^6 cells/ml.The life cycle timing of the clams in all three locations also occurred in tandem with the abundance of the microalgae cells. In Yan, the spawners released their eggs (between July 2015 to December 2015) during the highest range of cell densities between 2.38 x 10^6 cells/ml to 2.63 x 10^6 cells/ml. Similarly in both Lekir (March 2015 to August 2015) and Sekinchan (December 2015 to January 2016) the eggs were released when the cells density was between 2.0 x 10^6 cells/ml to 2.5 x 10^6 cells/ml and 2.0 x 10^6 cells/ml to 2.13 x 10^6 cells/ml respectively.

Table 4: List of microorganisms (phytoplanton, Zooplankton and protozoa) found in water samples from Kedah, Perak and Selangor.

Selangor	Perak	Kedah	Species	Group
	✓		*Actinocyclus normanii*	**Phytoplankton**
	✓		*Actinoptychus octonarius*	
✓			*Biddulphia capucina*	
		✓	*Ceratium furca*	
	✓		*Ceratoneis closterium*	
		✓	*Chaetoceros affinis*	
✓	✓	✓	*Chaetoceros constrictus*	
		✓	*Chaetoceros densus*	
	✓		*Closterium navicula*	
✓			*Coscinodiscus wailesii*	
		✓	*Dactyliosolen fragilissimus*	
		✓	*Entomoneis ornate*	
		✓	*Guinardia striata*	
✓	✓		*Gyrosigma acuminatum*	**Phytoplankton**
✓	✓	✓	*Isochrysis galbana*	
		✓	*Lauderia annulata*	
✓	✓	✓	*Melosira varians*	
	✓	✓	*Navicula capitatoradiata*	
✓	✓		*Navicula lanceolata*	
	✓	✓	*nitzschia acicularis*	
✓	✓		*Navicula gregaria*	
	✓		*Navicula radiosa*	
✓			*Navicula tripunctata*	
	✓		*Nitzschia dissipata*	
✓			*Nitzschia lorenziana*	
		✓	*Plagiotropis arizonica*	
	✓	✓	*Pleurosigma angulatum*	
		✓	*Prorocentrum micans*	
✓			*Rhizosolenia setigera*	
✓	✓		*Rhoicosphenia abbreviata*	
✓	✓	✓	*Skeletonema costatum*	
	✓		*Skeletonema marinoi*	
	✓		*Stephanodiscus reimeri*	
		✓	*Stephanopyxis nipponica*	
	✓		*Stephanopyxis turris*	
		✓	*Synedra capitata*	
✓	✓		*Trieres chinensis*	
✓			*Ulnaria ulna*	
		✓	*Daphnia sp.*	**Zooplankton**
✓	✓	✓	*Ciliate sp.*	**Protozoa**

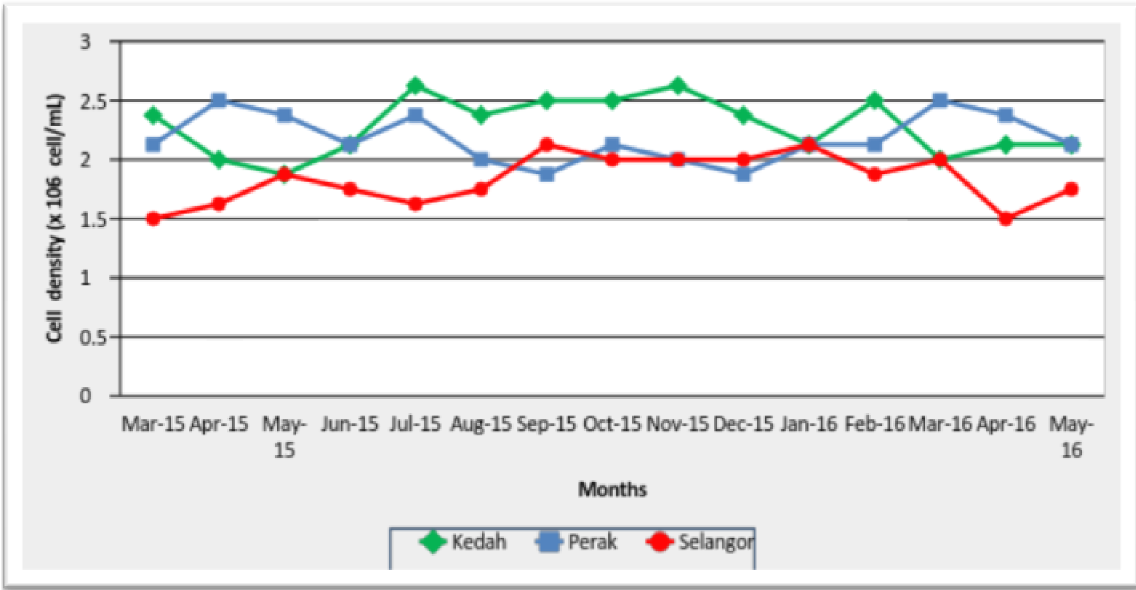


Figure 1: The density of microalgae (x 10^6 cells/mL) in water samples in Kedah, Perak and Selangor with time in months.

The range of physico-chemical parameters and nutrient content of water and soil in study locations are shown in table 5. Nutrient content in soil and water may affect the quality of microalgae. Carbon for example, may enhance the lipid production of microalgae [30]. Nitrogen serves as important element for the growth and formation of protein and nucleic acid in algal cell. Phosphate also important for the growth and metabolism of algae as it is a key component for the phospholipid [31]. Meanwhile, potassium is required for the growth and reproduction of algae. It also helps in photosynthesis process and in build up the cellulose [32]. The differences of these elements level in water and soil content resulting in the differences of microalgae abundance in the study locations. For instance, it can be observed that in Yan, it recorded highest value of C and NPK and consecutively the cells density at the average of 2.83 x 10^6 cells/ml. On the other hand, Lekir recorded higher N and P than Sekinchan, being the latter recorded higher value of C and K. The average cells density thus higher in Lekir (2.18 x 10^6 cells/ml) than in Sekinchan (1.83 x 10^6 cells/ml).

FAO suggested the following physico-chemicals parameters needed for optimum algal growth; salinity:20-24 mg/l, temperature: 16° C -35° C, and pH : 8.2-8.7 [33] and as pointed out by [34], feeding activity of matured *P. orientalis* was found to be at optimum level at temperature of 27°C and salinity 35 ppt. Meanwhile, a better rate of filtration was observed at temperature 28°C [35]. Stressor also can become a factor for slow developmental of *P. orientalis*. Minimization of stressors factors with a combination of quality algae diets led to faster gonadal development and directly faster the maturation of *P. orientalis* [30]. It is shown that the physico-chemicals parameters at the study sites were compatible for the algal growth where the temperature was between 30°C - 34°C, salinity between 20°C - 29°C, and pH between 8 - 8.5. A study by [36] on 108 marine invertebrates including 88 species of molluscs found that they survived well in DO ranges between 4.40 mg/l–4.73 mg/l suggesting the oxygen levels 4.3 mg/l -9.9 mg/l recorded at the study sites were sufficient for the clams.

Table 5: The range of physico-chemical parameters for the natural bed of *P. orientalis* in study sites.

Extractable µg/g		Total %		DO (mg/l)	pH	Salinity (ppt)	Temperature	Location
K	P	N	C					
1166 - 1374	159.6 - 196.7	0.13 - 0.16	1.11 – 1.38	6.02 – 8.66	8.11- 8.25	29.09 - 29.35	32.1 – 33.7	Kedah
475 – 769	102.9 – 113.4	0.05 – 0.11	0.76 - 0.94	4.32 - 9.93	7.76 - 8.48	20.08 - 21.68	30.3 - 34.4	Perak
759 – 841	96.6 – 142.8	0.04 – 0.06	6.14 – 8.11	5.9 - 6.27	7.93 - 8.42	24.71 - 26.23	31.9 - 33.3	Selangor

IV. Conclusion

The abundance of microalgea contributes towards the life cycle timing of the *P. orientalis* along with other abiotic factors such as temperature, salinity, pH, dissolved oxygen and nutrients content. Low nutrient level in water and soil resulted in low algal quality as its affect the biochemical composition of microalgae [37].This led in slower gonadal development and indirectly slower the maturation of *P. orientalis*. The growth rate of *P.orientalis* mainly is affected by the types and quality of microalgae which serve as a food source.

The cells density in all three study sites were found to be sufficient and accommodative for the development of the life cycle of the clams although the quality may be varied resulting different timing and growth rate. Yan, Kedah which was situated at the northern part of the country was more 'fertile' than Lekir, Perak and Sekinchan, Selangor. In Yan, high ranges of C and NPK had resulted the highest average of cells density of 2.83 x 10^6 cells/ml compared to Lekir and Sekinchan having average cells density of 2.18 x 10^6 cells/ml and 1.83 x 10^6 cells/ml respectively. Future study on the lifespan of *P. orientalis* will be a major interest to study. The lifespans has attributes to cellular survival during various stressors which called as 'cell resilience' [38]. Besides, according to [39], the lifespan of bivalve is important to maintain the population as it involve the early and late onset of maturation with semelparous or iteroparous reproduction.

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References

[1] Ablan, G. J., The diwal fishery of Occidental Negros. Philippine, *Journal of Sci*., *66*, 1938, 379-385.

[2] Amornjaruchit, S. (1988). Economically important molluscan shellfish of Thailand. In: Bivalve Mollusc Culture Research in Thailand, *ICLARM Tech Rep* 19. McCoy E. W., Chongpeepien T. (eds), pp 1-18. Dep. Fish. Bangkok, Thailand; ICLARM, Manila, Philippines and Deutsche Ges. Tech. Zusammenarb, GmbH, Eschborn, FRG.

[3] Davidson, A., Seafood of South-East Asia, *Federal Publications, Singapore*, 1976, 366

[4] Saraya, A. (1982). Thailand. In: Bivalve Culture in Asia and the Pacific. Davy, F.B., Graham, M. (Editors). pp73-78. Proc. Workshop held in Singapore on 16-19 February 1982. Int. Dev. Res. Center, Ottawa, Ontario, Canada.

[5] Tokrisna, R., Tugsinavisuitti, S., Kaoian, S., and Kantangkul, P. (1985). Marketing System of Shellfish Products. AFSSRN: Thailand Research Report. Department of Agricultural Economics, Faculty of Economics and Business Administration, Kasetsart University, Bangkok, Thailand.Mussel and Oyster Culture, 359.

[6] Young A., and Serna E. (1982). Philippines. In: Bivalve Culture in Asia and the Pacific. Davy F.B., and Graham M. (Editors), pp55-68. Proc. Workshop held in Singapore on 16-19 February 1982. Int. Dev. Res. Center, Ottawa, Ontario, Canada.

[7] Golez, M.S.M., Laureta L.V., Apines-Amar M.J.S., and Caipang C.M.A., Growth and survival of endangered angelwing clam, *Pholas orientalis* fed different algal diets, *Journal of AES Bioflux*, *3*, 2011, 237-242

[8] Ng, B.W. (2009). Biological aspects and the development of larvae and juvenile of the angelwing clam *Pholas orientalis* (Gmelin, 1791). M.Sc. Thesis. University Sains Malaysia

[9] Ramli, M.F.S. and Mohd Yusop, H., Identifying potential culture areas for Angelwing Clams (*Pholas orientalis*) in Malaysia, *Journal of Agriculture and Veterinary Science*, *Volume 9, Issue 12 Ver. II* , 2016, 50-5

[10] Broom, M. J., Gonad development and spawning in *Anadara granosa* (L.) (Bivalvia: Arcidae), *Aquaculture*, *30*, 1983, 211-219.

[11] Brown, D., Campos, B., and Urban, H. J., Reproduct-ive cycle of the bivalve clams *Semele solida* (Gray, 1828) (Semelidae) and *Gari solida* (Gray 1828) (Psammobiidae) from Chile, *Journal of Shellfish Research*, *21(2)*, 2002, 627-634.

[12] Suwanjarat, J. and Thongboon, L., Reproductive cycle of *Amusium pleuronestes* collected from a fish landing in Songkla, *Songklanakarin Journal of Science and Technology, 19 (4)*, 1997, 405-415.

[13] Drummond, L., Mulcahy, M. and Culloty, S., The reproductive biology of the Manila clam, *Ruditapes philippinarum*, from the North-West of Ireland. *Aquaculture*, *254*, 2005, 326-340.

[14] Tirado, C., Salas, C. and Marquez, I., Reproduction of *Venus verrucosa* L.,1758 (Bivalvia: Veneridae) in the littoral of Malaga (southern Spain), *Fishery Research*, *63*, 2003, 437-445.

[15] Delgado, M. and Camacho, A. P., Histological study of the gonadal development of *Ruditapes decussates* (L.) (Mollusca: Bivalvia) and its relationship with available food, *Science Marine*, *69(1)*, 2005, 87-97.

[16] Laruelle, F., Guillou, J. and Paulet, Y. M., Reproductive pattern of the clams, *Ruditapes decussatus* and *R. phillipinarum* on intertidal flats in Brittany, *Journal of Marine Biology Association of United Kingdom*, *74*, 1994, 351-366.

[17] Rodriguez-Rua, A., Prado, M. A., Romeo, Z. and Bruzon, M., The gametogenic cycle of *Scrobicularia plana* (da costa, 1778) (Mollusca: Bivalvia) in Guadalquivir estuary (Cadiz, SW Spain), *Aquaculture*, *217*, 2003, 157-166

[18] Suwanjarat, J., Pituksalee, C., and Thongchai, S., Reproductive cycle of *Anadara granosa* at Pattani Bay and its relationship with metal concentrations in the sediments, *Songklanakarin Journal of Science Technology*, *31(5)*, 2009, 471-479.

[19] Jones Jr, J. B.,*Laboratory guide for conducting soil tests and plant analysis* (CRC press, 2001)

[20] Kowalenko, C. G., Assessment of Leco CNS-2000 analyzer for simultaneously measuring total carbon, nitrogen, and sulphur in soil, *Communications in Soil Science and Plant Analysis*, *32(13-14)*, 2001, 2065-2078.

[21] Ronquillo, J. D., and Mckinley R. S., Developmental stages and potential mariculture for coastal rehabilitation of endangered Pacific angelwing clam, *Pholas orientalis*, *Aquaculture*, *256*, 2006, 180-191.

[22] Fish, J.D., and Fish, S., *A student's guide to the seashore*. (Second edition. Cambridge: Cambridge University Press, 1996)

[23] George, K., Richard, T., and James, S., *Introduction to Marine Biology* (4th edition. [ISBN 1133364462, 9781133364467], 2012).

[24] Ng, B., Tan, A.S.H., and Yasin, Z., Developmental stages, larval and post-larval growth of angelwing clam *Pholas orientalis, Journal of Aquaculture Resource*, *40*, 2009, 845-851.

[25] Bricelj, V.M., MacQuarrie, S.P., Schaffner, R.A., Differential effects of *Aureococcus anophagefferens* isolates (''brown tide'') in unialgal and mixed suspensions on bivalve feeding, *Marine Biology*, *139*, 2001, 605-616.

[26] Epifanio, C.E., 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), *Journal of Aquaculture*, *18*, 1979, 1-12.

[27] Greenfield, D.I., Lonsdale, D.J., and Cerrato, R.M., Linking phytoplankton community composition with juvenile phase growth in the northern quahog *Mercenaria mercenaria* (L), *Estuaries*, *28*, 2005, 241–251.

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

[29] Laureta, L.V., and Marasigan, E.T., Habitat and reproductive biology of angelwings, *Pholas orientalis* (Gmelin), *Journal of Shellfish Resource*, *19*, 2000, 19-22.

[30] Alam, A., Saleem, U., Sahib, A., Hamid, U.S., Saadia, A., Muhammad, S., Nazish, M., Influence of culture media and carbon sources on biomass productivity and oil content of the algae *Sirogonium sticticum, Temnogyra reflexa, Uronema elongatum,* and *Chroococcus turgidus, Journal of Botany* (Turki), 2015, 39:16.

[31] Harris, G.P., *Phytoplankton Ecology: Structure, Function and Fluctuation* (Chapman and Hall: New York, NY, USA., 1986)

[32] Wetman. (2011). Algae: Limiting nutrients. The Skeptical Aquarist. Available from World Wide Web http://www.skepticalaquarist.com/algae-limiting-nutrients. Accessed on June 2016.

[33] Lavens, P., &Sorgeloos, P., *Manual on the production and use of live food for aquaculture (No. 361)* (Food and Agriculture Organization (FAO), 1996)

[34] Alojepan M.J.L. (1999). Diet feeding patterns of angelwing clam *Pholas* (Monothyra) *orientalis* (Gmelin, 1790). Undergraduate Thesis. Division of Biological Sciences, College of Arts and Sciences, University of the Philippines in the Visayas, Iloilo, Philippines.

[35] Corda, D.R.G. (1998). Algal preference and filtration rate of the angelwing clam *Pholas orientalis* (Gmelin, 1790). Undergraduate Thesis. Division of Biological Sciences, College of Arts and Sciences, University of the Philippines in the Visayas, Iloilo, Philippines.

[36] Ponnusamy, K., Munilkumar, S., Das, S., Verma, A., Venkitesan, R., & Pal, A. K., Shellfish resources around Madras Atomic Power Station Kalpakkam, Southeast India, *Journal of Asia-Pacific Biodiversity*, *10(1)*, 2017, 118-123.

[37] Fernandez-Reiriz, M.J., Perez-Camacho, A., Ferreiro, M.J., Blanco, J., Planas, M., Campos, M.J., and Labarta, U., Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. *Journal of Aquaculture, 83*, 1989, 17-37

[38] Finch, C., Morgan, T., Longo, V., & de Magalhaes, J., Cell resilience in species lifespans: a link to inflammation?, *Aging Cell*, *9(4)*, 2010, 519–526.

[39] Doris A., Thomas B., Eva P., Bivalve models of aging and the determination of molluscan lifespans, *Experimental Gerontology, Elsevier*, *44 (5)*, 2009, 307