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SYMBIOTICALLY SNAPPER SHRIMP *ANCHISTUS CUSTOIDES* BRUCE, 1977 (DECAPODA: PALAEMONIDAE) INHABITING THE MANTLE CAVITY OF THE PEN SHELL (BIVALVIA: PINNIDAE)

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Abstract: Snapper shrimp is a symbiotic organism usually hidden under the rocks, sponges and pen shells in the seagrass and coral habitats. The relationship study within snapper shrimp and pen shell was conducted from Merambong shoal, one of the biggest seagrass beds in peninsular Malaysia. A total of 40 individual pen shells were collected randomly and four species of pen shells were identified. 40 *Anchistus custoides* were found inhabiting symbiotically in the mantle cavity of the pen shell as solitary males and females and heterosexual pairs. Pen shell, *Pinna bicolor* and *Atrina vexillum* recorded the highest average SH 217.79±53.15 mm, SV 2.62±1.36 dm^3 and SH 164.10-224.78 mm with the SV 1.18±0.43 dm^3, respectively compared to the other species. The size of *Anchistus custoides* ranged from 15.00 to 20.00 mm in length and it was determined to be female due to the presence of eggs in the pleopods. The length of the cephalothorax and its length were highly related (r_s=0.563, p≤0.01, N=40) and found wider in females. A little difference in size between the left and right chela in males of identical length was noticed, although the left chela is much bigger than the right. The significant relationship (r_s=0.450, p≤0.01, N=40) between the pen shell length and shrimp (male-female) length revealed that the size of the shell is important to be hosted the snapper shrimp in the shell cavity.

Keywords: Merambong shoal, pen shell, shell volume, snapper shrimp, symbiotic.

Introduction

The relationship between decapods and molluscs, known as symbiosis is a one-of-a-kind chance to investigate the evolutionary methods used by marine invertebrates (García-Ulloa *et al*., 2019). Some invertebrates included small decapods living in or on other organisms such as marine molluscs, anemones, polychaetes and echinoderms (Nizinski, 1989; Itani *et al*., 2002; Baeza *et al*., 2011; Baeza *et al*., 2013). They are an attractive method to determine the development of connections between decapods and molluscs since Pontonnid shrimps have developed a number of adaptations to deal with a symbiotic existence (Peiró & Mantelatto, 2011). These associations may arise when a guest organism is looking for a haven from its predators, investigates a new food niche or uses its host as a place for reproduction (Baeza, 2010). However, because of the different environmental conditions that impede direct observation and identification of the specific types of association, the relationship between a pen shell and a snapper shrimp is frequently not clearly defined (Radda & Milat, 2009; Overstreet & Lotz, 2016).

Because many species are composed of several different life stages, benthic habitats provide interesting systems for testing the working effect of different spatial scales (Munguia, 2004). Previous research has used the benthic creatures connected to the pen shells

(*Pinna bicolor*) as a model system to analyse the communities found in patchy environments (Keough, 1984a; 1984b; Butler, 1987). There have been reports of symbiosis between several different species of pontoniids and a diverse group of host taxa, which includes invertebrates such as corals, jellyfish, sponges and molluscs (Lee & Ko, 2011; Olliff, 2013; Dobson *et al*., 2014; García-Ulloa *et al*., 2019; Chow *et al*., 2021). One of the most well-researched symbiosis types involves bivalve hosts colonised by tiny shrimp (Baeza, 2008; Aucoin & Himmelman, 2010; Baeza *et al*., 2013). Important indications used to estimate the symbiotic relationship between a host and a guest are decapods' physical, sexual and reproductive characteristics in proportion to the size of the host (Baeza *et al*., 2015). In tropical and subtropical climates where they are found, the Pontoniinae family has more than 600 species that live within the first 100 meters of depth (De Grave & Fransen, 2013). The shells of many different kinds of marine bivalves, particularly those belonging to the Pinnidae family, served as homes for pontoniid shrimps (Kennedy *et al*., 2001; Aucoin & Himmelman, 2010). Many marine bivalves, particularly those belonging to the Pinnidae family are home to pontoniid shrimps inside their shells (Richardson *et al*., 1997; Kennedy *et al*., 2001; Rabaoui *et al*., 2008; Aucoin & Himmelman, 2010).

In the region known as the Indo-Pacific, the Pinnidae family may be found from southeastern Africa through Melanesia and New Zealand, all the way north to Japan and New South Wales and New Zealand (Butler & Keough, 1981; Poutiers, 1998). Additionally, pen shells may be found in the seas of the Mediterranean and the United States (Rosewater, 1961; 1982; Butler, 1987; Zavodnik *et al*., 1991; Butler *et al*., 1993; Munguia, 2004). The pen shell is an endemic species in the Mediterranean Sea (Katsanevakis, 2004) and the biggest Mediterranean bivalve as well as one of the largest in the world, reaching lengths of up to 120 cm (Zavodnik *et al*., 1991). In contrast, the horse mussel, known as *Atrina zelandica* is a huge pinnid bivalve that feeds on suspended particles and is found randomly spread around the coast of New Zealand (Cummings *et al*., 1998). In Malaysia, the distribution of pen shells (*Pinna* and *Atrina*) was reported at Merambong shoal and Tanjung Adang shoal (Johor), Bagan Panchor (Perak) and Merchang Lagoon (Terengganu) (Idris *et al*., 2008; 2009; Idris *et al*., 2012).

There is currently no extensive documentation on the symbiosis between the shrimp *Anchistus custoides* Bruce, 1977 (Decapoda: Palaemonidae) that occupy the mantle chamber of the shell (Bivalvia: Pinnidae) of Malaysian pen shells that have been reported. In Malaysia, most pen-shell studies focus on distribution and ecology (Idris *et al*., 2008; 2009). Most of the publications reported in the Indo-Pacific, Mediterranean and American regions a long time ago (Zavodnik, 1967; Butler & Brewster, 1979; Butler & Keough, 1981; Scheltema, 1983; Butler, 1987; Butler *et al*., 1993; Šiletić & Peharda, 2003; Katsanevakis, 2004). This study focuses on discovering symbiotic snapper shrimps in the pen shell mantle cavity, which may eventually help to know the characteristics and habitat information of snapper shrimp living inside the pen shell cavity in the seagrass bed of Malaysia.

Materials and Methods

During the time of low tide, a total of 40 different individuals of pen shells were collected at random from the Merambong shoal in the Johor Strait (N1^{o} 19' 55.62" E103^{o} 35' 57.75") (Figure 1). Merambong shoal is a sandy area with *Enhalus acoroides, Halophila ovalis, Thalassia hemprichii* and *Halodule uninervis* growing in seagrass meadows. Specimens were removed from their natural habitat using a hand scoop and specimens were placed into a plastic bag containing 10% formalin and labelled. Specimens were transported to the laboratory for further examination. In the laboratory, pen-shell specimens were emptied into a washing tray according to the labeled given and washed in distilled water over a 0.5 mm sieve to avoid the shrimp passing through with the running water.

Pen shells adductor muscles were sacrificed by having the back cut off to get the shrimp occupying the shell. The shrimp were identified based on the key features of Anker and De Grave (2016). The number of shrimps found on each pen shell was recorded and preserved in ethanol at a 70% concentration. The works of Richardson *et al*. (1997) were adopted to identify the presence of snapper shrimp inhabiting the pen shell. Using a MITUTOYO digital vernier calliper (±0.01 mm), the total body length (BL) (from the tip of the rostrum to the tip of the telson), cephalothorax length (CL) (from the tip of the rostrum to the posterior end of the carapace) and width of the widest part of the cephalothorax, and length of the right and left chelae (from the base of the dactyl and propodus to the tip of the claw) of the second pereiopods of each shrimp were measured. Morphometric relationships between the characteristic of shrimp (BL and CL) were analysed using Pearson's correlation (Bhujel, 2008).

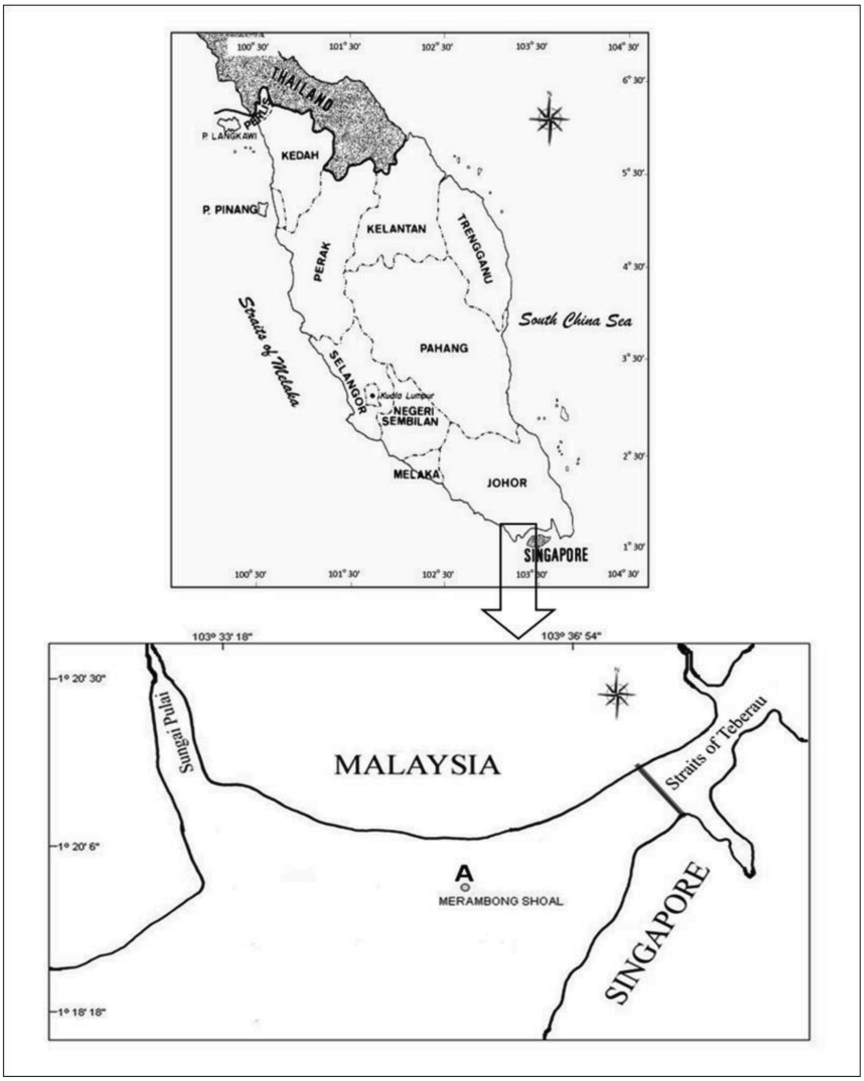

Figure 1: The map displayed the sample regions, including (A) Merambong shoal, which is located off the coast of Southwestern Johor in Malaysia

The shell height (SH) was determined by finding the point that was the farthest along the line perpendicular to the umbo. The shell length (SL) was determined by measuring the length of the perpendicular line to the umbo. When measuring shell width (SW), the distance from left to right was used as the reference point (Figure 2). The estimated volume of each pen shell (SV) was determined by multiplying the three morphometric variables according to Salas-Moya *et al*. (2014), Góngora-Gómez *et al*. (2015) and García-Ulloa *et al*. (2019) in order to comprehend a potential link between the accessible spaces of pen shell for the shrimp.

Results

Snapper shrimp in the pen-shell valve were semi-transparent and pale yellow (Figure 3). The snapper shrimp were found and identified from the Family Palaemoidae and genus *Anchistus* and species *custoides* Bruce, 1977 with 15 to 20 mm length (Figure 4). The broadest cephalothorax and the presence of eggs attached

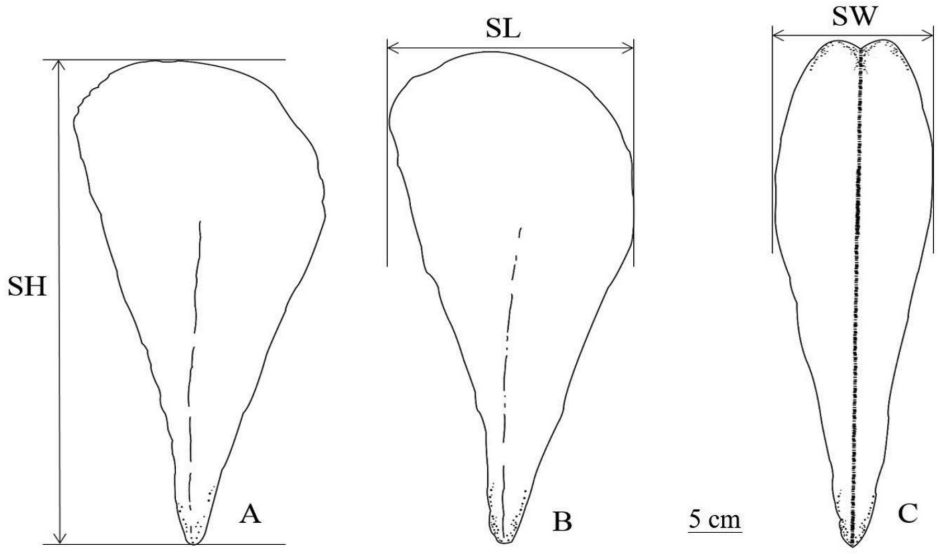

Figure 2: Morphometric measurement of pen shell (A), Shell Height (SH), (B) Shell Length (SL) and (C) Shell Width (SW)

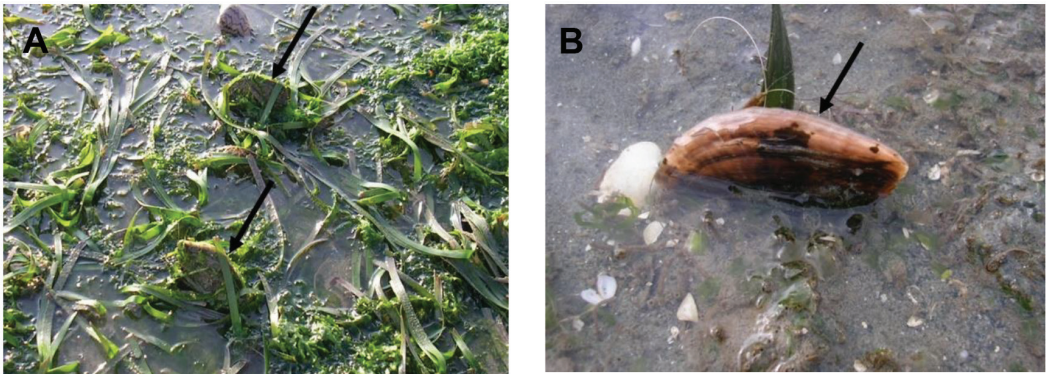

Figure 3: A few examples of pen-shell habitats in the study areas. A - Pen shells (arrow) from Merambong shoal found inhabited with seagrasses and seaweed. B - Pen shell inhabit with spoon grass *Halophila ovalis*

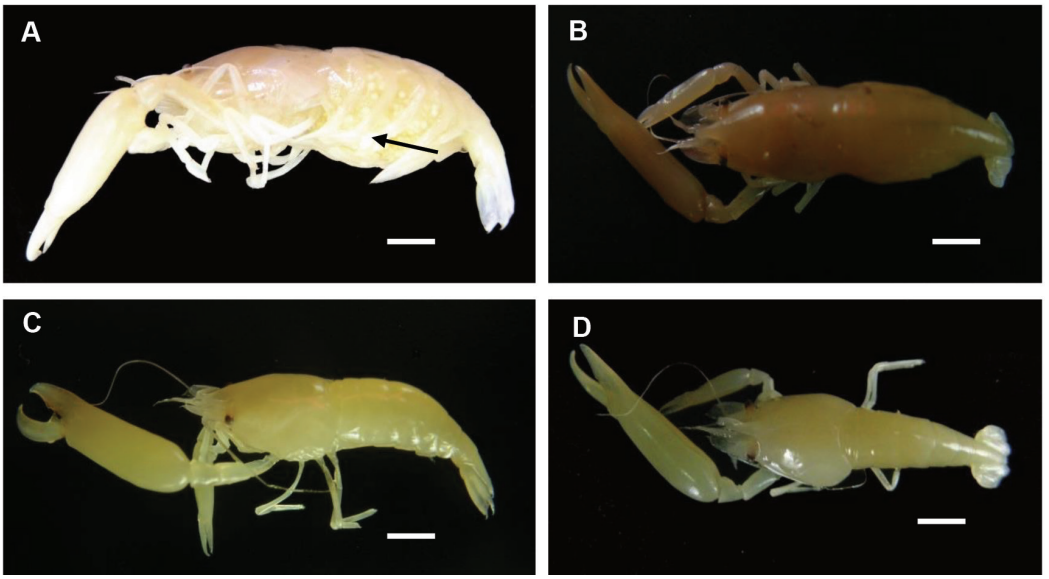

Figure 4: *Anchistus custoides* Bruce, 1977 found in the mantle cavity of the pen shells. (A) and (B) are female with (A) showing the presence of eggs attached to the pleopods and overlaying abdominal pleura (arrow). (C) Male showing pleopods with no overlying pleura and the difference in the size of the chela of the second periopods. Female (B) and male (D), dorsal view. Scale (5 mm)

to the pleopods determined female snapper shrimps. Usually, the width of the cephalothorax distinguishes males and females of *Anchistus custoides* [Figures 4 (B) and (D)]. The breadth of the cephalothorax was substantially connected to its length (r_s=0.563, $p \leq 0.01$, N=40) and females had broader cephalothoraxes [Figure 5 (A)]. The chelae on the second pereiopods of shrimp were not the same size and there was a correlation between the size of the chelae and sex [Figures 5 (B) and (C)]. The left chelae were greater than the right chelae of female and male shrimp. The average size of chelae of males (13.82±1.12 mm) and females (12.20 ±1.95 mm).

During this study, the symbiotic snapper shrimp was observed inhabiting the mantle cavity of the pen shell. Four species of pen shell from two genera (*Pinna* and *Atrina*) have been identified. The genus of *Pinna* comprising of *Pinna bicolor, Pinna muricata* and *Pinna deltodes*. At the same time, *Atrina vexillum* represented the genus *Atrina*. It was found that, out of the 40 pen shells, only 21 pen shells were found to be inhabited by the snapper shrimp (Table 1). A total of 40 individuals of the snapper shrimp have been recorded. *Pinna bicolor* recorded the highest number of shrimps inhabiting the shells with 13 shrimps.

Pinna muricata with recorded the higher SH averaged 283.94±34.5 mm while the lowest was *Pinna bicolor* 216.79±53.15 mm. *Pinna muricata* also recorded the highest average value for SL141.88±11.8 mm and *Pinna deltodes* recorded the lowest 105.50±27.0 mm. The highest SW was recorded by *Atrina vexillum* at 44.83±6.42 mm and *Pinna deltodes* recorded the lowest at 25.77±8.10 mm (Table 2). Based on the measurements for shell volume (SV) found, *Pinna bicolor* recorded a wider space value compared to other pen shells with the value of 2.62±1.36 dm^3. Therefore, the shrimp that inhabits *Pinna bicolor* are more numerous than the others because of the space provided. Part of the shrimp found inhabiting the pen shell solitary male (n=4) and solitary female (n=2). Typically, they occur as adult pairs inhabiting the mantle cavity.

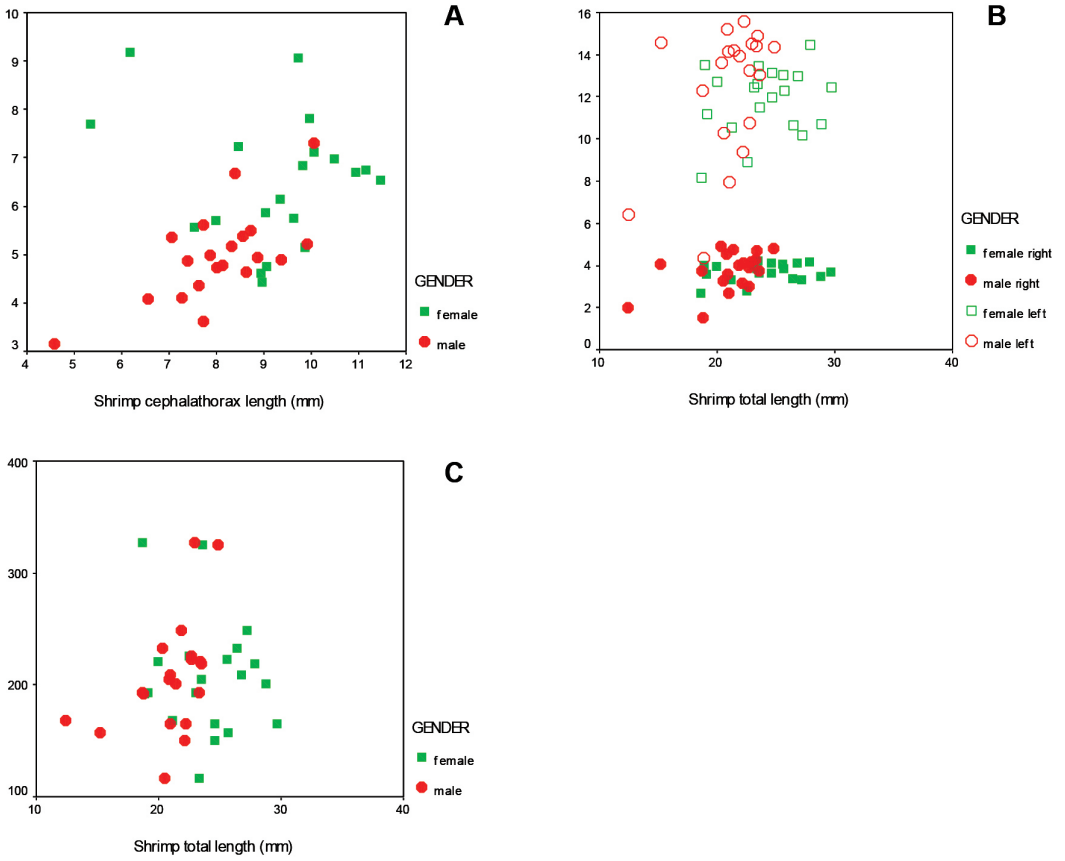

Figure 5: The proportion of breadth to the length between the cephalothorax of male and female *Anchistus custoides* (A), the correlation between the length of the right chela of the second pereiopod (represented by closed symbols) and the left chela of the second pereiopod (represented by open symbols) and the total body length of female and male pen shell inhabitants (B) and a comparison of the length of the female and male *Anchistus custoides* shells as well as their overall length (C)

Table 1: Distribution of snapper shrimp *Anchistus custoides* Bruce, 1977 in the mantle cavity of pen shells

Pen Shell Species	Number of Pen Shells Inhabited by Shrimps	Shrimp			
		Male	Female	Male and Female	Total Number of Shrimps
Pinna bicolor	7	0	1	6+6	13
Pinna muricata	4	1	0	3+3	7
Pinna deltodes	5	1	0	4+4	9
Atrina vexillum	7	2	1	4+4	11

Table 2: Means and ranges of shell measurements of four species of pen shells collected from Merambong shoal

Species	N	Shell Height (SH) (mm)		Shell Length (SL) (mm)		Shell Width (SW) (mm)		Shell Volume (SV) (dm^3)	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
Pinna bicolor	15	150.11-326.90	216.79± 53.15	65.35-150.89	107.62± 31.77	16.12-36.25	28.45± 8.16	0.19-5.18	2.62± 1.36
Pinna muricata	5	246.85-288.84	283.94± 34.51	127.57-153.59	141.88± 11.80	25.63-33.25	29.15± 4.18	0.95-1.41	1.16± 0.20
Pinna deltodes	10	115.57-276.81	203.44± 48.10	61.62-127.90	105.50± 20.27	15.36-32.12	25.77± 8.10	0.25-1.17	0.62± 0.36
Atrina vexillum	10	164.10-224.78	192.65± 22.74	107.83-155.61	131.07± 16.55	38.16-61.09	44.83± 6.42	0.74-1.52	1.18± 0.43

Note: N = number of samples

Studies found that the left chela of a female was somewhat bigger than the right chela, even though both were smaller than that of a male of comparable length, whose left chela was much larger than the right. A substantial correlation (r_s=0.450, p≤0.01, N=40) was obtained between the total length of the pen shells and the total length of the male and female shrimp [Figure 5 (C)]. This association suggests that the biggest individuals occurred in the bigger pen shell while the smallest individuals inhabited the smaller size of the pen shell.

Discussion

A number of studies have highlighted the relevance of the host-guest size association between shrimps and the various types of bivalve organisms. It was the first time that reported the snapper shrimp *Anchistus custoides* resided in the mantle cavity of pen shells in Malaysia seagrass habitat. In the sea of Hong Kong, it was observed that adult pairs of the pontoniine shrimps *Anchistus custos* and *Conchodytes monodactylus* inhabit *P. bicolor* (Morton, 1987). In another situation, a shrimp, *Conchodytes nipponensis* (DeHann) was found living in the mantle cavity of a specimen of *A. pectinata* from Korea (Lee & Ko, 2013) and Dutch New Guinea (Rosewater, 1961). Garcial-Ulloa *et al.* (2019) discovered that the pearl oyster shrimp *Pontonia margarita* lived in the mantle cavity of the *Pinna rugosa*.

This study found that the mantle cavity of the pen shell contained both male and female snapper shrimp. According to Richardson *et al*. (1997), female snapper shrimp may be distinguished by the presence of eggs connected to the pleopods or in their absence, by the wider look of the cephalothorax and the evident extent of the overlapping abdominal pleural partly covering the pleopods. Gracia-Ulloa *et al*. (2019) reported the female cephalothorax length was the widest compared to male cephalothorax length with an average of 9.48±0.16 mm and 5.05±0.76 mm, respectively, being similar to the cephalothorax length average (females = 8.21 ± 2.46 mm; males = 6.39±2.02 mm) reported by Cabrera-Pena and Solano-Lopez (1996). A similar observation was recorded from this study with an average of 8.80 ±0.71 mm and 6.27±0.37 mm for females and males.

Richardson *et al.* (1997) reported that the chelae of the second pereiopods of *P. pinnophylax* are of unequal size and there is also a difference in size of chelae in relationship to sex. The female's right claw is slightly larger than the left, although both are smaller than those of male *P. pinnophylax* of similar length

and whose right chela is substantially larger than the left. Richardson *et al.* (1997) found that the chelae of the second pereiopods of *P. pinnophylax* are not similar and there is also a size disparity between the chelae and the gander. Although the right claw of the female *P. pinnophylax* is marginally bigger than the left, both claws are much shorter than those of male *P. pinnophylax* of comparable length, whose right chela is significantly larger than the left. The presence study found the left chelae of *Anchistus custoides* was greater than right chelae and the average length of male chelae was greater than female. According to García-Ulloa *et al.* (2019), the maximum chelae length of the second pereiopod of *P. margarita* females was longer than their male counterparts with an average of 15.61±1.94 mm and 12.65±3.03 mm.

During the study conducted on the Merambong shoal, pen shell was found living in muddy sand areas and associated with seagrasses. Seagrass areas provide an important source of food for aquatic life. In the Sungai Pulai estuary, (Hossain *et al.*, 2018) reported ten species of seagrasses inhabiting the sandy-muddy area while Arina *et al.* (2020) recorded seven species (*Cymodocea serrulata*, *Halophila ovalis*, *Halodule pinifolia*, *Enhalus acoroides*, *Thalassia hemprichii*, *Halodule uninervis* and *Syringodium isoetifolium*) of seagrasses inhabiting the sandy-mud area in Merambong shoal. According to Idris *et al.* (2009), a total of seven species of pen shell have been recorded at Merambong shoal but from this study, only four species of pen shell have been recorded.

From the study conducted, it was found that the presence of *Anchistus custoides* inhabiting four species of pen shells. *Pinna bicolor* recorded higher space than three other species with a range size of 150.11-326.90 mm and an average of 216.79±53.15 mm with the SV 2.62±1.36 dm^3. García-Ulloa *et al*. (2019) reported the presence of shrimp in pen shells with SH ranging from 198 to 271 mm. However, there was no association between the size of the shrimp and the host shell. This research found that the average estimated volume for each rugose pen shell (1.26±0.76 dm^3) was lower than what was reported for *A. tuberculosa* (3.55±0.76 dm^3) by Góngora-Gómez *et al.* (2015) using the same morphometric computation. This would imply that the SV of the *Pinna bicolor* was big enough to accommodate not only a single *Anchistus custoides* but also a male and female. However, Baeza *et al.* (2013) conclude that a shell length of <175 mm is insufficient for the symbiotic shrimp. Aucoin and Himmelman (2010) came to a similar conclusion when they investigated the development of *Pontonia* sp. with its host, the pin shell *Pinna carnea*.

The investigations of the shrimps indicated that they react either violently or defensively along the shell edge, and they might serve as a warning to any curious predatory fish prevalent within the canopy of the seagrass meadow (Bell & Harmelin-Vivien, 1982; 1983). On the other hand, it is unclear whether the *Anchistus custoides* benefit from the association. In a study of the zoea development of *Pontonia pinnophylax*. Calafiore *et al.* (1991) found that the development from zoea stage VIII to the post-larval stage only occurred in the presence of adult mussels. In the absence of *Pinna*, the zoea continued to grow but they did not transform into juvenile shrimps.

According to Richardson *et al.* (1999), the snapper shrimp, pontoniine demonstrated a strong affinity for shade and they rapidly became immobilised in even a weak current flow. Therefore, the presence of these shrimp inside pinnids would provide them with the necessary shelter, shade and protection. The shrimp may get some of their nutrients from the pen shells. When *Pinna* is being fed in suspension, there is never a break in the creation of pseudofaeces on its part. In most cases, the contractions of the adductor muscle are responsible for the expulsion of pseudofeces; however, it seems that this is not the case in *Pinna* (Yonge, 1953). It is currently unclear whether or if shrimp are there, what function they play or what kind of influence they have on the pen shell. The presence of shrimp at the shell border and patrolling the mantle margin may operate as a

first line of defense, inhibiting predatory fish from grazing on the posterior edge of the mantle and shell (Richardson *et al.,* 1999). The precise nature of the interaction between the two species has not been scientifically established in this research. This is because *Anchistus custoides* represents the last link in a series of species that are all at risk of extinction. In the coastal seas of Johor, Malaysia, there is a kind of shrimp that lives inside of a pen shell, which is also an endangered bivalve.

Conclusion

A pair and single of adult snapper shrimp *Anchistus custoides* Bruce, 1977 with the ranges size of 15 to 20 mm in length have been recorded inhabiting the mantle cavity of pen shell in Merambong shoal seagrass beds. *Pinna bicolor* and *Atrina vexillum* recorded the highest number of shrimp inhabiting the shell with the average SH 150.11-326.90 mm with the SV 2.62±1.36 dm^3 and SH 164.10-224.78 mm with the SV 1.18±0.43 dm^3, respectively. It was observed that the shrimp was living symbiotically with the pen shells. Female shrimps were identified by the presence of eggs attached to the pleopods and the female size was greater than the male specimens.

Adult snapper shrimp *Anchistus custoides* has been found living in the mantle cavity of pen shells in the seagrass beds of Merambong shoal. Their lengths vary from 15 to 20 mm. A pair and a single adult snapper shrimp Anchistus custoides Bruce, 1977 have been reported. *Pinna bicolor* and *Atrina vexillum* reported the maximum number of shrimp occupying the shell, with an average shell height of 150.11-326.90 mm and a shell volume of 2.621.36 dm^3 for *Pinna bicolor* and SH 164.10-224.78 mm and SV 1.18±0.43 dm^3 for *Atrina vexillum*, respectively. It was also discovered that the shrimp lived symbiotically with the pen shells. It was possible to determine whether or not a shrimp was female by seeing whether or not it had eggs attached to its pleopods. Additionally, the size of female specimens was larger than that of male specimens.

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