

BIOLOGICAL ASPECTS AND THE DEVELOPMENT OF LARVAE AND JUVENILE OF THE ANGELWING CLAM PHOLAS ORIENTALIS (GMELIN, 1791)

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by

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LIST OF ABBREVATIONS

mm	Millimeter
m	Meter
μm	Micrometer
gL⁻¹	gram per liter
mgL⁻¹	Milligram per liter
°C	Degree Celsius
ppt	Part per thousand
%	Percentage
0	Degree
í.	Minute
mL	Milliliter
L	Liter
RM	Ringgit Malaysia
cm	Centimeter
Ν	North
E	East
DO	Dissolved Oxygen
nm	Nanometer
m ²	meter square
FTU	Formazin Turbidity
Ind.m ⁻²	Individual per meter square
NH ³	Ammonia
Temp	Temperature
mМ	millimole

Sal	Sanility
G	Gram
Ν	Number
3	Male
9	Female
Ve	Velum
Ft	Foot
day-1	Per day
m ⁻²	Per meter square
h	Hour
min	Minute
R	right
L	Left
f	Flange
r	Ridge
prov	Proviculum
l.h.s.	Lateral Hinge System
Q	equivalent
Ρ	Posterior
SR	Survival rate
SGR	Specific Growth rate
Nt	Number at the end of experiment
No	Number at the beginning of experiment
%day⁻¹	Percent per day

ASPEK-ASPEK BIOLOGI DAN PERKEMBANGAN LARVA DAN JUVENIL SIPUT MENTARANG *PHOLAS ORIENTALIS* (GMELIN, 1791)

ABSTRAK

Di dalam persekitaran semulajadi di Kuala Kedah, taburan kuantitatif *Pholas orientalis* telah ditentukan dengan menggunakan kuadrat bersaiz $1m^2$. *P. orientalis* didapati mempunyai taburan dan kelimpahan yang tertinggi di kawasan zon bawah intertidal di mana siput ini menggali ke dalam substrat lumpur sedalam 0.3m. Di dalam kajian ini, *P. orientalis* dijumpai pada bulan Julai, Ogos dan September 2006 dan saiznya di dalam julat 6.1 – 13.0cm. Analisis korelasi di antara kualiti air dan taburan *P. orientalis* didapati lemah dan menunjukkan kualiti air bukanlah faktor utama yang mempengaruhi taburan *P. orientalis* di kawasan kajian. Aktiviti manusia dan penerokaan secara berlebihan mungkin merupakan faktor utama yang mempengaruhi taburan dan kelimpahan *P. orientalis* di Kuala Kedah.

Dalam kajian ini juga, *P. orientalis* telah diaruhkan untuk membiak di dalam makmal dan larvanya berjaya dikultur sehingga ke peringkat juvenil. Perkembangan larva hingga ke peringkat juvenil telah dihuraikan. Telur yang telah disenyawakan berkembang sehingga ke peringkat 'straight-hinge' selepas 24 jam persenyawaan. Larva yang diberi makan *Isochrysis galbana* mencapai peringkat umbo (SL: 154.72µm, SH: 149.54 µm) dalam masa 13 hari dan mendap pada substrat sebagai juvenil (SL: 0.22 cm, SH: 0.14 cm) dalam masa 27 hari. Larva *P. orientalis* mencapai kadar tumbesaran purata

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spesifik sebanyak 58.59% cm per hari. Kolerasi antara panjang dan lebar cangkerang bagi larva dan juvenil boleh dikaitkan dengan persamaan linear y = 0.5969x + 77.69, $R^2 = 0.9743$ dan y = 0.2854x - 0.0105, $R^2 = 0.9527$, masing-masing. Penemuan ini telah dibandingkan dengan rekod spesies bivalvia yang lain.

Kesukaran pengecaman larva planktonik *P. orientalis* daripada sampel plankton boleh diselesaikan dengan penggunaan mikroskop cahaya dan mikroskop elektron pengimbas. Kajian ini membekalkan ciri-ciri morfologi dan perubahan morfologi cangkerang *P. orientalis* dengan lebih terperinci. Ciri-ciri morfologi pada larva dan umbo inilah yang memudahkan proses pengecaman untuk mengasingkan larva *P. orientalis* daripada larva bivalvia yang lain.

Tiga spesies mikroalga dan campurannya telah digunakan untuk menguji kesan makanan keatas tumbesaran dan kemandirian junenil *P. Orientalis.* Keputusan menunjukkan campuran *I. galbana* dan *C. gracilis* atau hanya *I. galbana* sahaja sesuai digunakan untuk ternakan juvenil siput mentarang *P. orientalis* di tempat penternakan. *Tetraselmis* sp. adalah diet yang tidak sesuai untuk menternak juvenil *P. orientalis.* Tumbesaran dan kadar kemandirian optimum telah dicapai di dalam diet campuran *I. galbana* and *C. gracilis.* Bagi *Tetraselmis* sp., 100% kematian telah dicatatkan pada minggu kelima. Penemuan ini telah dibandingkan dengan rekod spesies bivalvia yang lain dan implikasi penemuan ini dibincangkan bersama operasi pusat penternakan dan perkembangan kultur *P. orientalis* di Malaysia.

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BIOLOGICAL ASPECTS AND THE DEVELOPMENT OF LARVAE AND JUVENILE OF THE ANGELWING CLAM PHOLAS ORIENTALIS (GMELIN, 1791)

ABSTRACT

In the natural bed of Kuala Kedah, the quantitative distribution of *Pholas orientalis* was estimated (from July 2006 till July 2007) using quadrate of $1m^2$. *P. orientalis* were found most distributed and abundance in the lower intertidal zone, where the clams burrowed in compact muddy substrate to a depth of about 0.3m. The clams were found during July, August and September 2006 and the size of *P. orientalis* in this study ranged from 6.1 to 13.0 cm. The correlation results of the relationship between water quality, abundance and distribution of *P. orientalis* were weak, indicating that water quality is not the major factor affecting the distribution of *P. orientalis* in sampling site.

In this study also, *P. orientalis* was exposed to thermal shock to spawn in the laboratory and the larvae were successfully reared to juvenile stage. Larval development in *P. orientalis* from embryonic to settlement stages was described. The fertilized eggs developed into straight-hinge after 24h of fertilization. The larvae fed on diet of *Isochrysis galbana* reached the umbo stage (SL: 154.72 μ m, SH: 149.54 μ m) within 13 days and settling on a substratum as juvenile (SL: 0.22 cm, SH: 0.14 cm) within 27 days. The larvae showed an average specific growth rate of 58.59 % cm day⁻¹. Shell length and width correlation of larval and juveniles were corresponded to the

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linear equations y = 0.5969x + 77.69, $R^2 = 0.9743$ and y = 0.2854x - 0.0105, $R^2 = 0.9527$, respectively.

The difficulties in the identification of *P. orientalis* planktonic larvae from plankton sample could be resolved by using light microscope and scanning electron microscope. This study provided morphological characteristics and diagnostic features of the larval and umbo which have led to the development of an identification method for separating *P. orientalis* larvae from those of other bivalves larvae occurring in plankton samples.

Three species of microalgae and their combination were used to test the effect of diet on the growth and survival of the *P. orientalis* juveniles. Results showed that mixed diets of *I. galbana* and *C. gracilis* or just *I. galbana* alone were suitable for hatchery rearing of juvenile Angelwing clam *P. orientalis*. *Tetraselmis* sp. as a single diet is not suitable to feed the juveniles of *P. orientalis*. Optimum growth and survival rate were observed in mixed diets of *I. galbana* and *C. gracilis* sp., 100% mortality was recorded on week five. The results were compared to those reported for other bivalve species; and the implication of these findings was discussed in relation to hatchery operations and development of *P. orientalis* culture in Malaysia.

CHAPTER ONE:

GENERAL INTRODUCTION

1.1 Pholadidae in General

Marine bivalves of the family Pholadidea, subfamily Pholadidea (Lamarck, 1809), are found mostly in the tidal flats of Southest Asia. In Malaysia, *Pholas orientalis* (Plate 1.1) is known locally as "Mentarang" (Malay), "Pim" (Thai), "Diwal" (Philippines) or "Angelwing clam" (English). It is commercially important and highly esteemed among the Malay communities due to its juicy and sweetly meat but yet poorly understood. It has a long siphon out of the mud during feeding and in Philippines it is found burrowing in sticky, soft sandy muddy bottoms with the ambient water temperature and salinity ranged between 28-30°C and between 30-35 ppt respectively (Ronquillo and Mckinley, 2006).



Plate 1.1 Pholas orientalis harvested from the wild

Ronquillo and Mckinley (2006) stated that *P. orientalis* is one of the most expensive bivalves in the Philippines's market and its high market demand in Taiwan and Hong Kong. In Malaysia the price of this species varies with their sizes. The biggest size command a higher price (RM6/ kilo) in Perlis and Kedah in the year 2006 but it costs about RM8 to RM9/ kilo in Penang since the late 1990s. Almost the entire angel-wing clams that are available in the Penang's wet market are purchased from the neighboring country (Thailand) or state (Perlis and Kedah).

According to Truman (2006), destruction of the natural habitat, unregulated and over fishing of *P. orientalis* may cause *P. orientalis* to vanish in lloilo City, Philippines's environment and from time to time, it begun to disappear when the prawn industry was rising. The similar phenomenon is also happening in our environment In Malaysia. Almost all *P. orientalis* in Malaysian wet market were catches from wild but since the late 1990s, angel-wing clam have been hard to find and is considered a seasonal species in Penang's wet market and recent survey indicated the presence of this species can only be recorded in Perlis, Kuala Kedah, Kuala Limau, Yan, Penaga, Telok Ayer Tawar and Kuala Selangor (Kosmo, 2007). Apart from these areas, no other site has been identified for the collection of angelwings in the country.

1.2 Taxonomy and morphology

Pholas orientalis (Gmelin, 1791) belongs to the Phylum Mollusca (Plate 1.2). The following outline of the taxonomy illustrates the position of *P.orientalis* within the animal kingdom.

Kingdom	:	Animalia
Phylum	:	Mollusca
Class	:	Bivalvia
Famili	:	Pholadidae
Genus	:	Pholas
Species	:	Pholas orientalis (Gmelin, 1791)



Plate 1.2 External shells of Pholas orientalis

Pholas (Monothyra) *orientalis* (Gmelin 1791) has a long twin bi-system siphon (inhalant and exhalant) and it has a habit of extending its siphon (Plate 1.3) out of the mud during filter feeding. *P. orientalis* was found to burrow in compact mud to a depth of over 30cm (Laureta and Marasigan, 2000; Ronquillo and Mckinley, 2006) and it was found almost in patches. The burrowing tendency is to protect themselves from predators and the adverse effects of the physical environment. Its valves are generally equal in

size, white in color and the shells are very fragile. The body shape of *P. orientalis* is elliptical and elongated with tapering of the posterior end with the shell length from 6cm to 13cm.



Plate 1.3 Inhalant and exhalant siphon of Pholas orientalis

Mature spawners had a creamy colored-gonad. The gonad for male spawners was light beige in color while reddish brown in female. The siphon or bodies (mantle) of female angelwing spawners were light reddish in color while light yellowish for male spawner.

1.3 Factors affecting the distribution of angelwing clam

In general, most of the *P. orientalis* clams that are sold in the wet market are harvested from wild. An early survey by Wan Abdul Aziz (1987) showed that angelwing clam was indigenous to the coastal water of Tanjong Bungah, Penaga, Kuala Kedah and Kuala Perlis, Malaysia. A more recent survey indicated the presence of this species in the coastal area of Penaga, Telok Ayer Tawar, Kuala Kedah, Yan, Kuala Limau, Kuala Perlis and Salak Bernam in Kuala Selangor (Figure 1.1). Apart from these areas, no other sites have been identified for the collection of the angelwing clams in the country.



Figure1.1 Map of Peninsular Malaysia showing the locations where *Pholas orientalis* can be found

Although it is difficult to identify the main factors affecting the distribution of angelwing clams and its population thus based on the high level of correlation among different factors such geomorphology, current, nutrient availability, water quality, sedimentation, human activity and predator may affect the distribution of angelwing clams.

Currently, the researchers in the Philippines are actively studying this endangered species but none in Malaysia. Therefore, it is necessary to study this species before it is vanished in Malaysian water. The main aim of this study is to elucidate and understand this endangered species as it can be one of the potential culture species for Malaysian aquaculture industries and hopefully in longer term *P. orientalis* will be identified as an option for Malaysian Food Industries. In addition, due to its decline in natural abundance, hatchery rearing and grow out may help to conserve this species.

CHAPTER TWO:

SIZE AND LENGTH FREQUENCY DISTRIBUTION AND POPULATION ABUNDANCE OF PHOLAS ORIENTALIS

2.1 Introduction

Pholas orientalis has been reported in Japan, Australia, India, Papua New Guinea, Malaysia and the Philippines (Figure 2.1). They can be found in film mud, clay and shale. In Malaysia, it can be found mainly in mudflat.



Figure 2.1. *Pholas orientalis* occurrences distribution map (modified from Global Biodiversity Information Facility)

Various studies have been carried out on its ecology reproductive biology (Laureta and Marasigan, 2000), development stages (Ronquillo and McKinley, 2006) and induced spawning and development (Wan Abdul Aziz, 1987). However, knowledge on the distribution, abundance and population structure of this clam remains scarce.

In the 1990s to 2000s in Malaysia due to pollution, siltation, unregulated gathering, over fishing and destruction of the natural habitats, the indigenous population of *P. orientalis* is rapidly vanishing in the Malaysian waters. To date, very limited publications on *P. orientalis* was published. There was transplantation and rehabilitation of *P. orientalis* in the Philippines but the effort failed and the reason was unknown and therefore proper distribution, reliable estimates and insight into the population structure and its controlling factors are urgently needed. Only when such basic knowledge is available will its rehabilitation and transplantation make sense.

2.2 Objectives

The objectives of investigation of *P. orientalis* are as below:

- i) To describe the habitat of *P. orientalis*; and
- ii) To describe the changes in abundance, size and length frequency distribution of *P. orientalis* across the study area

2.3 Materials and method

2.3.1 The study area

P. orientalis samples were collected monthly from July 2006 to July 2007 from Kuala Kedah. The study location was in the Straits of Malacca at latitude N 6°05' and longitude E 100°17' on the North of Peninsular Malaysia (Figure 2.2).



Figure 2.2. Map of Kuala Kedah sampling site

2.3.2 Ambient water quality measurement

Environment parameters (dissolved oxygen, salinity, water temperature, turbidity, ammonia concentration, nitrate concentration and

orthophosphate concentration) were measured from the sampling site. The sampling was done once a month during spring tide.

2.3.2.1 Water temperature (°C), Salinity (ppt) and Dissolved oxygen (mgL⁻¹)

Physical parameters of seawater such as seawater temperature, salinity and dissolved oxygen were measured using the following equipment shown in Table 2.1.

Table 2.1 The equipment and unit of physical parameters of seawater measured in the sampling site.

Parameters	Equipment	Unit
Seawater temperature	DO meter YSI 51	°C
Salinity	ATAGO Hand Refractometer	ppt
Dissolved oxygen	DO Meter YSI 51	mgL⁻¹

The sea bottom salinity, temperature and dissolved oxygen of seawater were measured before or after the low tide using the refractometer and DO meter which had been calibrated.

2.3.2.2 Water Nutrient Analysis

The water samples (500mL water sample) were placed into small ice chest filled with ice and brought back to the laboratory for water nutrient analysis (phosphate, ammonia and nitrate concentration). Phosphate, nitrate and ammonia concentrations were measured using HACH Kit Spectrophotometer Model DREL 2000. Phosphate, nitrate and ammonia results are presented in milligram per liter (mgL⁻¹). The methods for nutrient analysis using HACH Kit Spectrophotometer Model DREL 2000 is shown in Appendix 1. The reagents and wavelengths used in the analysis are shown in Table 2.2.

Table 2.2. Reagent and wavelength of nutrient analysis using HACH Kit Spectrophotometer Model DREL 2000.

Parameter mgL ⁻¹	Sensitive range	Wavelengths (nm)	Method	Reagent
Phosphate (PO ₄ -3)	0 – 2.50	890	Ascorbic acid	PhosVer 3
Nitrate (NO ₃ ⁻)	0 – 0.40	507	Cadmium reduction	NitriVer 3 NitraVer 6
Ammonia (NH ₃)	0 - 0.50	665	Salicylate	Ammonia Salicylate ammonia sianurat

2.3.2.3 Turbidity measurement

Turbidity of seawater was measured using HACH Kit Spectrophotometer Model DREL 2000 with the deionized water as the blank at wavelength of 507nm. The procedure for turbidity analysis is shown in Appendix 1.

2.3.2.4 Sediment size analysis

Sediment size analysis was not carried out in this study but it was carried out by Chew (2007) at the same sampling area and also at the same sampling period. Her data will be used in this study to determine the effect of sediment on the distribution of the clams.

2.3.3 Collectiong of sample

During spring tide, the sampling site (100m from the upper tidal zone) was divided into three zones (Zone I, II and III) within the intertidal zone where each zone had the same width (33m).

Zone I was named as upper zone where it was exposed for the longest during low tide, followed by zone II (middle zone) which was 33.33m from zone I and then followed by zone III (lower zone). Lower zone was the zone with the least exposure to sun during low tide.

1m² quadrate was used to sample (Figure 2.3) and three replicates were collected from each zone. *P. orientalis* samples within the sampling quadrate (30 cm in deep) were collected and transferred to the Muka Head Marine Research Station, Universiti Sains Malaysia.

The collected samples were brushed to remove mud and wiped with absorbent cloth to remove water. The wet weight of each sample was measured by using electronic balance to the nearest 0.0001g while length was measured from the anterior part to the posterior part by using caliper (Plate 2.1). Number of samples in each quadrate was recorded. This information would provide the size distribution, population abundance and length-weight correlation.



Figure 2.3. View of sampling location



Plate 2.1. *Pholas orientalis* showing general exterior features and method of shell length measurement.

The length-weight correlation equation for *P. orientalis* was derived and plotted with the aid of statistical package Sigmaplot intent to obtain the population dynamics.

2.4 Results

2.4.1 Habitat of Pholas orientalis

The characteristic of the natural bed of *Pholas orientalis* in Kuala Kedah, Kedah are shown in Table 2.3. *P. orientalis* from Kuala Kedah were found to burrow in mud at the sampling site (upper to lower zone). No specimen was found after the lower zone (zone III). There is only one vegetation (mangrove trees) found in the sampling area. According to Chew (2007), the particles size for the natural bed of *P. orientalis* was about $< 62\mu$ m.

Study site	Habitat type	Substrate type	Vegetation	
Kuala Kedah, Kedah	intertidal	Muddy flat [particles size <62µm (Chew,2007)]	Mangrove	

Table 2.3 Ecological information on the natural bed of *Pholas orientalis* in Kuala Kedah.

2.4.2 Water quality measurement

The trends of physico-chemical parameter (dissolved oxygen, salinity, water temperature, turbidity, ammonia concentration, nitrate concentration and orthophosphate concentration) at the sampling location are shown in Figure 2.4 and Table 2.4.



Figure 2.4. Physico-chemical parameters of the sampling location in Kuala Kedah

	- Month -	Water Quality Parameter						
Year		Physical			Chemical			
		Dissolved oxygen (mgL ⁻¹)	Salinity (ppt)	Temperature (°C)	Turbidity (FTU)	Ammonium (mgL ⁻¹)	Orthophosphate (mgL ⁻¹)	Nitrate (mgL ⁻¹)
	July	5.20 ^d	30.00 ^a	27.00 ^a	50.67 ^{bc}	0.26 ^{fg}	0.31 ^b	0.05 ^c
	August	6.40 ^c	29.00 ^a	30.00 ^a	73.33 ^{bc}	0.40 ^d	0.21 ^{bc}	0.21 ^{bc}
2006	September	8.10 ^b	25.00 ^a	30.00 ^a	294.67 ^a	0.49 ^c	0.21 ^{bc}	0.21 ^{bc}
	October	6.60 ^c	28.00 ^a	29.00 ^a	349.00 ^a	0.95 ^a	0.51 ^a	0.51 ^a
	November	8.70 ^a	28.00 ^a	32.00 ^a	16.00 ^{bc}	0.67 ^b	0.27 ^b	0.27 ^b
	December	4.80 ^{de}	31.00 ^a	27.00 ^a	20.33 ^{bc}	0.24 ^{gh}	0.07 ^c	0.07 ^c
	January	6.80 ^c	30.00 ^a	31.00 ^a	24.33 ^{bc}	0.28 ^{fg}	0.09 ^{bc}	0.09 ^{bc}
	February	6.70 ^c	31.00 ^a	28.00 ^a	15.00 ^{bc}	0.13 ^{ij}	0.07 ^c	0.07 ^c
	March	6.70 ^c	30.00 ^a	35.00 ^a	98.67 ^{bc}	0.30 ^{ef}	0.17 ^{bc}	0.17 ^{bc}
2007	April	4.60 ^e	30.00 ^a	28.00 ^a	22.33 ^{bc}	0.19 ^{hi}	0.09 ^{bc}	0.09 ^{bc}
	May	3.30 ^f	30.00 ^a	29.00 ^a	3.67 ^c	0.13 ^{ij}	0.03 ^c	0.03 ^c
	June	4.70 ^e	30.00 ^a	29.00 ^a	114.33 ^b	0.34 ^e	0.18 ^{bc}	0.18 ^{bc}
	July	4.50 ^e	30.00 ^a	30.00 ^a	3.00 ^c	0.09 ^j	0.02 ^c	0.02 ^c

Table 2.4 Water quality values at the sampling location in Kuala Kedah from July 2006 to July 2007

Mean value that are significantly different (P < 0.05) according to LSD test are indicated by different alphabets.

Dissolved oxygen (DO) in the sampling site showed significant differences between the whole sampling period (P<0.05). The lowest DO was recorded in May 2007 with the DO level of $3.22 \pm 0.21 \text{ mgL}^{-1}$, while the highest DO level ($8.7 \pm 0.14 \text{ mgL}^{-1}$) was observed in November 2006 (Figure 2.4 A).

There were no significant differences (P>0.05) in salinity throughout the sampling period. December 2006 showed the highest salinity (31.00 ± 1.41 ppt) and the lowest salinity was recorded in September 2006 with 25.00 ± 0.00 ppt. The difference between the highest and the lowest was 6.0ppt and the average salinity was 29.42±1.66 ppt (Figure 2.4 B).

Temperature during the sampling period showed no significant differences (P>0.05). The lowest temperature was recorded in January 2006 and December 2006 (both showing temperature of 27.00 \pm 0.00°C). Meanwhile, the highest temperature was 34.8 \pm 1.41°C, recorded in March 2007. The difference was 7.8°C (Figure 2.4 C).

The highest nutrient concentration was observed in October 2006 for ammonia ($0.95 \pm 0.03 \text{ mgL}^{-1}$) and nitrate ($0.75 \pm 0.11 \text{ mgL}^{-1}$) where both of these nutrients showed significant differences (P<0.05) between the sampling period. Ammonia ($0.09 \pm 0.00 \text{ mg/L}$) and nitrate concentration ($0.02 \pm 0.01 \text{ mg/L}$) were the lowest in July 2007 (Figure 2.4 E&G).

Water quality measurement showed that turbidity (Figure 2.4 D) and orthophosphate (Figure 2.4 F) exhibited significant differences (P<0.05) between the months during the sampling period (July 2006 – July 2007). The highest turbidity

level was observed in October 2006 (349.00 ± 215.06 FTU) and the lowest turbidity was recorded in July 2007 (3.00 ± 1.00 FTU). The highest orthophosphate concentration was recorded in August 2006 (7.29 ± 7.77 mgL⁻¹) and July 2007 was the lowest ($0.08 \pm 0.05 \text{ mgL}^{-1}$).

2.4.3 Sediment size

Generally the particle size of the sampling location was found less than 62µm. According to Chew (2007), during the sampling period, most of the sampling months were dominated by mud and clay (Figure 2.5). Sand content was high at the upper, middle and lower zone in July 2006 (upper: 12.92%; middle: 13.88%; lower: 11.27%) and the values increased in December 2006 (upper: 28.59; middle: 43.93%; lower: 17.94%). The mud content in the sampling location followed the same trend which increased from July (upper: 44.67%; middle: 44.7%; lower: 22.71) to December (upper: 51.12%; middle: 37.4%; lower: 48.22%) within three zones. In contrast, the clay content was found decreasing in three sampling zones from July 2006 (upper: 42.41%; middle 41.76%; lower: 66.02%) to December 2006(upper: 20.29%; middle: 18.67%; lower: 33.83).





2.4.4 Size and length frequency distribution and population abundance of *Pholas orientalis*

In this study, the *Pholas orientalis* samples were only found in the sampling area in 4 months namely June, July, August and September. There were 861 individuals found in the sampling site for June 2006; followed by July 2006 with 23 individuals; August 2006 (14 individuals) and September 2006 (3 individuals). After September 2006, no *Pholas orientalis* sample was found after September 2006 until July 2007. The length frequency distribution of subpopulation within a population of *Pholas orientalis* are illustrated in Figure 2.5a and Figure 2.5b.

Most of the shells in the population ranged from the length group of 9.1 - 10.0cm with only a small number of larger and smaller shells observed (x ≤ 3.1 -4.0 cm x ≥ 12.1 -13.0 cm where x = shell length). The patterns of the length group were shifted to the left (from June to September) where the length of the shell was getting smaller.



Figure 2.6 a. Length frequency distribution of Pholas orientalis.



Figure 2.6 b. Length frequency distribution of *Pholas orientalis*.



Figure 2.7. Distribution of individual clams at different zones.