

## Abundance and size distribution of female *Scylla olivacea* in Klong Ngao mangrove swamp, Ranong Province, Thailand

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**Abstract:** The abundance and size distribution of female *Scylla olivacea* were investigated in Klong Ngao mangrove swamp, Ranong Province, Thailand using collapsible traps during the period of October 2003 to September 2004. *Scylla olivacea* formed about 99.76% of the mud crab catch. Both mature and immature female crabs were caught throughout the year. The variation in CPUE of immature females was not correlated with either temperature or salinity whereas the CPUE of mature females was positively correlated with temperature but not salinity. The abundance patterns of mature female with respect to monsoon timings at different sites show that the sites located in landward areas were very different from other sites that located near the Andaman Sea which are end route to migration and aggregation locations for mature female crabs. About 75% of the female catches in 2003–04 consisted of individuals with a carapace width less than that of mature individuals (~9.5 cm). The median size of female crabs caught was 8.25 cm, which is smaller than that for female crabs caught in 1988–89 where the median carapace width was 9.50 cm.

**Keywords:** Abundance; Size distribution; female *Scylla olivacea*; Klong Ngao mangrove swamp, Thailand

### 1. Introduction

Mud crabs of the genus *Scylla* (also known as the mangrove crab) occur from tropical to warm temperate zones in the Pacific and Indian Oceans. They are commonly associated with mangrove swamps and nearby inter-tidal and sub-tidal muddy habitats where they feed predominantly on mollusks and other less mobile invertebrates (KEENAN, 1999; HILL, 1980). Within their inter-tidal habitat mud crabs hide in an extensive burrow system which offers protection from predators (MACINTOSH, 1988). Mud crabs occupy a wide range of habitats ranging from the inter-tidal to the sub-tidal zone. Generally, they prefer to live in the mangroves of estuarine areas. Each stage of the

mud crab, especially the juveniles, seeks shelter in mangrove areas where they are to be found in small creeks and channels under stones, in sea grass beds and even between roots or pneumatophores of mangrove trees (HILL *et al.*, 1982).

The mud crab, *Scylla* spp., represents a valuable component of small scale coastal fisheries in many countries in tropical and subtropical Asia. It is likely that the mud crab population is now facing an increased fishing pressure which targets all size class, from juveniles to adults. These crabs are used in either pond culture for production of mature females for a premium Market or in soft shell mud crab culture (PRIPANAPONG, 1995; CHOLIK, 1999). A decline in mud crab landings and a high percentage of small size classes in mud crab catches have been reported over the last two decade due to the tremendous increase in fishing efficiency and effort and the reduction in mangrove forest habitat (JIRAPUNPIPAT and PRADISSAN, 2005). The main reason for mangrove loss has been wood extraction,

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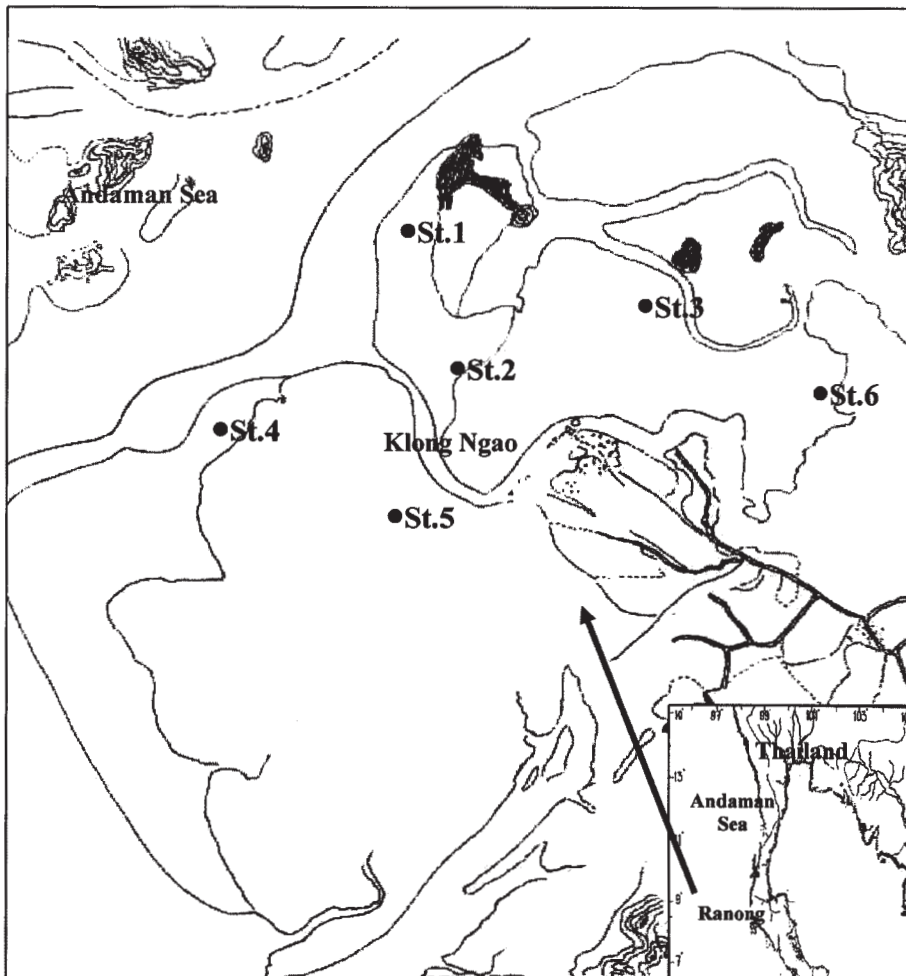


Fig. 1. Study area at Klong Ngao mangrove swamp, Ranong Province, Thailand showing the sampling station 1-6, Inset shows the location of the study area on the west coast of Thailand.

conversion of mangrove areas to agricultural and coastal aquaculture and tin mining. Meanwhile the demand for mud crabs has increased considerably. To conserve the mud crab stock some countries have imposed minimum landing sizes (ROBERTSON and KRUGER, 1994). Furthermore, restoration of mangrove habitat is now widely practiced in the south East Asia (MACINTOSH *et al.*, 2002) but little is known about the recovery potential of mud crabs

Ranong province in the western coast of Thailand is well known as the main area for orange mud crab (*Scylla olivacea*) or black mud crab collection by local fishermen. Klong Ngao is a mangrove-fringed shallow tidal creek

located within the northern part of Ranong where the mud crab fishery is the main income of local fishermen (MOSER, *et al.*, 2005).

The mangrove forests in Ranong Province have been rapidly decreasing in area from 367,900 Ha in 1961 to only 168,682 Ha in 1993, a decrease of 54% (KHEMNARK, 1995), while the total annual catch had declined from 109 tons in 1988-89 to 65 tons in 1994-95 (JIRAPUNPIPAT and PRADISSAN, 2005). Furthermore, feedback from local fishermen that the size and relative abundance (catch per unit of effort) of mud crab have decreased in recent years. Therefore a large area of mangrove forest has been replanted with the aim of bringing the ecosystem

back to its original condition. In addition, the Department of Fisheries, Thailand (DOF) has developed a project for mud crab stock enhancement, with Klong Ngao mangrove forest being selected as a pilot project. The project started in 2001 with the release of  $\sim 200$  million megalopa crab larvae to the mangrove forest. In spite of the comprehensive project, scientific assessment of the recovery project is still insufficient. Thus, little is known about the abundance and size range of mud crab caught after reforestation and stock enhancement in the area. Moreover, it is said that environmental condition of local habitat is important for recovering the mud crab population, while the relationship between the abundance and environmental factors is also unknown. This paper investigates the current state of abundance; include variation in abundance related to the environment factors, size distribution and long-term changes in median size of female mud crabs (*Scylla olivacea*) caught in Klong Ngao mangrove forest. From this quantitative analysis, we will discuss some effectively of the recovery programs for the mud crab stock such as rehabilitation of the mangrove forest and stock enhancement.

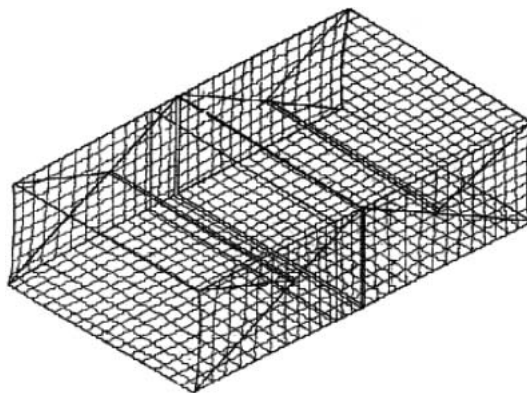


Fig. 2. Collapsible trap for catching the mud crab in Klong Ngao mangrove swamp, Ranong Province, Thailand.

## 2. Materials and methods

### 2.1. Study area and collection of samples

The study was carried out in a mangrove forest called Ngao located in Ranong province. It lies between latitude  $9^{\circ}21'$  to  $10^{\circ}42'$  north and longitude  $98^{\circ}24'$  to  $98^{\circ}56'$  east, and covers an area of approximately 30 square kilometers (Fig. 1). Ngao canal or Klong Ngao as it is called locally, runs from east to west and divides the mangrove forest into two parts. The

Table 1. CPUE (number of crabs per trap) of mature and immature females *Scylla olivacea* by station and month

Site	Month	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
St.1	No. of traps	75	80	85	85	80	85	85	70	73	81	80	72
	Mature	0.040	0.025	0.024	0.012	0.025	0.059	0.071	0.086	0.151	0.037	0.013	0.000
	Immature	0.213	0.025	0.200	0.094	0.150	0.247	0.141	0.671	0.493	0.247	0.038	0.042
St.2	No. of traps	95	105	105	105	76	90	105	105	98	98	98	91
	Mature	0.042	0.019	0.000	0.010	0.000	0.144	0.057	0.105	0.020	0.112	0.041	0.077
	Immature	0.179	0.029	0.038	0.067	0.118	0.122	0.133	0.305	0.316	0.235	0.082	0.099
St.3	No. of traps	75	70	80	80	80	80	80	70	70	85	80	80
	Mature	0.080	0.000	0.000	0.025	0.038	0.025	0.050	0.029	0.100	0.071	0.063	0.038
	Immature	0.120	0.100	0.038	0.100	0.125	0.113	0.138	0.429	0.386	0.212	0.025	0.225
St.4	No. of traps	80	83	85	71	85	85	75	86	86	87	89	88
	Mature	0.075	0.012	0.012	0.014	0.024	0.141	0.107	0.023	0.023	0.126	0.034	0.023
	Immature	0.275	0.120	0.082	0.225	0.306	0.235	0.187	0.523	0.547	0.322	0.124	0.239
St.5	No. of traps	85	85	85	85	85	85	85	85	85	85	85	75
	Mature	0.024	0.012	0.000	0.012	0.024	0.082	0.118	0.071	0.059	0.071	0.035	0.027
	Immature	0.153	0.012	0.047	0.059	0.212	0.094	0.106	0.306	0.282	0.153	0.212	0.080
St.6	No. of traps	60	60	50	50	60	60	60	60	50	50	50	40
	Mature	0.000	0.000	0.000	0.000	0.000	0.050	0.050	0.017	0.020	0.040	0.040	0.000
	Immature	0.233	0.250	0.180	0.280	0.400	0.217	0.317	0.167	0.280	0.160	0.060	0.100

local climate is strongly influenced by two monsoon seasons: the southwest monsoon from May to September and the northeast monsoon from November to February.

Six stations were established in different areas as shown in Fig.1. Sampling was conducted monthly for 12 months during October 2003 to September 2004 using collapsible traps (Fig. 2). Measurements of surface temperature and salinity were also taken at these times. The number of traps used at each station varied between 40–105 traps (Table 1) depending on the weather condition. Fresh trash fish was used as bait in the traps. The standard size of the collapsible traps was 27 cm width; 40 cm length and 12 cm height with two funnel entrances at opposite side. The metal frame of the trap was covered with a strong fishing net of stretched mesh diameter 4 cm. Crabs were collected during spring tides when the water level was high enough to cover the mangrove habitat. Traps were set in the morning at low tide and then collected during the subsequent high tide. At each station the size (external carapace width) and weight of crabs were recorded. Sexes were identified and female crabs from each station were grouped into immature and mature females according to the shape of abdomen, mature crabs as determined by a larger, more rounded abdomen than the immature crab.

## 2.2 Data analysis

### 2.2.1 Abundance and assemblage pattern

At each station, the relative abundance (catch per unit of effort; CPUE) of mature and immature female crabs was estimated in terms of number of crabs caught per trap. Pearson correlation was used to identify the relationship between CPUE of immature and mature female crab and salinity and temperature.

The CPUE of mature and immature females during the inter-monsoon period between the southwest and northeast monsoon (October); the north east monsoon (November to February); the inter-monsoon period between northeast and southwest monsoon (March to April) and southwest monsoon (May to September) were estimated. Assemblage patterns of abundance at different sites were analyzed by cluster analysis. Square Euclidian distances among

sampling stations were calculated and UPGMA (Unweighted Pair-Group Method Using Arithmetic Average) were used to determine clusters.

### 2.2.2 Size distribution and long term change of median size

The recent size distribution of both immature and mature females *S. olivacea* was analyzed using catch data during October 2003–September 2004. In addition, the long-term changes in size distribution and median size were compared by female *S. olivacea* caught in Klong Ngao mangrove swamp for the period of 15 years. The size of female *S. olivacea* data during April 1988 – March 1989 and July 1994 – June 1995 were previously available from Cheewasedtham and Sudthongkong pers. comm. and the data during October 2003–September 2004 was from the present sampling. The crab data during 1988–1989 was collected by crab lift net while the data during 1994–95 and 2003–2004 were collected by collapsible pot. There was no difference in the size of mud crabs caught by the two forms of fishing gear (JIRAPUNIPAT and PRADISSAN, 2005). The SPSS version 10 (Statistical Package for the Social Sciences) was used in all analyses.

## 3. Results

### Abundance and assemblage patterns

The dominant species of mud crab was *Scylla olivacea* which accounted for 99.76% of the total annual mud crab caught by collapsible trap. The female catch of *S. olivacea* was made up of 19% mature and 81% immature crabs (Table 1). Relative abundance was defined as the mean number of *S. olivacea* caught per trap (CPUE). Mean surface water temperature varied from 23 to 32°C while salinity varied from between 23 to 34 ppt. The variation in CPUE of immature females was not correlated with either temperature or salinity whereas the CPUE of mature females was positively correlated with temperature but not salinity (Spearman,  $P=0.03$ ). The CPUE of immature female crabs caught throughout the year was greater than that of mature females. The maximum CPUE of immature crabs was found in May at station 1 with a value of 0.67 crabs per trap but the highest average CPUE for immature crabs was

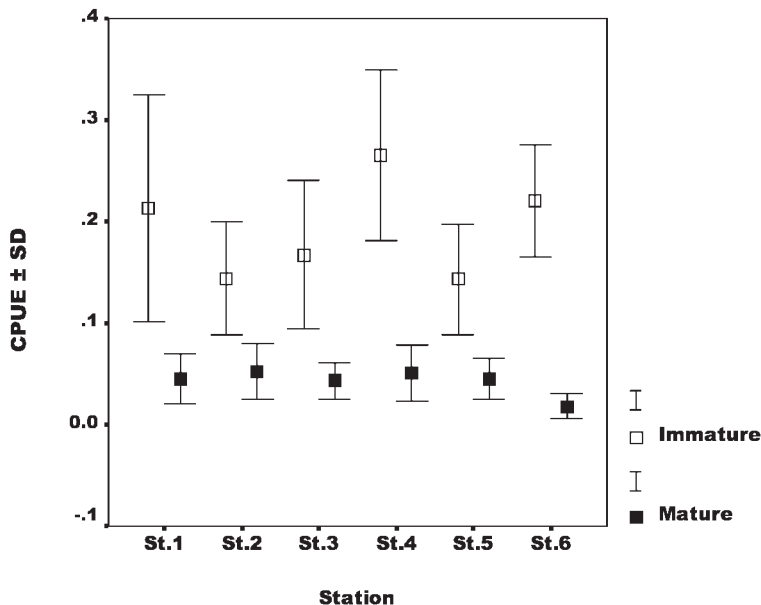


Fig. 3. Mean CPUE (number of crabs per trap) and standard deviation for immature and mature females *Scylla olivacea* by station.

at station 4 (Fig. 3 and Table 1).

Fig. 4A shows the abundance pattern of immature female crabs. The dendrogram reveals three distinct clusters: stations 2, 5, which are near to Klong Ngao and station 3 which was located more landward, appeared in the first cluster while the second cluster comprised stations 1 and 4, (sites close to Andaman Sea). The third cluster included only station 6 which was located in a more landward position that was not connected to the sea. The first two clusters follow the same seasonal pattern with different magnitudes while the third cluster shows a different pattern from the first two clusters (Fig.4B).

The CPUE of mature female crabs varied from 0.01 to 0.15 crabs per trap (Table 1). The monthly CPUE of mature female crabs from each station showed similar trends with slight differences in the duration of maximal values. High CPUE levels were found in June at station 1 and in March at station 2 and 4 with values of 0.15 and 0.14 crabs per trap respectively. The lowest CPUE values were found between September and February for all stations especially station 6 where mature females were not caught during the period October to February

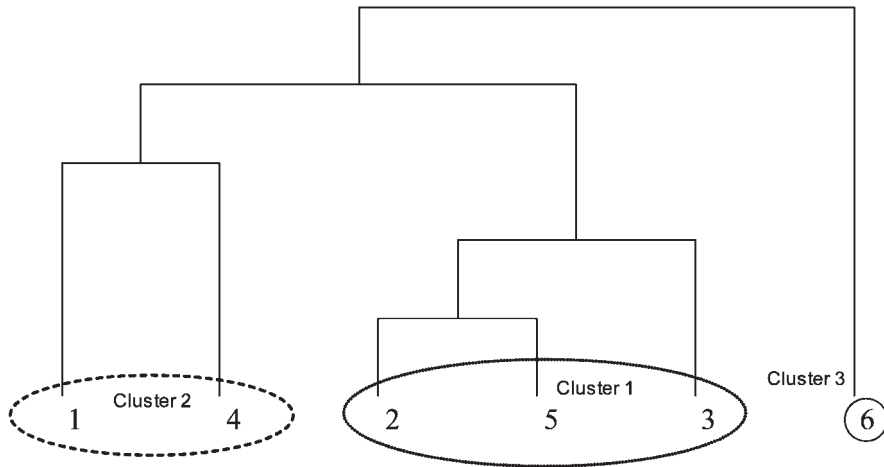
and during September. The average CPUE values for mature female crabs at stations 1 to 5 were greater than that for station 6 (Fig. 3).

The abundance pattern of mature female crab at each site revealed two obvious distinct clusters and another loosely cluster as shown in Fig. 5A. Station 6 which located in the most landward position appeared in the first cluster; station 3 which was intermediate between inner and outer landward positions appeared in the second cluster and the other stations (1, 2, 4 and 5) (located near the Andaman Sea and close to Klong Ngao) are included in the third loosely cluster. Although three clusters are unclear on the dendrogram, seasonal trend of CPUE among these clusters is obviously different (Fig. 5B). The CPUE values of mature female crabs during different monsoon periods showed varying patterns with quite different magnitudes apart from the northeast monsoon period when CPUE values were of similar magnitude.

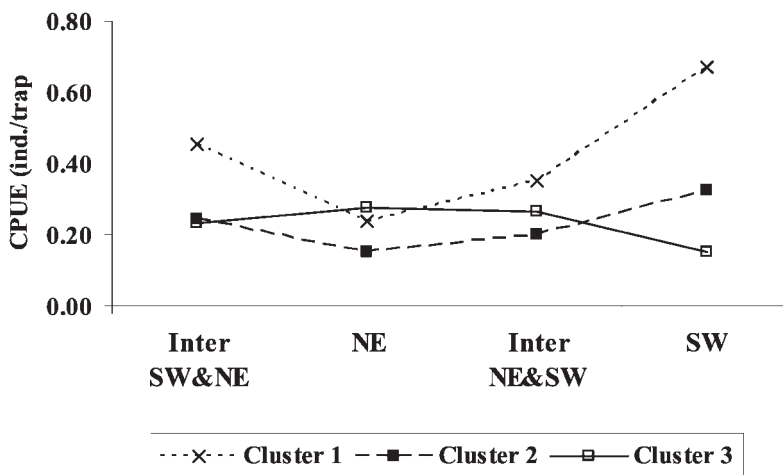
#### *Size distribution and long term change in median size*

The size distribution of immature females ranged from 4.0 to 11.0 cm while mature females ranged from 8.5 to 14.0 cm. Fig.6 shows

### Rescale Distance Cluster Combine



(A)



(B)

Fig. 4. (A) Dendrogram obtained from cluster analysis on abundance of immature female at 6 stations. (B) The abundance of immature female; cluster 1 include station 2, 3 and 5; cluster 2 include station 1 and 4 and cluster 3 include station 6 versus monsoon season (inter SW&NE; inter-monsoon period between southwest and northeast monsoon in October, NE; north east monsoon during November to February, Inter NE&SW; inter-monsoon period between northeast and southwest monsoon during March to April and SW; southwest monsoon during May to September) as derived from cluster analysis.

the size distribution of *Scylla olivacea* over a 15y period during 1988–1989, 1994–1995 and 2003–2004. The size distribution of female crabs caught during 2003–2004 ranged from 4.0 to 14.0 cm with a median size of 8.25 cm. The size distribution of female crabs caught during 1994

–95 varied from 3.5 to 13.0 cm with a median size of 8.0 cm. The sizes of female mud crabs caught during 2003–04 were a little larger when compared to those of female crabs caught in 1994–95. The box plot showed that about 75% of the crabs caught during 1994–95 and 2003–04

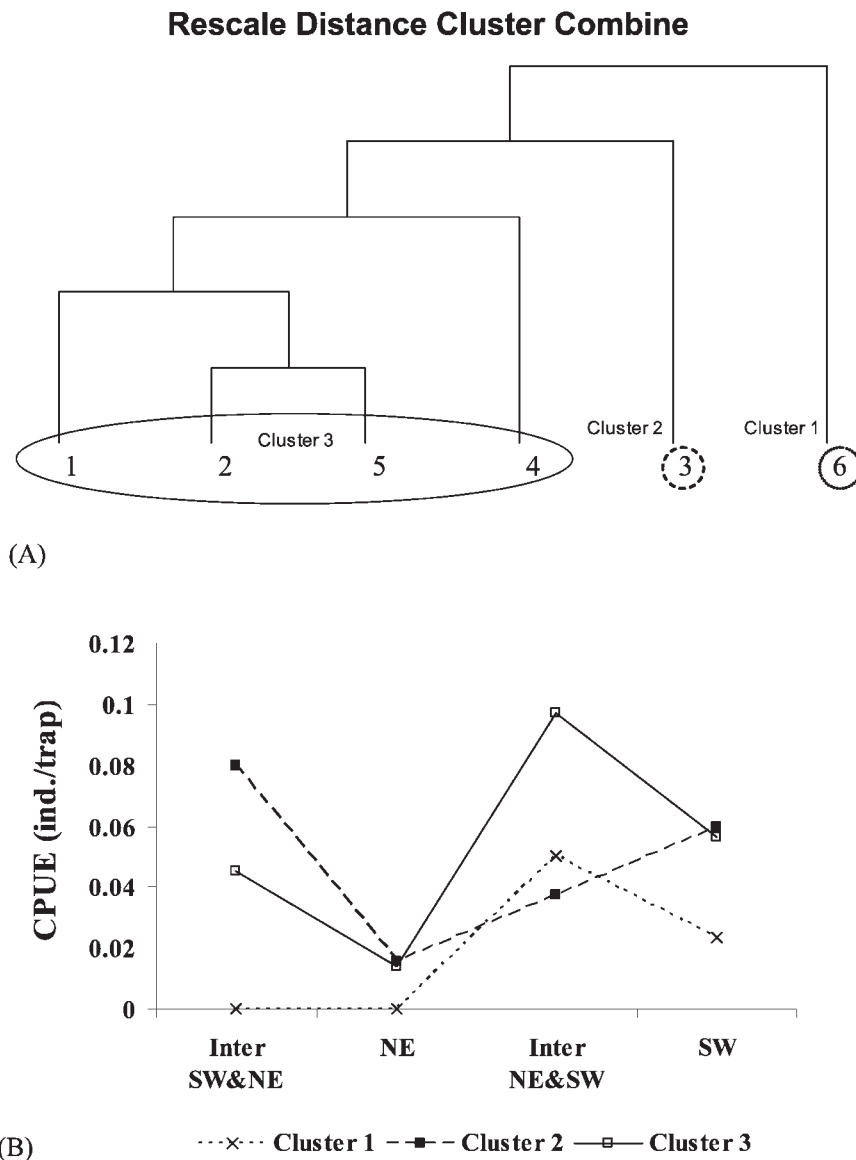


Fig. 5. (A) Dendrogram obtained from cluster analysis on abundance of mature female at 6 stations. (B) The abundance of mature female (cluster 1 include station 6; cluster 2 include station 3 and cluster 3 include station 1, 2, 4 and 5 versus monsoon season (inter SW&NE; inter-monsoon period between southwest and northeast monsoon in October, NE; north east monsoon during November to February, Inter NE&SW; inter-monsoon period between northeast and southwest monsoon during March to April and SW; southwest monsoon during May to September) as derived from cluster analysis.

were small sized with carapace width less than the mean size at 50% first maturity ( $CW_{50}=9.55$  cm, JIRAPUNPIPAT 2008). Few female crabs were observed with a carapace width size greater than 12.0 cm. The size distribution of

female crabs caught during 1988–89 ranged from 6.0–14.5 cm with a median size of 9.5 cm; about half of the female catch over this period was larger in size than the 50% mature individuals (9.55 cm).



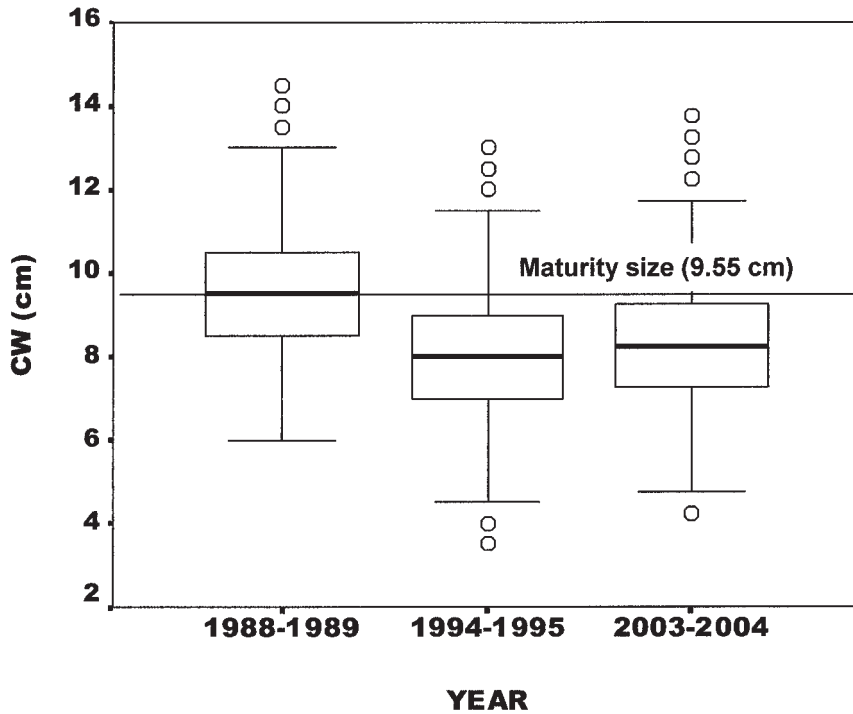


Fig. 6. Box plot of the size distribution of female crabs caught in Klong Ngao (the box lower and upper limits represent the 25th and 75th percentile, the black horizontal line inside the box represents the median and the lower and upper limits of the T-bars represent the 10th and 90th percentile respectively, with values beyond the range represented by open circles). Maturity size is referred from JIRAPUNPIPAT (2008) .

#### 4. Discussion

Since there was no information on abundance of female mud crab in Klong Ngao mangrove swamp before this study, the CPUE value was used as an index of abundance even though it is not representative of the entire population. In this study the collapsible traps were thought to provide the best estimation of CPUE as they are the main fishing gear for catching mud crab in Klong Ngao mangrove swamp. Although size distributions of samples caught by collapsible pots were skewed in other crabs (ARCHDALE and KUWAHARA 2005; ARCHDALE *et al.* 2006a, b), there was no difference in the size of the mud crabs sampled between the traps and another fishing gear (JIRAPUNPIPAT and PRADISSAN, 2005). Moreover, the CPUE by the traps demonstrated seasonal distributions of both immature and mature females are correlated with two environmental factors; monsoon timing and temperature.

The CPUE of mature females was positively correlated with temperature but not salinity. This probably because crabs were captured by means of traps bait with fish which relies on a feeding response by the crabs. Feeding by decapod crustaceans is affected both by environment factors such as temperature and physiological factors such as molt condition (WILLIAMS and HILL, 1980). MILLER (1990) also reported that the catchability of crustaceans often increases with temperature. In contrast, the CPUE of *S. serrata* in Australia was negatively correlated with salinity (24-35 ppt) but positively correlated with temperature (WILLIAMS and HILL 1982). However this is not the case in all examples as MARK *et al.* (2006) found that there was no correlation between CPUE of *S. olivacea* and salinity and temperature in Buswan mangrove, Philippines.

Both immature and mature female crabs were observed throughout the year though no



berried females were caught by collapsible traps. However, the berried female crabs were frequently caught by trawl and push net during October to December at offshore sites from the Klong Ngao mangrove swamp (CHEEWASEDTHAM, pers. obs. and POOVACHIRANON, 1987). Furthermore, HEASMAN *et al.*, (1985) reported that the berried females are less susceptible to conventional fishing methods, e.g., baited trap, as they stop feeding when they migrate offshore. Our data support the conclusion that berried female *S. olivacea* migrates offshore for spawning.

It is notable that few mature females were found during October to February and September in landward areas. This is likely due to the fact that during the period September to December when *S. olivacea* is spawning mature females move from landward sites to areas close to the sea in order to migrate offshore for spawning. This behavior is consistent with the abundance patterns of mature female with respect to monsoon timings at different sites e.g. results from station 6 (Fig. 5. B) were very different from other sites. Stations 1 and 4, located near the Andaman Sea, are end route to migration and aggregation locations for mature female crabs.

The largest size of immature crabs was 11.0 cm while a mature female was 14.0 cm. Similarly, the largest size of immature *S. olivacea* in Ban Don Bay, Thailand was found to be 11.8 cm while the largest size of mature measured 17.1 cm in Ban Don Bay (OVERTON and MACINTOSH, 2002). The size distribution of female *S. olivacea* caught during 2003–2004 ranged from 4.0 to 14.0 cm with the median size being 8.25 cm. The size distributions of female crabs caught during 1994–95 and 2003–2004 were similar although the size range of female crabs caught in 2003–04 was a little larger than those of female crab caught in 1994–95. About 75% of the crabs caught during 1994–95 and 2003–04 were small sized with a carapace width less than the mean size at 50% first maturity ( $CW_{50}$ =9.55 cm, JIRAPUNPIPAT, 2008); a few female crabs were observed with a carapace width > 12.0 cm. In contrast, the size distribution of female crabs caught during 1988–89 was

much larger than those caught during the two subsequent periods. The median size of female crab caught in 1988–89 was 9.5 cm which is close to  $CW_{50}$  while the median size of female crabs caught during 1995–96 and 2003–04 were 8.00 and 8.25 cm, respectively. Such results suggest an increase in the percentage of small crabs and a decrease in the annual median carapace width of crab landings with few female crabs being caught which are larger than 12.0 cm. The data may indicate a decline in the abundance of larger sized mud crabs. The increase in number of smaller crabs may be a consequence of good recruitment, but traps are selective and smaller crabs tend to avoid entering traps when bigger crab are already inside (ARCHDALE *et al.*, 2007). The increase in number of smaller crab may be the result of there being fewer large crabs on the ground, allowing the smaller crabs to enter the traps. Alternatively market conditions may be responsible for this result since there has been a tremendous increase in demand for soft shell mud crab with the fishermen catching smaller sized crabs. However the size of mud crabs caught in 2003–04 was little different from that of crabs caught in 1995–96. This indicates that both rehabilitation of the mangrove forest and stock enhancement may be effective in enhancing the mud crab stock in Klong Ngao mangrove swamp. However, if heavy fishing pressure and the utilization of all size class mud crab still continue then it is inevitable that the mud crab stock will decline. To be effective in restoring fisheries, both habitat rehabilitation and some form of fishery control such as limiting effort or imposing a minimum landing size of mud crab are necessary. One specific recommendation for the recovery is that the minimum size for *Scylla olivacea* capture in Klong Ngao mangrove swamp Ranong province should be 9.5 cm external carapace width (ECW), which was proposed by JIRAPUNPIPAT (2008).

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# Laboratory assessment of the motion behaviour of intertidal gastropods

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**Abstract:** The motion behaviour of three coexisting species of intertidal gastropods, *Bembicium melanostomum*, *Austrocochlea porcata* and *Nerita atramentosa*, was quantitatively studied in the laboratory in the absence of any abiotic and biotic cues. The behavioural properties used to assess movement pathways were the speed of the displacements and two measures of path complexity, the net to gross displacement ratio (NGDR) and the turning angle. *B. melanostomum* displacements were significantly slower than *A. porcata* and *N. atramentosa*, and significantly more complex than those of *N. atramentosa*. These differences cannot be explained by abiotic (e.g. topographic complexity) or biotic factors (e.g. competition for food, space). The movement patterns observed in the laboratory are, however, consistent with the feeding patterns previously reported for these species. The origin of these similarities is discussed in relation to inborn and acquired behaviours. The importance of the differences in the displacements between closely-related species is finally discussed in relation to the niche differentiation process.

**Keywords:** *gastropods, motion behaviour, innate, acquired*

## 1. Introduction

Dispersal behaviour is a key process influencing the structure and function of ecosystems (CHAPMAN, 2000b; MORALES and ELLNER, 2002). Understanding the factors affecting motion behaviour is then important to improve our knowledge of the distributions (STAFFORD and DAVIES, 2005), abundances and dispersion patterns of coexisting species (CHAPMAN, 2000b) as well as community diversity (KERR *et al.*, 2002; DAVIDSON *et al.*, 2004). Most animal movement studies have essentially investigated the role of environmental factors (e.g. changing tidal regime, time of immersion, pneumatophore density, DAVIES *et al.*, 2006; SEURONT *et al.*, 2007; BISHOP *et al.*, 2007) on motion behaviour within different species. Particularly, intraspecific variability has received increasing attention over the last decade (MARSHALL and KEOUGH, 1994; ERLANDSSON

and KOSTYLEV, 1995; CHAPMAN, 1998; PARDO and JOHNSON, 2004, 2006; NG and WILLIAMS, 2006; RAJASEKHARAN and CROWE, 2007). Quantitative comparative studies about intraspecific motion behaviour have been investigated in different habitats (CHAPMAN and UNDERWOOD, 1994; CROWE, 1999; PARDO and JOHNSON, 2006; RAJASEKHARAN and CROWE, 2007) to assess the relative importance of intrinsic characteristics (e.g. sex, body size; PARDO and JOHNSON, 2004) and the flexibility of the behaviour (CHAPMAN, 2000b). Only a few quantitative comparative studies of movement patterns have, however, been done between species (CHAPMAN, 2000a; MICHEL *et al.*, 2007) although interspecific variability can be large even among ecologically similar species (LEVINGS and GARRITY, 1983; CHAPMAN, 2000a).

Intertidal rocky shores are particularly suited to compare closely-related species which exploit the same resource and occupy the same area (CHAPMAN, 2000a). Herbivorous grazing gastropods inhabiting rocky intertidal shores typically compete for resources and space (ESPINOSA *et al.*, 2006). Dispersal and different

movement patterns have been shown to be a short term response to competition (TILMAN, 1994; WILSON *et al.*, 1999; CROWE and UNDERWOOD, 1998; BYERS, 2000; ESPINOSA *et al.*, 2006). However, it is still not well understood whether these differences in motion behaviour are innate or caused by acquired experiences (PYKE, 1984).

In this context, this work investigates the motion behaviour of three ecologically-related species of intertidal gastropods, *Nerita atramentosa*, *Bembicium melanostomum* and *Austrocochlea porcata*, which are found at the same level of intertidal rocky shores in South Australia. More specifically, to improve our understanding of the determinism of the motion behaviour of coexisting species, the aims of this study are (i) to quantify the motion behaviour of three coexistent species observed from continuous measurements at small spatial-scales, (ii) to assess whether interspecific variability in movement pattern is maintained in the absence of any environmental cues, and (iii) to investigate the part of innate and acquired in the motion behaviour.

## 2. Materials and methods

### 2.1. Sampling site and individuals collection

Snails were collected in February 2007 at low tide on a moderately exposed rocky shore situated at Marino Rocks, South Australia (35°02'40"S-138°30'30"E). Individuals were sampled at the mid-level shore on a platform characterized by a simple topography (i.e. flat, smooth rocky substrate with a few shallow pits and grooves). The salinity and temperature at the time of sampling were 35 and ~25 °C respectively. Three species of snails coexisting on this platform were collected, a species found in Australia and New Zealand, *Nerita atramentosa* (REEVE, 1855), and two exclusively Australian species, *Bembicium melanostomum* (GMELIN, 1791) and *Austrocochlea porcata* (ADAMS, 1851). Individuals of each species are herbivorous grazers (EDGAR, 1997) and as such are expected to compete for food and space. Ten individuals were investigated for each species. It was ensured that each individual belonged to the same body size class (15.5 ± 0.9 mm; x ± SE). Prior to the behavioural experiments,

individuals were acclimatized in aquaria under experimental conditions, where temperature = 19 °C and salinity = 38, for 36 h.

### 2.2. Behavioural observations and analysis

Experiments were conducted in an opaque tank (2 × 1.10 m) to avoid the potential bias of phototaxis (PETRAITIS, 1982; HAMILTON and WINTER, 1982), a dim light positioned above the centre of the tank was used to provide a steady light intensity (0.32 μE m<sup>-2</sup> s<sup>-1</sup>). Between each trial, the tank was emptied and washed to remove the mucus of the previous individual, then refilled with seawater of constant salinity and temperature (S = 38, T ~ 19°C) to a depth of 4 cm to completely submerge individuals. Trajectories of each active individual were recorded for one hour using a digital camera (DV Sony DCR-PC120E) placed above the tank. Movements were subsequently plotted onto tracing paper attached to a TV screen by carrying forward the successive positions every 15 seconds. Then, trajectories were computerised in order to quantify the motion behaviour.

Motion behaviour was assessed using the motion speed as well as two indexes of complexity, namely the net-to-gross displacement rate (NGDR) and the turning angle (TA). The speed  $v$  (cm min<sup>-1</sup>) was calculated as  $v = d \times f$  where  $f$  is the frequency of observation (15 seconds) and  $d$  is the displaced distance estimated from the plotted coordinates  $(x_t, y_t)$ ,  $(x_{t+1}, y_{t+1})$ , at time  $t$  and  $t+1$ , respectively, as  $d = [(x_{t+1} - x_t)^2 + (y_{t+1} - y_t)^2]^{1/2}$ . The NGDR (SEURONT *et al.*, 2004b) was calculated as  $NGDR = ND/GD$ , where  $ND$  (Net Displacement) represents the linear distance between the first and the last positions of an individual, and  $GD$  (Gross Displacement) the actual distance travelled and calculated by the sum of the distances of the successive moves (Fig. 1). NGDR gives information about the linearity of a trajectory; higher values of NGDR show a straight displacement, while smaller values indicate the presence of curviness in the path. Finally, the turning angle  $\theta_e$  is defined as the change in direction from one vector of movement to the next (JERDE and VISSCHER, 2005) and calculated as  $\theta_e = 180 - ((180 * \pi) \times \theta)$ , where  $\theta = \arccos(\vec{A} \cdot \vec{B} / \|\vec{A}\| \|\vec{B}\|)$ ,  $\vec{A}$  is the

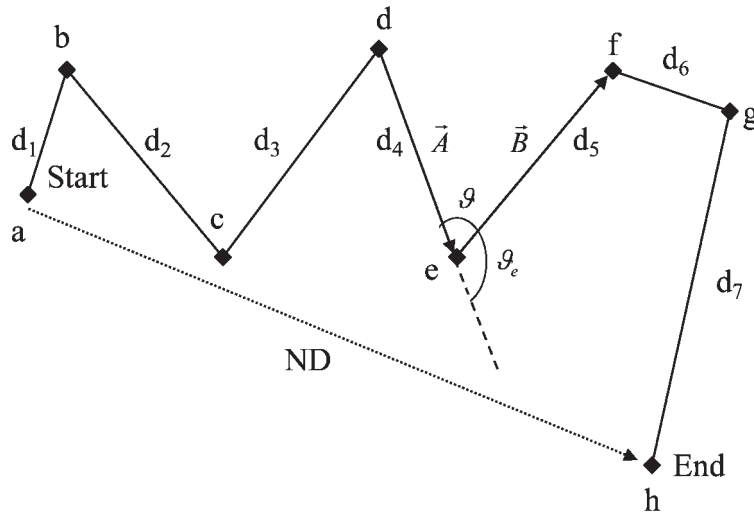


Fig. 1. Schematic illustration of the principles to estimate turning angle and NGDR. The turning angle  $\theta_e$  corresponds to the change in direction between the successive vectors of movement  $\vec{A}$  and  $\vec{B}$ . The NGDR is the ratio between the net displacement ND (i.e. the straight line between the start and the end of the trajectory) and the actual distance GD, the gross displacement (i.e. the sum of the distances  $d_i$ ).

vector between location  $d$  and location  $e$ ,  $\vec{B}$  is the vector between location  $e$  and location  $f$  (Fig. 1). The norms  $\|\vec{A}\|$  and  $\|\vec{B}\|$  are the lengths of the vectors  $\vec{A}$  and  $\vec{B}$  (Fig. 1). Low mean turning angles indicate weaker changes in the direction between successive moves than the high mean turning angles.

### 2.3. Statistical analyses

The distribution of speed and complexity indexes were non-normally distributed, accordingly we used non-parametric statistical analyses. Interspecific comparisons of the parameters were done with the Kruskal-Wallis test followed by a multiple comparison procedure based on the Tukey test to identify distinct groups of measurements.

### 3. Results

A two-dimensional illustration of the typical paths exhibited by the three species of gastropods is given in Fig. 2. Clear dissimilarities in the movement patterns of the three species were apparent. More specifically, at a qualitative level, *B. melanostomum* appeared most dissimilar amongst the 3 species. *B. melanostomum* individuals displacements were tortuous with frequent changes in direction,

resulting in paths typically formed by the presence of small loops and a relatively restricted search area (Fig. 2). In contrast, the motion patterns of *N. atramentosa* and *A. porcata* were similar (Fig. 2), with a larger area typically explored than by *B. melanostomum* over the same time of observation. They travelled rectilinearly with large curves and loops around the whole field of view (Fig. 2).

Statistically significant differences were observed between the three species for speed, NGDR and turning angle (KW test,  $p < 0.05$ ). *B. melanostomum* was significantly ( $p < 0.05$ ) slower than the two others species (Fig. 3a, b), averaging  $3.66 \pm 0.21 \text{ cm min}^{-1}$  ( $\bar{x} \pm \text{SE}$ ). The speed of *N. atramentosa* and *A. porcata* were not significantly different ( $p > 0.05$ ), however, on average *N. atramentosa* ( $8.7 \pm 0.5 \text{ cm min}^{-1}$ ) was faster than *A. porcata* ( $7.8 \pm 0.8 \text{ cm min}^{-1}$ ). The movements of *B. melanostomum* were significantly ( $p < 0.05$ ) less linear and more sinuous (NGDR =  $0.3 \pm 0.1$ , TA =  $26.7 \pm 1.0$  degrees;  $\bar{x} \pm \text{SE}$ ) than *N. atramentosa* (NGDR =  $0.7 \pm 0.1$ , TA =  $17.6 \pm 1.8$  degrees; Fig. 3c). The complexity of *A. porcata* trajectories (NGDR =  $0.4 \pm 0.1$ , TA =  $20.2 \pm 1.3$  degrees) did not differ significantly from those of the two others species.



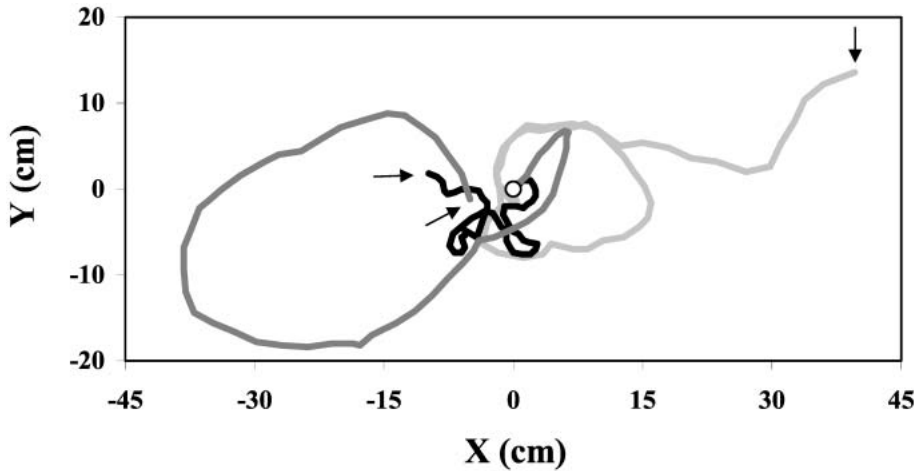


Fig. 2. Motion behaviour of *A. porcata* (dark grey), *N. atramentosa* (light grey) and *B. melanostomum* (black). Time of observation : 14 minutes. The initial and final locations are respectively represented by the white point and the black arrows.

#### 4. Discussion

*Nerita atramentosa*, *Austrocochlea porcata* and a congeneric species of *Bembicium melanostomum* (i.e. *B. nanum*) have also been found to move differently in the field over different periods of time (i.e. two 24 h periods, UNDERWOOD, 1977; 24 h, 1 and 2 weeks; CHAPMAN, 2000a). UNDERWOOD (1977) found that *N. atramentosa* and *A. porcata* moved similar distances but significantly further than *B. nanum*. In contrast, CHAPMAN (2000a) found that over 24 h, *A. porcata* dispersed further than the two others species. These differences have been related to topographic complexity, specific characteristics, interspecific interactions and feeding activities (CHAPMAN, 2000a). These differences could also be related to the different scales of observations, i.e. 48 and 24 h respectively in UNDERWOOD (1977) and CHAPMAN (2000a), as the distance travelled has been acknowledged as a scale-dependent metric (SEURONT *et al.*, 2004a). The patterns observed here cannot be thought as a behavioural response to abiotic factors as the complexity of the topography (CHAPMAN, 2000a) or biotic factors as the feeding (UNDERWOOD, 1977), since the experiments were done in controlled conditions in the absence of any cues after 36 h of starvation.

It is suggested that the observed behaviours

are instead (i) a reminiscence of the previous trophic conditions encountered in the field, i.e. an acquired behaviour and/or (ii) an innate behaviour inherited through natural selection at the evolutionary scale. The differences observed in the motion behaviour of *N. atramentosa*, *A. porcata* and *B. melanostomum* in the laboratory are consistent with their feeding ecology. *N. atramentosa* and *A. constricta*, a congeneric species of *A. porcata*, graze preferentially on microalgae (UNDERWOOD, 1978; QUINN and RYAN, 1989). In contrast, *B. nanum*, a congeneric species of *B. melanostomum* is more suitable to graze on macroalgae (QUINN and RYAN, 1989). *N. atramentosa* and *A. porcata*, and *B. melanostomum* are then expected to have developed foraging extensive and intensive search strategies, respectively. This is consistent with both the highly convoluted paths exhibited by *B. melanostomum*, and the more rectilinear paths of *N. atramentosa* and *A. porcata* that cover larger areas over the same duration (Figs. 2 and 3). This resource partitioning (WILSON and RICHARDS, 2000) constitutes a niche differentiation which favours coexistence (LEIBOLD and MCPHEEK, 2006) between the consumers of microalgae (i.e. *A. porcata* and *N. atramentosa*) and those of macroalgae (*B. melanostomum*).

It is also likely that the motion patterns

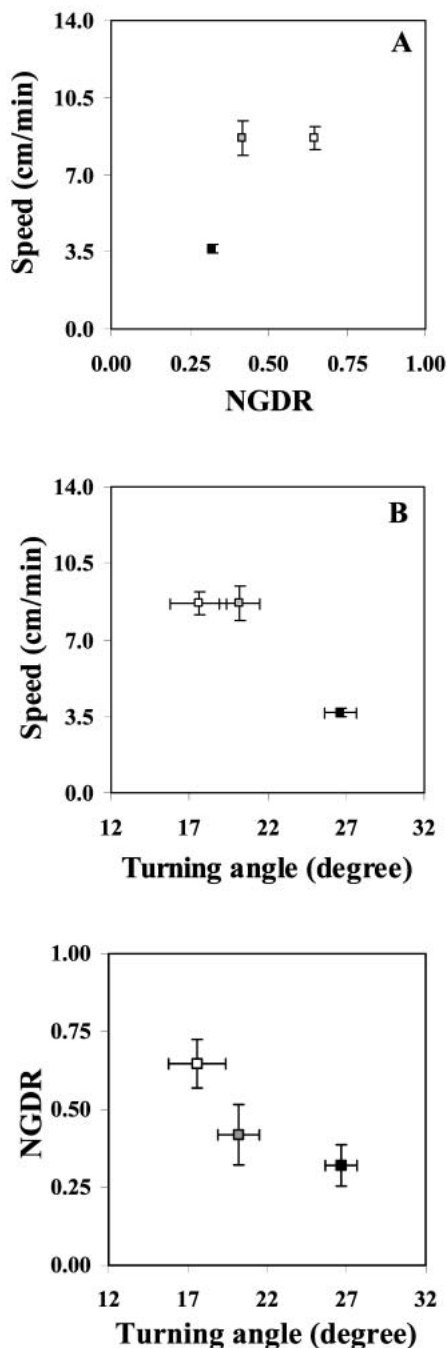


Fig. 3. Fig. 3. Speed as a function of net-to-gross displacement ratios (A) and turning angle (B). Net-to-gross displacement ratios as a function of turning angle (C) observed in *A. porcata* (grey), *N. atramentosa* (white) and *B. melanostomum* (black). Values are means and errors bars are standard errors.

observed in the laboratory are related to the spatial distribution of the preferential resources. Food items, notably microalgae, are heterogeneously distributed throughout the environment (SEURONT and SPILMONT, 2002; KLAASSEN *et al.* 2006). The related optimal foraging strategy would be to move linearly and to travel rapidly over long distances to maximize the chance of encounter with high food densities (HUGUES, 1980; ERLANDSSON and KOSTYLEV, 1995). We observed this typical motion behaviour in *A. porcata* and *N. atramentosa* (Fig. 2) which consume preferentially microalgae. Resource abundance can also influence the movement patterns of consumers. If the food is abundant at one location (e.g. the blade of a macroalgae), the forager will concentrate effort in this area (PYKE, 1984), thus moving tortuously. This is consistent with the area restricted displacements of *B. melanostomum* (Fig. 2). Finally, the differences observed between the motion behaviour of *A. porcata* and *N. atramentosa* may suggest an additional level of niche differentiation through specific behavioural adaptation. While these two species are both microphytobenthos grazers, they might have coevolved to exploit different levels of food patches, thus to minimise resource competition. However, our knowledge of gastropod spatial memory, learning performance and individuals abilities to collect and store information is still poor, and suggests that this area of research is still in its early age.

## 5. Conclusion

Quantitative comparisons of the motion behaviour of coexisting intertidal gastropods are still scarce in the literature. The present work complements the few studies conducted in the field which showed interspecific differences in the motion behaviour in response to abiotic and biotic factors. We found that interspecific differences in movement patterns still exist in three species of intertidal gastropods coexisting on the same rocky shore, even after 36 h of acclimation in the laboratory in the absence of any cues. The motion behaviour observed in the laboratory for *N. atramentosa*, *A. porcata* and *B. melanostomum* is consistent with their

feeding ecology and the recognised spatial properties of their resource. It is then consequently hypothesised that the observed patterns may be the result of acquired and/or innate properties driven by the history of the tested individuals and by natural selection, respectively. A thorough understanding of the determinism of gastropod motion behaviour is, however, still lacking, and the complexities highlighted by these experiments stress the need for further experiments to assess the relative part of innate and acquired in gastropod motion behaviour.

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# Preliminary assessment for age estimation of wild population of mud crab (*Scylla olivacea*) in Pak Phanang Bay, Thailand, using histologically quantified lipofuscin as age marker

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**Abstract:** The age structure of wild mud crab (*Scylla olivacea*), one of the most important mangrove fisheries resource in the Southeast Asian region, was explored using autofluorescent age pigment, lipofuscin. Samples were collected from the mangrove swamp area in Pak Phanang Bay, Thailand. The carapace width-frequency distribution did not show any distinct modes of the sample population. Lipofuscin concentration in the olfactory lobe cell mass (OLCM) of the brain was measured using image analysis of fluorescent micrographs and its concentration showed positive correlation with carapace width. The lipofuscin concentration (% of area fraction) ranged from 0.09 to 0.28 with the formation of three modes. Strong correlation was found between lipofuscin concentration and mode numbers observed in the lipofuscin concentration histogram ( $R^2=0.99$ ) and when modes were considered as distinct ages, the lipofuscin accumulation rate showed almost constant (0.07% of area fraction) in each year. Although, existence of wide size ranged population in a lipofuscin concentration mode, the analysis suggested that *S. olivacea* live in the mangrove ecosystem at best of 2<sup>+</sup> year class.

**Keywords:** Mud crab, lipofuscin quantification, age estimation, mangrove ecosystem

## 1. Introduction

Mud crabs of the genus *Scylla*, commercially important and conspicuous crustacean found in intertidal and subtidal coastal habitats (KEENAN *et al.*, 1998), are traditionally exploited in a number of ways by artisanal fishermen (MACHINTOSH *et al.*, 1993). Mud crabs provide basic source of income for coastal fishing communities throughout the Indo-Pacific region, especially in Thailand (MOSER *et al.*,

2002). However, this important resource is presently vulnerable and proper management is becoming a key issue. Understanding the age structure of wild population of mud crabs is undoubtedly necessary for better stock management. But difficulties in age determination in crustaceans are apparent due to high variability in growth rates and molting frequencies. It is also impossible to use permanent hard body parts as growth indicator, which is frequently used in other animals, because of the crustacean's molting properties. Thus, growth parameters in crustaceans have been traditionally assessed either by tagging and recapture experiments, (MOSER *et al.*, 2002; VAY *et al.*, 2007) or using specimens cultured in captive condition (PLAUT and FISHELSON, 1991; HILL, 1992). Recently, quantitative studies of lipofuscin have encouraged researchers to determine age on the basis of chronological deposition of lipofuscin in neuron cell masses.

Lipofuscin is a lipopigment that is produced in secondary lysosomes as a result of cellular metabolism (DOWSON and HARRIS, 1981). Since

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the formation of lipofuscin is dependent upon metabolism, it should increase in concentration as long as the cell is alive (HARVEY *et al.*, 1999). The universal property of lipofuscin is the emission of yellow to greenish autofluorescence when excited with ultraviolet or blue light (SOHAL and WOLFE, 1986; BRUNK *et al.*, 1992). These characteristics have given the lead to measurements of the autofluorescence and to quantify the amount of lipofuscin accumulated by the cells for application in the determination of ages (DOWSON 1982, MARZABADI *et al.*, 1992). Although lipofuscin are likely to form in all postmitotic cells (SHEEHY, 1989), most cells turnover at different rates, which is difficult to follow over the lifespan of an organism. Nervous tissues are special as they divide and are replaced very slowly in all organisms. Thus, nervous cells can accumulate lipofuscin for relatively longer periods, hence suggesting their usability for measuring age. Pioneering work in the analysis of extractable fluorescent age pigments using spectrofluorometry was done by ETTERS HANK (1983 and 1985), who used lipofuscin for aging crustaceans but was criticized on the spectrofluorometric technique in the following years (NICOL, 1987; HILL and WOMERSLEY, 1991; SHEEHY, 1996). Later, promising results in aging crustaceans were achieved by in situ quantification of lipofuscin granules on histological sections of nervous tissue using fluorescence microscope (SHEEHY, 1989). SHEEHY (1990a) was the first to confirm the widespread occurrence of lipofuscin-like fluorescent material in the brain of crustaceans and also to find a broad correlation between the adult body size of the species and the occurrence of the fluorescence. In fact, morphological lipofuscin has been found to occur in (associated with) the neuron soma in all cell masses of the brain and eyestalks of decapod crustaceans (SHEEHY, 1989 and 1990a; SHEEHY and WICKINS, 1994, SHEEHY *et al.*, 1996), being particularly conspicuous in the globule cell masses associated with the olfactory lobe.

To date, the quantification of lipofuscin method were successfully applied in many studies of wild population of crustaceans (*Cherax cuspidatus*, SHEEHY, 1989; *Notocrangon antarcticus*, BLUHM and BREY, 2001; *Waldeckia*

*obesa*, BLUHM *et al.*, 2001; *Oratosquilla oratoria*, KODAMA *et al.*, 2005) and in captive condition (*Cherax quadricarinatus*, SHEEHY, 1990b and SHEEHY *et al.*, 1994; *Euphausia superba*, NICOL *et al.*, 1991; *Homarus gammarus*, SHEEHY *et al.*, 1996; *Marsupenaeus japonicus*, VILA *et al.*, 2000; Dendrobranchiate shrimps, MEDINA *et al.*, 2000; *Homarus gammarus*, UGLEM *et al.*, 2005).

In genus *Scylla*, although lipofuscin accumulation has been reported in nerve cell masses in the brain (SHEEHY, 1990a), lipofuscin concentration has never been used as an age marker. However, the wide application of this technique on other crustaceans to estimate population age encouraged us to apply histological lipofuscin quantification methods for wild population of mud crabs. The present study was conducted to gain a deeper knowledge in the existence of lipofuscin in mud crabs (*Scylla olivacea*) and to use the lipofuscin quantification technique to assess the age of wild population in the tropical mangrove forest, Pak Phanang, Thailand.

## 2. Materials and methods

### 2.1 Study site

Pak Phanang Bay is located in Nakhon Si Thammarat province in the southeastern part of Thailand, covering an area of 126 km<sup>2</sup>. The eastern side of the bay is largely occupied by mangrove forest (approximately 90 km<sup>2</sup>) and an extensive mudflat (1–3 km wide) emerges at low tide. The present study was conducted within the eastern mangroves that cover 6994 ha, 82% of the total Pak Phanang district mangroves (Fig. 1). THAMPANYA *et al.* (2002) mentioned that there are three distinct seasons; hot-dry season (February–May), rainy season (June–September) and the highest rainfall period of monsoon season (October–January) with water temperature ranges between 25 and 36°C. The average rainfall in this area ranges about 2000–3000 mm and salinity fluctuates between 1–25 ppt (BOROMTHANARATH *et al.*, 1991). Crab fishing is conducted throughout the year within the mangrove channels as well as associated channels connected with the bay.



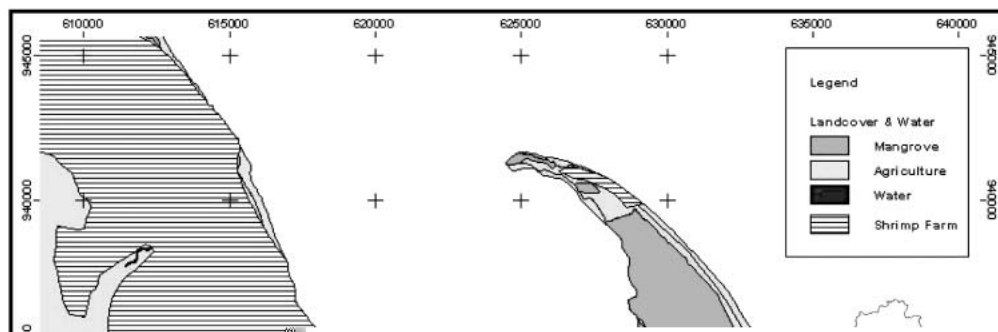


Fig. 1. Study area, Pak Phanang mangrove ecosystem and the sampling place (fishermen settlement) inside of the mangrove.

## 2.2 Samples

Crab trap is the main gear using for mud crab fishery in the Pak Phanang mangrove swamps. Crabs also captured by bare hand and as bi-catch of channel trap that used for shrimp fishing. Samples were collected randomly from the middlemen traders in the mangrove communities on October and November in 2006 and on April and May in 2007. The live crabs were brought back to the laboratory where internal carapace width (ICW: the distance across the carapace between the eight and ninth anterolateral spines) were measured using digital caliper. Crab species were identified from their color and external morphology as suggested by KEENAN *et al.* (1998). Three species (*Scylla olivacea*, *S. paramamosain*, and *S. serrata*) were identified with *S. olivacea* accounting for the highest composition of 46%. The present study on lipofuscin analysis focused on the May 2007 samples where 43% (21 out of 45) were identified as *S. olivacea*. Mud

crab recruitment is year round but since mature females were observed to migrate offshore mostly from June (fishermen's experience), samples from May is expected to contain various age classes. Crab samples were ice-shocked to anaesthetize the animal. The head part (containing the brain) was then dissected out and was fixed in 10% neutral buffered formalin. After 10 days of fixation, the brain was isolated and preserved in 70% ethanol for histological observation.

## 2.3 Identification and quantification of lipofuscin

The brain samples were dehydrated in ascending ethanol concentrations from 70% to 100%, transferred to lemosol and embedded in paraffin. Serial vertical cross sections of the samples were cut at  $5\mu\text{m}$ . For confirmation of the position of the olfactory lobe (OL), some of the histological samples were stained with haematoxylin-eosin and then examined under

the optical microscope. Longitudinal serial sections were prepared from the left side of brain in dorsal view position. All sections were de-waxed through three 10-min xylene changes and mounted without staining. The observed different clusters of cell bodies were numbered according to SANDEMAN *et al.* (1992). The number 10 cell mass (corresponding to olfactory lobe cell mass; OLCM) was used for fluorescent concentration analysis in the present study as it was large and clearly visible.

Fluorescent microscope (Olympus-BX51, Japan) was used to detect autofluorescence of lipofuscin. The histological sections of OLCM at the left side of the brain were excited at a 488 nm excitation wavelength and images were taken with  $40\times$  lenses. A total of 10 central most OLCM digital images were taken from each brain with a resolution of  $512 \times 512$  pixels. The images were edited and quantified lipofuscin concentration using Photoshop CS2 image processing software. The outline of the OLCM in the image was traced manually to select the area of analysis and then maximizing the contrast of lipofuscin by using gray-scale thresholding binary image. We used ImageJ software (National Institute of Health, USA) to measure the area fraction (%) of lipofuscin granule in earlier outlined OLCM area. The geometric average area fraction was calculated from the 5–10 sections of an individual and then used for statistical treatments.

#### 2.4 Modal analysis

The Kolmogorov-Smirnov test (SOKAL and ROHLF, 1995) was conducted to identify any difference in frequency distributions between the sexes. An internal carapace width-frequency distribution (ICFD) was established from the size-data of 121 specimens, using class interval of 5 mm. A lipofuscin concentration-frequency distribution (LFD) was constructed from samples (21 specimens; May 2007) used for the pigment concentration analysis with 0.02% class interval. Potential cluster of samples were identified by plotting ICW against lipofuscin concentration. The observed clusters were further substantiated and the mean values of the peaks were estimated with the Hass elblad's (HASSELBLAD, 1966) methods as

supportive information.

### 3. Results

#### 3.1 Carapace width-frequency

A total of 61 males and 60 females were sampled during the study period and the length frequency distribution is showed in figure 2. Numbers of immature (<94 mm ICW; KOOLKALYA *et al.*, 2006) and mature crabs were 84 (69%) and 37 (31%), respectively for all the samples while in May 2007 samples used in the lipofuscin study contained 12 (57%) and 9 (43%), respectively. The male-female distribution did not differ significantly (Kolmogorov-Smirnov test,  $P > 0.05$ ) in any month.

Though the immature crabs (<94 mm ICW) were noted in each sampling time, small crabs (<70 mm ICW) were noticed to be more abundant in October 2006. In the ICFD, one to three modes were observed but the numbers and position of modes were not consistent over the sam-

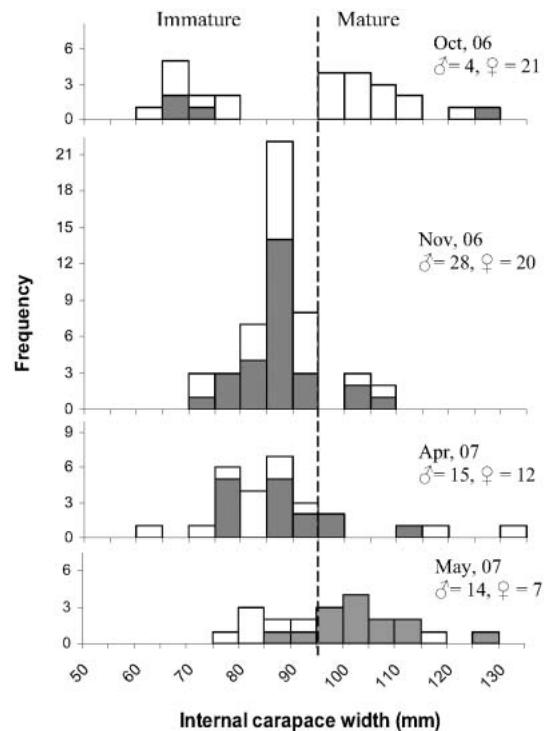


Fig. 2. Size-frequency distributions for samples of *Scylla olivacea* examined from Pak Phanang mangrove ecosystem during October 2006 to May 2007. The dash lines show division between immature and mature crabs.

pling months (Fig. 2). Some modes were not visually obvious.

### 3.2 Morphology of OLCM and lipofuscin

The olfactory lobes are clearly delineated as spheres lying on each side of the brain (Fig. 3A). The OLCM lies posterior to ventral of olfactory lobe in the brain of *S. olivacea*. These neuron groups are easily distinguishable from other neuronal aggregates because they consist of crescent-shaped, compact clusters of small-sized globule cells (Fig. 3B). Lipofuscin was identified by its bright yellow autofluorescence and by its round or irregular granular shape, usually  $=2\ \mu\text{m}$  in diameter, which sometimes formed in aggregates of several granules (Fig. 4A & B). In the present study, although we found different clusters of cell bodies in the

crab brain, OLCM was selected for the lipofuscin study because of its relatively large size and clear indication of position.

### 3.3 Lipofuscin concentration

There was no significant difference between the sexes in the lipofuscin concentration frequency distribution (Kolmogorov-Smirnov test,  $P > 0.05$ ), hence sexes were not treated separately in further analysis. Lipofuscin concentrations varied between 0.09 and 0.28% area fraction. Lipofuscin concentration progressively increased with the increase in ICW and the relation could be linearly regressed ( $L = 0.0024\ \text{ICW} - 0.07$ ;  $R^2 = 0.38$ ,  $P < 0.05$ ) that showed three clusters in the sample population (Fig. 5). Adjacent lipofuscin groups did not overlap largely. Each cluster was numbered in

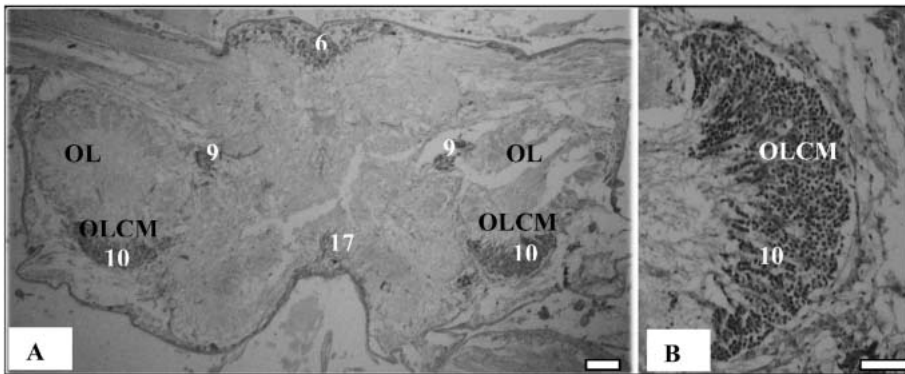


Fig. 3. A horizontal section through the brain of *Scylla olivacea*. A, The olfactory lobe (OL) lying in each side of the brain and the olfactory lobe cell mass (OLCM); scale bar =  $200\ \mu\text{m}$ . B, The close view of the OLCM; scale bar =  $100\ \mu\text{m}$ . The different clusters of cell bodies were numbered according to the SANDEMAN *et al.*, 1992.

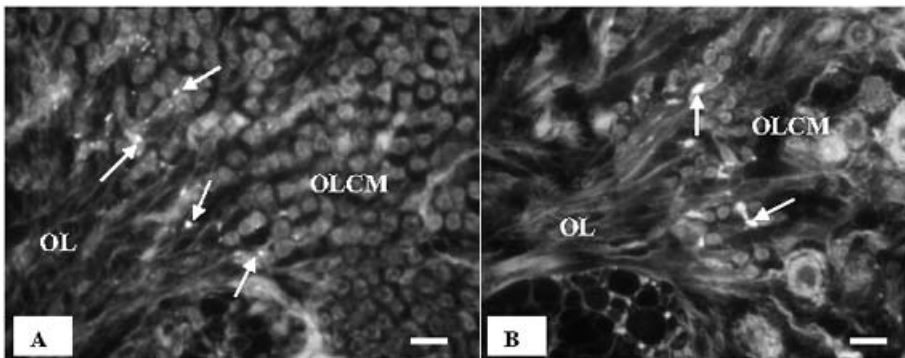


Fig. 4. The accumulated fluorescent lipofuscin granules (some arrowed) in the olfactory lobe cell mass of *Scylla olivacea*. A, Common granule type lipofuscin and B, aggregated lipofuscin granules. Scale bar =  $10\ \mu\text{m}$ .

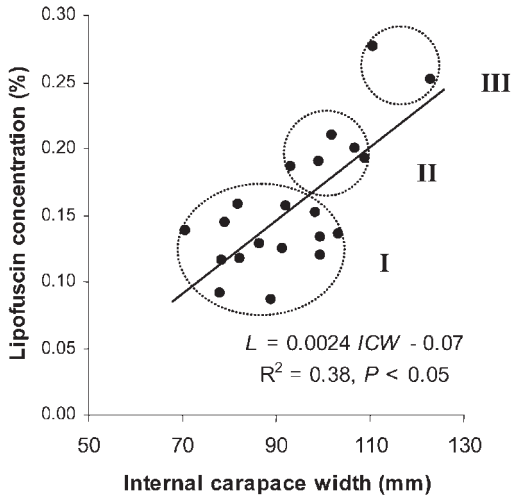


Fig. 5. Scatter plot of lipofuscin concentration against internal carapace width of *Scylla olivacea* collected from Pak Phanang mangrove ecosystem, Thailand, during May 2007. The linear relationship of lipofuscin concentration with growth and possible clusters (dotted circle) of population regarding lipofuscin accumulation.

ascending order as Mode M (M = I, II, III). The mean values of the three peaks I, II, and III in the lipofuscin concentration distribution were  $0.14 \pm 0.02$ ,  $0.21 \pm 0.01$ ,  $0.28 \pm 0.01$  respectively (Fig. 6). The relationship between lipofuscin concentration  $L$  and mode numbers  $M$  is shown in figure 7 and the linear regression equation defining the relationship is  $L = 0.07 M + 0.07$  ( $R^2 = 0.99, P < 0.05$ ). Despite a positive correlation between ICW and lipofuscin concentration (correlation coefficient  $r = 0.72; P < 0.05$ ), there was a considerable dispersion of ICW within each lipofuscin groups. Several of the lipofuscin groups were noticed in each size class (Fig. 8).

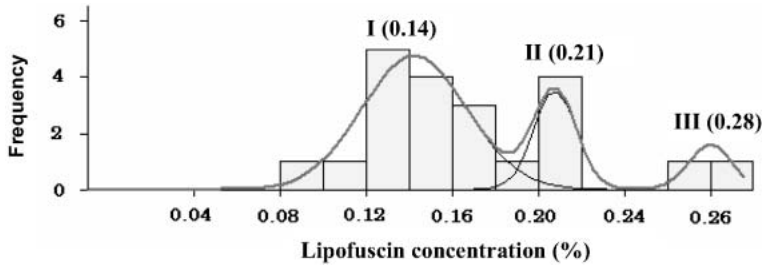


Fig. 6. Frequency distribution of the lipofuscin concentration of *Scylla olivacea* collected from Pak Phanang mangrove ecosystem, Thailand, in May 2007. The potential mean values in parenthesis of each peak estimated by Hasselblad's method.

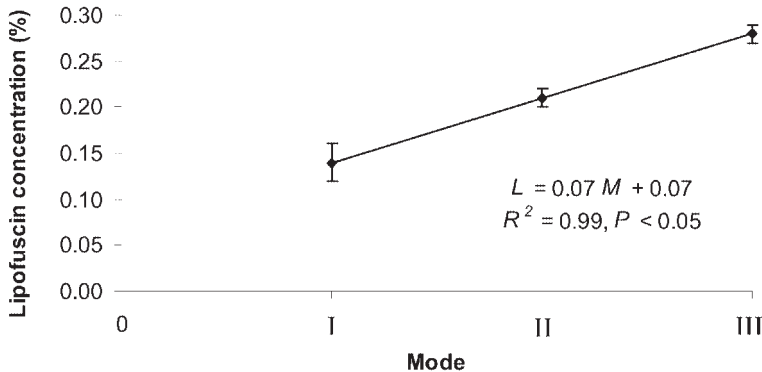


Fig. 7. Relationship between lipofuscin concentration and number of modes of lipofuscin concentrations of *Scylla olivacea* collected from Pak Phanang Bay, Thailand. Vertical bars show 95% confidence limits.

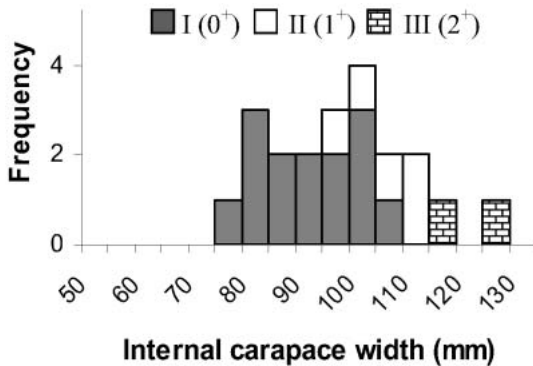


Fig. 8. The internal carapace width-frequency distribution of *Scylla olivacea* samples during May 2007. The modes developed by the lipofuscin analysis are applied to the ICW histogram. Presumable age groups are also shown in parentheses.

#### 4. Discussion

No clear continuous mode groups can be differentiated from the carapace width-frequency analysis to estimate the wild population age (Fig. 2). There is no continuity of modes and hence age determination of *S. olivacea* is difficult. This discontinuity may be caused by the different growth rates of individuals in a cohort from fast growing to slow growing individuals (MOSER *et al.*, 2002). A cohort starting in a small size class will have some individuals reaching the largest size class very quickly, while the majority still remains in the medium or lower size classes. Moreover, in general, the increment of carapace width of crustaceans varies between individuals under-going the same molt (HARTNOLL and ABELE, 1982) and the interval between successive molts becomes longer as age increases, particularly after sexual maturity (ABE, 1982; KODAMA *et al.*, 2005). The size range among all male and female *S. olivacea* recorded in the study was larger (70–123 mm ICW), particularly, when compared with the result by MOSER *et al.* (2005) from the Ranong province, Thailand. In the present study, samples were taken from commercial middlemen, hence the smaller crabs less than the commercial size (50 mm, 4–5 months aged; MOSER *et al.*, 2005) were not included in the present analysis.

The position of olfactory and the neuron cell

masses of *S. olivacea* were similar to those reported in *S. serrata* (SANDEMAN *et al.*, 1992). The morphology of the OLCM and globuli cells of *S. olivacea* is also similar with other brachyuran (SANDEMAN *et al.*, 1992 and 1993). Lipofuscin granules in *S. olivacea* were not greater in numbers or light intensity compared with other species (*Homarus gammarus*, SHEEHY *et al.*, 1996; *Marsupenaeus japonicus*, VILA *et al.*, 2000; *Oratosquilla oratoria*, KODAMA *et al.*, 2005). However, dense aggregations of lipofuscin granules (Fig. 4B) were often found similar to the species of *Birgus latro* (SHEEHY, 1990a).

Although microscope-based quantification of lipofuscin is rather time-consuming and labor intensive (SHEEHY, 1990b and 1996; VILA *et al.*, 2000; BLUHM and BREY, 2001), this method can give very precise measurements (SHEEHY, 2002) than other biochemical extractable lipofuscin quantification (ETTERSHANK, 1985) for crustacean age determination. SHEEHY (1996) reported that there was no relationship between intensity of autofluorescence of extracted lipofuscin and in situ lipofuscin concentration based on microscope observation of same tissue. He concluded that the extraction of lipofuscin was not stable and recommended to avoid quantification by chemical extraction. Moreover, SHEEHY *et al.* (1998) and KODAMA *et al.* (2006) verified the ageing technique of wild crustacean population using lipofuscin quantification by microscopic method. This encouraged us to apply the same procedure for wild mud crab population for the first time.

Lipofuscin accumulates in postmitotic nerve cells where cellular metabolic activity is high (SHEEHY, 1989). There were some specific nerve cell mass in different parts of the crustacean species that have been used for the purpose of histological lipofuscin quantification such as OLCM (SHEEHY, 1989; SHEEHY and WICKINS, 1994; VILA *et al.*, 2000; BLUHM and BREY, 2001), protocerebral bridge cell mass (KODAMA *et al.*, 2005) and eyestalk ganglia (SHEEHY *et al.*, 1996). In decapod species, the lipofuscin concentration is especially dense in the OLCM (SHEEHY, 1989; SHEEHY and WICKINS, 1994; VILA *et al.*, 2000; BLUHM and BREY, 2001). In the present study, we also noticed the high



concentration of lipofuscin granules in the OLCM of the brain of *S. olivacea*. We, therefore, used the OLCM for the lipofuscin quantification and hence to confirm its usefulness in age determination of *S. olivacea*.

In the present study, we could not find any difference in accumulation of lipofuscin between sexes. In other studies, differences were not found between male and female in the aspect of lipofuscin accumulation with growing age in other crustaceans like *Marsupenaeus japonicus* (VILA *et al.*, 2000), *Homarus gammarus* (SHEEHY *et al.*, 1996; UGLEM *et al.*, 2005); *Cherax quadricarinatus* (SHEEHY, 1992) and *Oratosquilla oratoria* (KODAMA *et al.*, 2005). Thus, the combination of data from both sexes was used for analysis in this study.

We observed a linear relationship between size and lipofuscin concentration in the samples in May 2007 (Fig. 5). From this, we can conclude that lipofuscin concentration increases with growth of *S. olivacea* as in other species (SHEEHY, 1990b; SHEEHY *et al.*, 1998; KODAMA *et al.*, 2005). When we compared the distribution in size and lipofuscin concentration of the samples, obvious breaks existed in lipofuscin concentration, which were not found in the size distribution (Fig. 5). In the regression analysis between order of peaks and mode numbers (Fig. 7), a higher regression coefficient was observed indicating that the peaks have the same interval with the lipofuscin accumulation period. Those results strongly support that the order of peaks indicating the order of age as shown in previous studies in wild population of other crustaceans (SHEEHY *et al.*, 1998; BLUHM and BREY, 2001; KODAMA *et al.* 2005) and show the applicability of microscopic quantification of lipofuscin as a tool for cohort analysis and age determination.

Despite the continuous year round recruitment of *S. olivacea* (MOSER *et al.*, 2002 and 2005) in Thailand, there were some periodic peaks (MOSER and MACHINTOSH, 2001). They noticed periodic portunid larvae recruitment during dry to wet (October-November) and wet to dry (March to April) seasons in Klong Ngao mangrove, Ranong province. The present study area showed different seasonal pattern, dry season in February-May, rainy season

starting in June, and heavy monsoon rains begin in October and prolonged until January (THAMPANYA *et al.*, 2002). Fishermen stated that mud crab recruitment period in Pak Phanang mangrove is from September to February (heavy rain monsoon). However, age group recruit in the mangrove system in September are not caught until February in the subsequent year due to lowest commercial crab size limit for *S. olivacea* (50 mm ICW; MOSER *et al.*, 2005).

On the other hand, periodic spawning period was also noticed for *S. olivacea*. This is around June and August-October in Ranong province (TONGDEE, 2001) and June-November in the Andaman sea, Thailand (KOOLKALYA *et al.*, 2006). Moreover, higher number of female crabs was noticed in commercial catch in May to July (MOSER *et al.*, 2005) while higher number of mature females can be observed in July-November (KOOLKALYA *et al.*, 2006) following the migration to offshore region for spawning. In the present study, female number decreased in May, 2007 that may be attributed to the migration of females to offshore for spawning in May. This is also supported by fishermen's observation of the crab's life cycle in the Pak Phanang mangroves. Crab larvae those are spawned around June reaching a 1-year age by next June.

*Scylla olivacea* takes 3-4 weeks of larval development (MOSER *et al.*, 2005) and do not enter into the mangroves until the Instar 1 stage (MOSER and MACHINTOSH, 2001). In the Instar 1 stage, crabs settle in the mangrove ecosystem for at least 1 month old. *Scylla olivacea* takes 3-4 months to reach the smallest size (50 mm ICW) to be caught by commercial fishermen and another 4-5 months to reach sexual maturity (>90 mm ICW; MOSER *et al.*, 2005). Therefore, the 1st lipofuscin mode and/or the youngest age group caught in May 2007 were 9 to 10-months-old (0<sup>+</sup> year). Also, 70-90 mm ICW classes are composed of the 1st lipofuscin mode (Fig. 8), suggesting that the 1st lipofuscin mode corresponds to the 0<sup>+</sup> year age group.

It was difficult to infer the 2nd and 3rd modes of lipofuscin as distinct age groups from our results. In other crustaceans, lipofuscin

accumulates in nerve cell masses at an almost constant accumulation rate in rearing experiments (SHEEHY *et al.*, 1996) as well as from wild populations (BLUHM and BREY, 2001; KODAMA *et al.* 2005). SHEEHY *et al.* (1998) proved that annual accumulation rate of lipofuscin in western rock lobster *Panulirus cygnus* was constant in both wild and laboratory-reared specimens. When we consider the modes of lipofuscin as an age classes, the regression equation (Fig. 7) indicate that lipofuscin accumulation in OLCM of *S. olivacea* was at an almost constant annual accumulation rate of  $7.0 \times 10^{-3}\%$  volume fraction that could be afforded that each of the groups corresponded to a distinct age class.

Moreover, regularly spaced modes in lipofuscin concentration histogram in wild population of other crustacean species have been observed in other studies, in which relationship between lipofuscin modes and age was established (SHEEHY *et al.*, 1998; BLUHM and BREY, 2001; KODAMA *et al.* 2005 and 2006). The present study also showed modes in lipofuscin concentration histogram with strong linear relationship between modes. Therefore, it would be presumable to regard groups I, II, and III as a distinct age class of 0<sup>+</sup>, 1<sup>+</sup> and 2<sup>+</sup>, respectively that also supports the average 3-year life expectancy of mud crabs (HEASMAN, 1980).

When we accepted the hypothesis that the cluster in figure 5B and/or the peaks in figure 6 as cohort of the age class 0<sup>+</sup>, 1<sup>+</sup> and 2<sup>+</sup>, respectively, there are considerable overlaps in the size between different age groups (Fig. 8). A possible explanation for the wide range of sizes can be partially explained by the long spawning period of the species in tropical area. However, the overlap of the size among size classes cannot be explained only by the long spawning season. Another explanation for the wide size class in same age group is the wide variation of growth rates in the same age group as reported by MOSER *et al.* (2002).

Conclusively, the present study showed the possible application of the lipofuscin microscopic observation for age determination of *S. olivacea*. The weakness of the present study for the validation of this method is small sample size and lack of seasonal movement of

lipofuscin cohorts. For future validation purposes, a year-round observation of the lipofuscin cohorts and/or examination of lipofuscin concentrations of specimens with known ages are recommended.

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資 料

## 第 45 卷第 4 号掲載欧文論文の和文要旨

Kanchana Jirapunpipat, 横田賢史, 渡邊精一: タイ国クローン・ングオ・ラグーンにおけるアカテノコギリガザミ雌の資源量とサイズ分布

タイ国クローン・ングオ・ラグーンにおけるアカテノコギリガザミ *Scylla olivacea* の雌の相対資源量とサイズ分布について調査した。2003年10月から2004年12月の間にカニ籠により漁獲されたノコギリガザミ類全体でアカテノコギリガザミは99.76%を占めた。成熟雌と未成熟雌はともに年間を通じて漁獲された。未成熟雌のCPUE(努力量あたり漁獲量)は水温と塩分のいずれにも相関は無かったが、成熟雌のCPUEは水温に正の相関が見られた。モンスーンの時期が異なる生殖海域間の成熟雌の資源量の月別変動を比較したところ、沿岸域の成熟雌の季節的変動は、成熟雌が密集し回遊ルートの末端にあたるアングマン湾側の生息域と異なる傾向を示した。2003-04年を通じて漁獲された雌の75%は甲幅9.5cm以上の成熟個体であった。また、この期間に漁獲された雌の中央値は8.5cmで、1988-89年に漁獲された雌の中央値9.5cmよりも小さかった。

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Coraline Chapperon: Laboratory assessment of the motion behaviour of intertidal gastropods

潮間帯に共存する3種の腹足類 (*Bembicium melanostomum*, *Austrocochlea porcata*, および *Nerita atramentosa*) の行動様式が、非生物的刺激も生物的刺激も存在しない条件下の実験室で定量的に調べられた。移動経路の評価に用いられた行動学的特性は、移動の速さ、経路の複雑さ、純移動距離と総移動距離との比 (Net to Gross Displacement Ratio: NGDR) および変針角度である。*B. melanostomum* の移動は、*A. porcata* と *N. atramentosa* のどちらよりも有意に遅く、*N. atramentosa* よりも有意に複雑であった。これらの差異は、非生物的要因(すなわち、地形の複雑さ)によっても生物的要因(すなわち、餌と空間をめぐる競争)によっても説明できない。しかし、実験室で観察された行動様式は、これら3種についての既に報告されている摂餌様式と矛盾しない。本論文では、このような類似性の起源を、先天的および後天的行動と関連させて議論する。最後に、極めて近縁な種の間で移動様式が異なることの重要性を、ニッチの分化過程と関連づけて議論する。

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ムハンマド・シェラズール・イスラム<sup>1</sup>、児玉圭太<sup>2</sup>・黒倉寿<sup>1</sup>: Lipofuscin蓄積量を用いた *Scylla olivacea* の年齢推定法の検討

自家蛍光色素lipofuscinの神経細胞内の蓄積量によって、ノコギリガザミ属の一種 *Scylla olivacea* の年齢推定を行う手法について検討した。分析に供したサンプルはタイ国南部パックパナン湾のマングローブ汽水域で集めた。ホリマリン固定された嗅葉細胞の切片を、顕微鏡によって撮影し、コンピュータ画像解析システムによってlipofuscinの蓄積量を測定した。甲幅長の分布には複数のピークは見られなかったのに対して、lipofuscinの蓄積量には3つのピークが見られた。また、甲幅長とLipofuscin蓄積量の間には相関がみられた。それぞれのピークに蓄積量の低い方から番号をあたえ、そのモードの値をグループ番号に回帰させたところ、 $R^2=0.99$ で回帰することができた。以上のことから、Lipofuscin蓄積量を年齢形質として使うことができるものと考えられた。漁獲サイズ、加入サイズ、および、成熟雌の生態などを考慮すると、初めのピークは0<sup>+</sup>の年齢と推定され、この地域におけるこの種の平均寿命は2<sup>+</sup>と推測された。

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## 学 会 記 事

1. 2007年11月5日(月)日仏会館会議室において、幹事会が開かれた。

### 審議事項

- 1) 総合シンポ「日仏(学術)交流のルネッサンス」(2008・9・26~28)への開催準備
- 2) 日仏海洋シンポジウム「Marseille+Paris 2008」
- 3) 名誉会員の推薦
- 4) 20・21年度評議員(24名:無記名連記)の選出および会長選挙の実施
- 5) 学会賞・論文賞の推薦(15名)委員
- 6) バックナンバーのDVD化を笹川日仏財団に共同事業として申請

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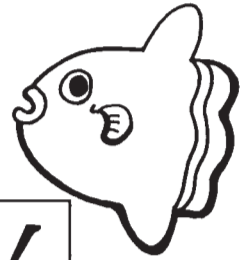
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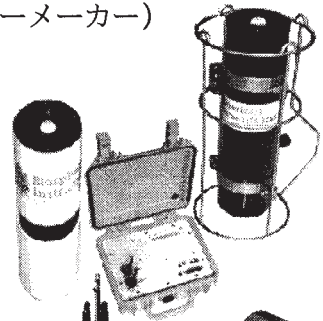
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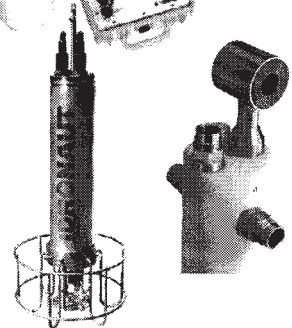
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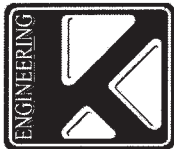
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- 24 ビット分解・RS インタフェース内蔵ロガー
- 6 項目測定



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# 日仏海洋学会入会申込書

(正会員・学生会員)

	年度より入会	年	月	日申込
氏名				
ローマ字		年	月	日生
住所 〒				
勤務先 機関名				
電話	E-mail:			
自宅住所 〒				
電話	E-mail:			
紹介会員氏名				
送付金額	円	送金方法		
会誌の送り先 (希望する方に○をつける)		勤務先          自宅		

(以下は学会事務局用)

受付	名簿	会費	あて名	学会
	原簿	原簿	カード	記事

入会申込書送付先：〒150-0013 東京都渋谷区恵比寿 3-9-25

(財) 日仏会館内

日 仏 海 洋 学 会

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