

Comparative morphology and function of feeding appendages in food intake behaviour of the whiteleg shrimp, *Litopenaeus vannamei*, and the giant freshwater prawn, *Macrobrachium rosenbergii*

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Abstract

Combining feeding appendage morphology and behavioural observation of the motion pattern of the feeding appendages clarified many aspects underlying the feeding processes of the giant freshwater prawn (*Macrobrachium rosenbergii*) and the marine whiteleg shrimp (*Litopenaeus vannamei*) in aquaria. The food intake behaviour was video recorded during eating pellet food, and pieces of fresh squid and fish. While *M. rosenbergii* took pellet one by one, *L. vannamei* picked up many pellets at one time and held them at the mouth with the 3rd maxilliped endopods and the 1st walking legs. Both species used the right chelate walking legs rather than the left walking legs to pick up the food. The 3rd walking legs of *L. vannamei* were longest and heaviest among the chelate walking legs but their major role was not for feeding but for feeding contests often in the form of aggression. While *M. rosenbergii* easily crunched pellets by the mandibles, *L. vannamei* did not crunch pellets due to the softer and not so strong mandibles and frequently spat out them, indicating that the present hard pellets are not suitable for *L. vannamei*. Both the species kept a piece of elastic fresh squid or fish flesh at the mouth and tore the food into small pieces with the help of repeated pulling down motion of the 3rd maxilliped endopods. However, the mandible teeth of the two species were not sharp enough to gnaw off the fibrous muscle in one bite. The 2nd and 3rd maxilliped endopods were used for holding food at the mouth and did not contribute to mastication of food. The 2nd and 3rd maxilliped exopods exhibited the horizontal fanning motion, which caused a unidirectional water flow moving backwards in the gill chamber (visualized with milk). The maxilliped exopods were found to contribute not to feeding but ventilation. Based on the results obtained, development of softer pellets was recommended for *L. vannamei*.

Keywords: Mouthparts, Walking legs, Video observation, Ventilation, Pellet food

Introduction

Feeding behaviour includes searching, detection, orientation, grasping, manipulation and ingestion of the food. Understanding feeding behaviour is important for formulated feed development. From the searching to the ingestion processes various appendages and sensory systems are involved. Information on the sensory basis of shrimp feeding provides the means for assessment of the effectiveness of food items in terms of smell, taste, size, and colour. Kawamura *et al.* (2017) documented the sensory basis of the feeding behaviour of the giant freshwater prawn (*Macrobrachium rosenbergii*) and the marine whiteleg shrimp (*Litopenaeus vannamei*) by using selective sensory ablation techniques in the laboratory.

Food is normally the largest single expenditure item in the operation of a shrimp farm (Rhodes, 2000). In formulating shrimp foods, the nutritional quality, palatability, and cost effectiveness are important considerations for commercial success. Nutritional and palatability investigations on shrimp have been confined to

empirical dietary trials and the data so generated have elucidated their nutritional requirements and the palatability of feeds (Tantikitti, 2014). However, more research is still necessary, particularly on the feed intake behaviour of shrimp; grasping, manipulation and ingestion of food.

The analyses of gut contents of *M. rosenbergii* and *L. vannamei* sampled from the wild showed some preference for food (Costa and Wanninayake, 1986; Varadharajan and Pushparajan, 2013). While the selection of food takes place throughout the feeding behaviour (Kawamura *et al.*, 2017), the most important selection would be done through the food intake behaviour which involves the function of the feeding appendages such as the mouthparts and chelate walking legs. Morphology of the mouthparts has been studied in many shrimp and crab species and the function of the mouthparts in feeding was based on the morphology, specially the presence and structure of setae examined by light microscopy or electron microscopy (Sahlmann *et al.* 2011; Rocha *et al.*, 2016, 2017; Yam, 2016).

On the other hand, feeding behaviour was grossly and video observed to analyze the function of mouthpart appendages. For this purpose, several video recording techniques were employed, Barker and Gibson (1977) conducted cine-photography of the European lobster (*Homarus gammarus*) in a glass tank and analyzed feeding mechanism in slow motion. In another experiment the live specimens of the Eastern King prawn (*Penaeus plebejus*) were held in water with the ventral surface upwards and movements of the appendages were recorded on movie films (Suthers, 1984). Individual phyllosoma of the packhorse lobster (*Jasus verreauxi*) were tethered with adhesive to fine wire at the posterior dorsal surface of the cephalothorax and then placed in a glass beaker containing 10 mL water (Cox and Johnston, 2003). The deep-sea lobsters (*Metanephrops formosanus* and *M. armatus*) were allowed to move freely in a small 2 L tank and filming was done from below the tank and from the side (Sahlmann et al., 2011). These techniques for video observations are not always applied to other crustacean species such as *M. rosenbergii* and *L. vannamei* which do not take food at all in a seriously stressed condition.

Understanding the morphology and function of the feeding appendages provides useful information on feeding habits and prey characteristics. For example, the mandible is involved in the initial breakdown of food and, therefore, has a large functional significance and thus its form may provide insights into the diet or feeding mode of the species (Ashelby et al., 2015). Analyses of the functioning of crustacean mouthparts have been few largely as the result of the difficulty of making direct observations on live animals (Hunt et al., 1992). In spite of large number of studies of morphology of mouthpart appendages of decapod crustaceans as mentioned above, the function of the appendages is not understood well due to insufficient observation of motion of the appendages during feeding. In the present work, morphology was examined by light microscopy and function of the chelate walking legs and the mouthparts in food intake was examined by video observations for two decapod species of different habitats, the freshwater *M. rosenbergii* and the marine *L. vannamei* freely moving in aquaria. This baseline information will be used to infer possible changes in dietary preference and ingestion capabilities, which are key considerations when formulating artificial diets for culture.

Materials and Methods

The experiments were conducted in Borneo Marine Research Institute (BMRI), Universiti Malaysia Sabah. All the experimental animals were cared and handled following the guidelines by the World Health Organization (WHO, Geneva, Switzerland); the Malaysian Code of Practice for The Care And Use of Animals For Scientific Purposes; and the Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Research (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute to Laboratory Animal Research 2011).

Test animals

Juveniles and adults of *M. rosenbergii* were obtained from BMRI. They had been reared with formulated pellet feed (Sheng Long- Royal Dragon, DT313, Vietnam). Postlarvae of *L. vannamei* were supplied by the BV Shrimp Farm, Tuaran, Sabah and reared in the shrimp hatchery of the BMRI with formulated powder feed (Gold Coin, Malaysia) and then pellet feed (Gold Coin, Starter pellet 904, Malaysia).

Morphology of feeding appendages

Test animals were anesthetized in ice water before dissection. The feeding appendages (the walking legs, maxillipeds and mandibles) were surgically removed and placed in a petri dish filled with freshwater and their morphology was studied under a dissection microscope (Nikon SMZ 645, Tokyo, Japan) and photomicrographed with a colour CCD camera (Olympus Tough TG-3, Olympus Corporation, Tokyo, Japan). The length measurements were done with a digital caliper (SINWA, Sanjo, Japan) to 0.01 mm.

Video observation

Video recording of food intake behaviour of the freely moving *M. rosenbergii* and *L. vannamei* was done with the CCD camera with temporal resolution of 0.017 s, corresponding with 60 frames per second. Two aquaria (60 × 45 cm, 31 cm high, 20 cm water depth) were used for one species, each contained three specimens. The aquaria were placed in the shaded shrimp hatchery and video recording was carried out under natural light conditions in daytime. Since the mouthparts are typically directed ventrally, a mirror (21 × 28 cm) was placed under each aquarium and mirror images of the motion of the mouthparts and the chelate walking legs were video recorded ventrally with the hand-held CCD camera (Figure 1). When a shrimp came near the tank walls and lifted the head up, direct frontal view video recording was done from outside of the aquaria (Figure 1). Recording with the hand-held CCD camera enabled to follow moving shrimp. Before recordings, experimental animals were starved for at least 18 h. Total number of video recording was 319 and total recording time was 4 h 23 min 50 s (Table 1). The video recordings were played back repeatedly and analyzed for motion pattern and function of the feeding appendages on a computer screen.

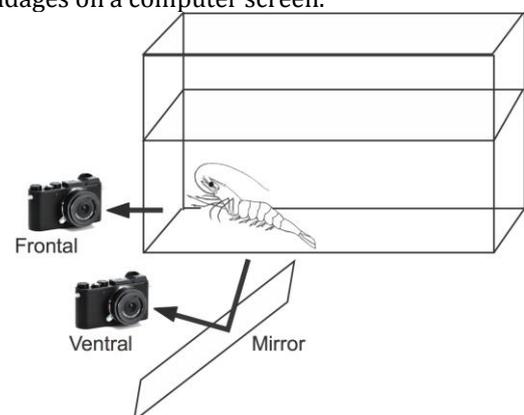


Figure 1. Method of video recording of the ventral and frontal views of *Macrobrachium rosenbergii* and *Litopenaeus vannamei* in an aquarium. Mirror images of ventral views were recorded.

Table 1. Number and size of specimens used, number of video recordings and total recording time by shrimp species and food.

Shrimp	No. of individuals	Size	Food	No. of video recordings	Toal video recording time
<i>Macrobrachium rosenbergii</i>	Adults: 1 male and 2 females	Adults: 11.5-14.6 cm TL, 21.0-28.0 g BW	Large pellet	48	68 min 54 s
			Squid piece	21	37 min 45 s
	Juveniles: 2 males and 1 female	Juvenile: 4.10-5.30 cm TL, 0.50-0.90 g Bw	Large pellet	21	21 min 49 s
			Small pellet	46	30 min 01 s
				Sub total	2 h 33 min 56 s
<i>Litopenaeus vannamei</i>	Adults: 2 males and 2 females	Adults: 13.0-14.0 cm TL, 10.5-12.5 g BW	Large pellet	11	9 min 08 s
			Squid piece	38	14 min 29 s
			Fish piece	50	19 min 53 s
	Juveniles: 4 males and 6 female	Juvenile: 9.0-10.8 cm TL, 6.0-8.0 g Bw	Large pellet	51	35 min 07 s
			Squid piece	22	14 min 08 s
			Fish piece	8	17 min 09 s
				Sub total	1 h 49 min 54 s
				Total	4 h 23 min 50 s

Table 2. *Litopenaeus Vannmaei*. Length of left and right walking legs.

Total length (mm)	Walking leg	Left (mm)	Right (mm)	Difference (left-right, mm)
Specimen 1 115.61	1st	20.18	24.19	-4.01
	2nd	30.81	33.48	-2.67
	3rd	42.31	43.76	-1.45
	4th	32.43	35.19	-2.76
	5th	35.78	38.06	-2.28
Specimen 2 127.72	1st	26.67	27.93	-1.26
	2nd	35.93	34.59	1.34
	3rd	47.84	47.73	0.11
	4th	38.15	37.71	0.44
	5th	41.91	40.89	1.02
Specimen 3 134.01	1st	22.91	23.51	-0.60
	2nd	31.13	32.39	-1.26
	3rd	43.80	41.24	2.56
	4th	33.09	30.59	2.50
	5th	36.11	34.74	1.37
Specimen 4 132.08	1st	Lost	24.01	
	2nd	35.31	32.74	2.57
	3rd	45.94	45.29	0.65
	4th	35.96	34.67	1.29
	5th	39.93	38.99	0.94
Specimen 5 117.35	1st	24.81	23.14	1.67
	2nd	32.12	32.24	-0.03
	3rd	44.59	43.15	1.44
	4th	25.62	32.11	-6.49
	5th	37.00	37.08	-0.08
Specimen 6 114.27	1st	25.12	25.94	-0.82
	2nd	33.73	33.71	0.02
	3rd	46.89	43.22	3.67
	4th	35.71	33.81	1.9
	5th	41.17	37.69	3.48

During the experiment, water quality parameters were: Temperature at 25–28°C, salinity 27–28 ppt, dissolved oxygen (DO) 5.9–7.5 mg/L, pH 7.9–8.8 for *L. vannamei* and 25–28°C, 0.02–0.04 ppt, 5.9–7.5 mg/L, 6.9–8.2 pH for *M. rosenbergii*, respectively.

Food used

The motion pattern of the feeding appendages could be altered depending on food types with different physical characteristics such as hardness, texture and size. Therefore, three different types of food were used: pellet food (*L. vannamei* feed, Sheng Long Bio-tech International Co., LTD, Long An Province, Vietnam), small pieces of squid mantle (*Loligo* sp.) and fish flesh, bigeye scad (*Selar crumenophthalmus*). And pellets of different sizes were used for *M. rosenbergii* juveniles: larger (length 4.67 ± 0.11 mm, mean \pm SD, $n = 20$) and smaller (approximately juvenile's eye size, 2.35 ± 0.07 mm, $n = 20$), the diameter of the pellets was 1.80 mm (Figure 2). The size of the pellets was measured to 0.01 mm with the digital caliper.



Figure 2. Pellets of two sizes (large 4.67 mm long: small 2.35 mm long) used in the experiment on food intake behaviour of *Macrobrachium rosenbergii* juveniles.

As elastic foods, pieces of fresh squid mantle (*Loligo* spp.) (0.2–0.4 g and 0.3–0.7 g in wet weight for juvenile and adult *L. vannamei*, respectively) and pieces of fish flesh (*Selar crumenophthalmus*) (0.03–0.06 g and 0.3–0.9 g for the juvenile and adult *M. rosenbergii* respectively) were used.

Visualization of current produced by exopod motion

M. rosenbergii and *L. vannamei* vigorously moved the fan-like shaped exopods of the maxillipeds which probably produce respiratory current. The respiratory current was reported to play an important role in the feeding of the lobster (*Porcellana longicornis*) (Nicol, 1932). To confirm this possibility, water movement around and in the gill chamber

of *L. vannamei* was visualized with milk pipetted in front of the rostrum and the movement of the milk plume was video recorded (12 video recordings; total recording time, 6 min 41 s) (Figure 3). The movement of the milk plume was analyzed on a computer screen.

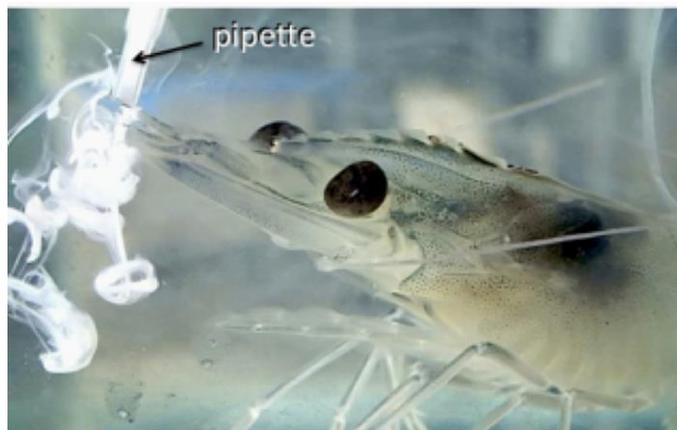


Figure 3. Milk, a highly visible white liquid, pipetted in front of *Litopenaeus vannamei* to visualize water flow.

Statistical analysis

In this experiment, the paired *t*-test was used for the comparison of the length of the right and left walking legs, the *t*-test was used to statistically analyze the pellet mastication time for *M. rosenbergii* juveniles and also food handling times expended for tearing between fish and squid foods for *M. rosenbergii* and *L. vannamei*. The χ^2 -test was used to compare the frequency of use of right and left walking legs. The level of significance was set at $\alpha = 0.05$.

Results

Morphology of feeding appendages

Since there is no discernible difference in the morphology of the feeding appendages, except for the 2nd walking legs of *M. rosenbergii*, the feeding appendages were shown only for male or female.

Walking legs

Photographs of the pincers and dactyls of the walking legs of *M. rosenbergii* and *L. vannamei* are shown in Figure 4.

M. rosenbergii: The 2nd walking leg is extremely longer and heavier than the others and the 1st one is the second longest. The dactyls of the 3rd, 4th and 5th walking legs bear thick serrata setae along the outer edge, but there is no seta along the inner edge (Figure 4F, H, J).

L. vannamei has walking legs with similar length but the 1st walking leg is the shortest and the third one is the longest (Table 2). The difference of the 3rd walking legs between left (mean, 49.93mm) and right (mean, 48.69 mm) is statistically significant (paired *t*-test, *df* = 6, *t* = 2.374, *P* = 0.049) indicating that the left 3rd walking leg is longer than the right one. The mean difference of the other walking legs (1st, 2nd, 4th and 5th) between left and right (-0.16 mm) is

not statistically significant (95% confidence interval, -1.692 – 0.632 mm).

1st walking leg: Both species have a pair of chelate first walking legs. The pincers and carpus, segment connecting with the pincers, bear setae and the gap is narrower in both species (Figure 4A, B). The pincer is three times longer in *M. rosenbergii* than in *L. vannamei*.

2nd walking leg: Both species have a pair of chelate second walking legs. The pincers bear setae (Figure 4C, D, E).

3rd walking leg: *L. vannamei* has chelate third walking leg but *M. rosenbergii* does not. The pincers bear setae (Fig. 4G) and the dactyl of *L. vannamei* bears thick setae (Figure 4F).

4th and 5th walking legs: Dactyls of these walking legs bear thick setae in *M. rosenbergii* while those bear fringing thin setae in *L. vannamei* (Figure 4H–K).

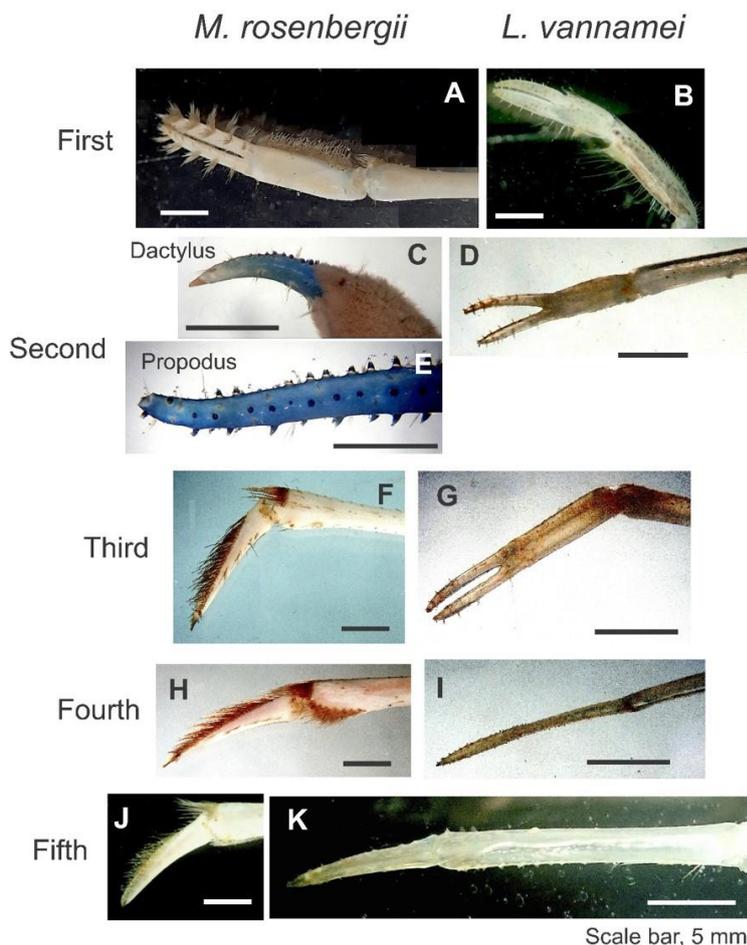


Figure 4. *Macrobrachium rosenbergii* walking legs have small pincers on the end of the first pair (A), large pincers on the second elongated pair (C, E), and pointed tips on the posterior three pairs (F, H, J). *Litopenaeus vannamei* walking legs have small pincers on the first three pairs (B, D, G) and pointed tips on the posterior two pairs (I, K).

Mouthparts

The general structure of the mouthparts is similar in both species. Ventral view of the mouthparts is shown for *M. rosenbergii* (total length 158 mm) and *L. vannamei* (142 mm) in Figure 5. The 1st (innermost), 2nd and 3rd (outermost) maxillipeds are arranged in front of the mandibles.

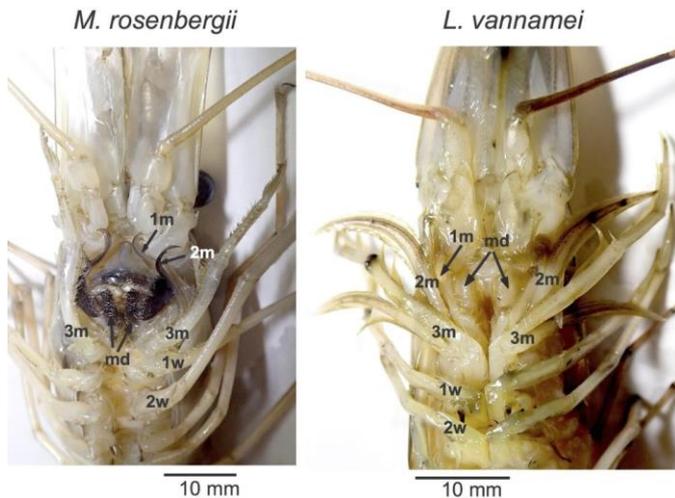


Figure 5. Mouth parts of *Macrobrachium rosenbergii* and *Litopenaeus vannamei*: paired maxillipeds 1m, 2m, and 3m; and paired mandibles md. The black pigmentation of the mouthparts of *M. rosenbergii* shown in this photograph is not common in this species. The first two walking legs 1w and 2w are shown for orientation.

1st maxilliped: In both species, the basis and exopod are flat and bear setae along the edge. It consists of the exopod and endopod (Figure 6A, B).

2nd maxilliped: The exopod is small in *M. rosenbergii* (Figure 6C) but large and bears fan-like plumed setae in *L. vannamei* (Figure 6D).

3rd maxilliped: The 3rd maxilliped of *M. rosenbergii* differs greatly from that of *L. vannamei*. The endopod is slender, long and resembles the walking legs in both species (Figure 6E, F). The exopods is large and bears fan-like plumed setae similarly to that of the 2nd maxilliped in *L. vannamei* (Figure 6F).

Mandible: Figure 7 shows mandibles for the female *M. rosenbergii* (total length 145 mm) and the female *L. vannamei* (total length 140 mm). The mandible is spindle-shaped and consisted of 3 teeth and a molar (a grinding process) and is connected with the strong mandibular muscle in *M. rosenbergii*. The mandible of *L. vannamei* lacks the hard teeth and the molar and the mandibular palp is flattened and its rim is packed with pappose setae resembling that of tiger prawn (*Penaeus monodon*) (Garm, 2004). The structure of mandible of *M. rosenbergii* is hard compared to that of *L. vannamei* which is a bit softer. The incisor process of *M. rosenbergii* is larger and much heavier (5.86 mm, 0.694 g) compared to that of *L. vannamei* (3.96 mm, 0.035 g).

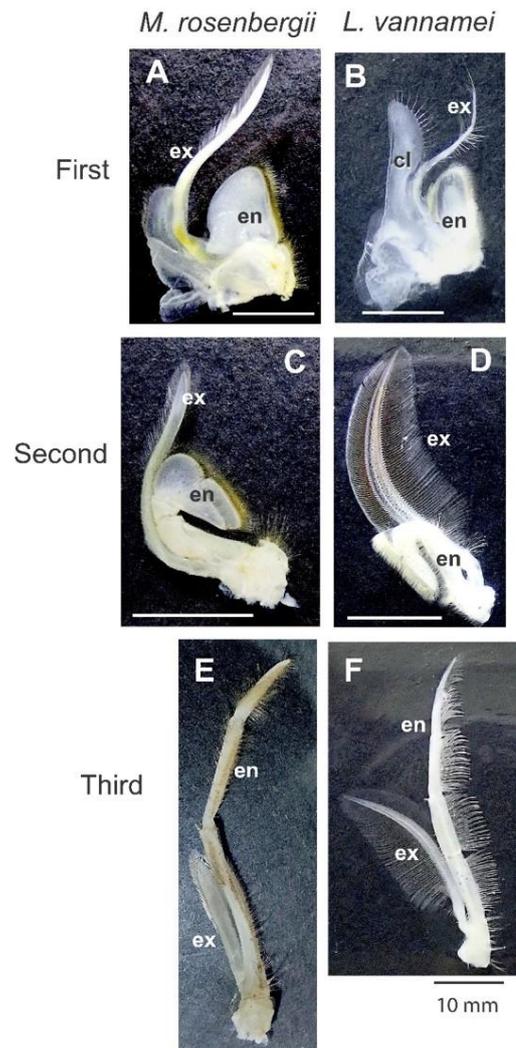


Figure 6. Photomicrographs of the three right-side maxillipeds of *Macrobrachium rosenbergii* (A, C, E) and *Litopenaeus vannamei* (B, D, F) showing the distal exopods (ex), the proximal endopods (en), the caridean lobe (cl), and feather-like setae.

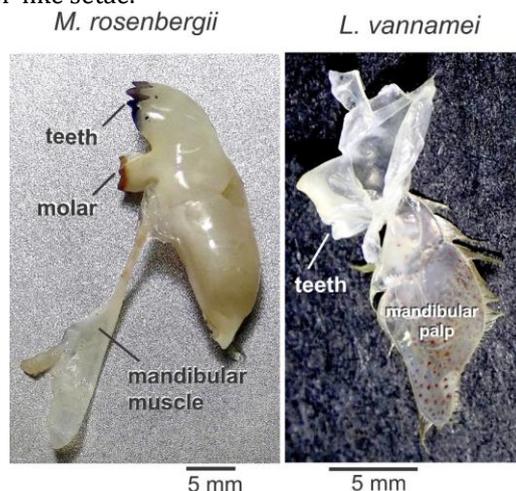


Figure 7. Photographs of the dissected mandibles of *Macrobrachium rosenbergii* and *Litopenaeus vannamei*. The incisor process of *M. rosenbergii* is larger and much heavier (5.86 mm, 0.694 g) compared to that of *L. vannamei* (3.96 mm, 0.035 g).

Motion pattern of feeding appendages during food intake

Pellet food

M. rosenbergii

Searching of food was done with the chelate walking legs; the 2nd walking legs were extended forwards and moved slowly sidewise and the 1st walking legs were actively moved in front of the mouth. The endopods of the 3rd maxillipeds were kept extended forward during the searching.

M. rosenbergii adults picked up one or two pellets with the 1st and 2nd pincers (Figure 8A, B) and conveyed one to the mouth and crunched with the mandibles (Figure 8C). Immediately after the pellet was held with the mandibles the empty pincer was again employed for picking up another pellet, and an actively feeding specimen frequently held three pellets, one in each pincer, while at the same time there was already one pellet in its mouth (Figure 8A). Sometime one was in the mouth and second one was held with the pair of 3rd maxilliped endopods at the mouth (never more than two at the mouth). In a video, an actively feeding adult crunched 12 pellets in 79 s.

The juvenile *M. rosenbergii* picked up a pellet of conventional size with the 1st and 2nd pincers and conveyed it to the mouth, held it with a pair of the 3rd maxilliped endopods, shredded it with a pair of mandibles only a small part or half and spat out the remained mass, and quitted feeding. They did not crunch the pellets. When the pellets were small, they often held 2 or 3 pellets at the mouth but masticated one by one. The juveniles often spat out the small pellets without consuming all and quitted eating.

Figure 9 compares *M. rosenbergii* juveniles' handling time (time from a convey to the next convey) between the pellets of large and small sizes. The time for the larger pellets was 20–178 s (median 59.5 s) and 19–213 s (median 45 s) for the small pellets. While the median time was shorter in the smaller pellets than that in the larger pellets, the difference was statistically insignificant (t -test after log transformation, $t = 1.433$, $P = 0.803$), indicating that the handling time was not significantly affected by the size of pellets. However, the juveniles spat out 55% of the larger pellet ($n = 20$) and 7 % of the smaller pellets ($n = 46$) without consuming all.

While they used both the right and left pincers in picking up pellets, the right pincers were used significantly more often than the left ones (adults, 85:53; juveniles, 44:16) (χ^2 -test, $0.005 < P < 0.010$) (Table 3). The adults used the 1st pincers significantly more often than the 2nd ones (109:28) (χ^2 -test, $P < 0.005$) (Table 3) while the juveniles used the right 2nd pincers most often (χ^2 -test, $P < 0.005$) (Table 3). It was often observed that the adults picked up a pellet with the 2nd pincer and passed it to the 1st pincer which conveyed it to the mouth.

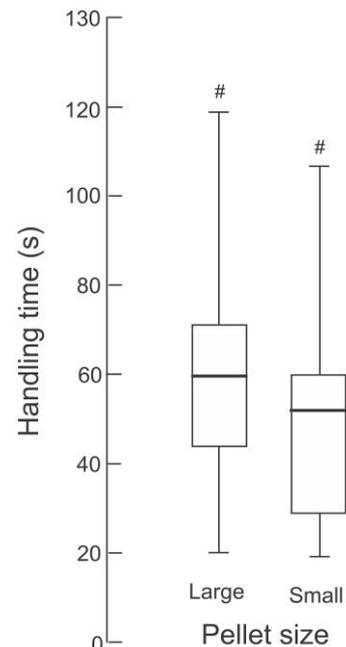


Figure 9. *Macrobrachium rosenbergii* juveniles show no significant difference in handling times between large (length 4.67 ± 0.11 mm, mean \pm SD mm) and small (2.35 ± 0.07 mm) pellets.

L. vannamei

L. vannamei searched for food with the chelate walking legs by probing tank bottom and picked up pellets with the 1st, 2nd and 3rd pincers (Figure 10), mostly the 1st pincer followed by the 2nd one, and the 3rd one played least role in picking up pellets (88:44:10) (χ^2 -test, $P < 0.025$) (Table 4). They picked up pellets more frequently with right pincers than the left ones (93:45) (χ^2 -test, $P < 0.005$) (Table 4). The adults picked up a pellet with right 2nd pincer and tossed to the 1st pincer which conveyed it to the mouth. Juveniles also used mostly the 1st pincers followed by the 2nd ones and the 3rd ones played a least role in picking up pellets (62:19:3) (χ^2 -test, $P < 0.005$) (Table 4).

The 3rd walking legs were found to be used for feeding contests aggression between opponents in the video observations. *L. vannamei* aggressively extended the 3rd walking legs toward an opponent each other and violently moved them and fought each other until one of them withdrew.

L. vannamei adults held 1 to 8 pellets at one time (Figure 10D) with a pair of the 3rd maxilliped endopods at the mouth but only one was inserted vertically into the mouth. While the mandibles continuously vibrated and simultaneously flicked each other, this action shredded only a small part and the remained mass was spat out. Immediately after spitting out the remained mass, a next pellet was conveyed to the mouth. *L. vannamei* adults and juveniles repeated this feeding behaviour in most cases. In a video, a pellet was not crushed after 39 s flicking motion of the mandibles. During this motion, complete maceration of the pellets was not observed.



Figure 8. *Macrobrachium rosenbergii* handling pellets. A, Pincers of right and left walking legs 2w picking up pellets (indicated by *). B, A pellet being passed by right 2w to right 1w. C, The paddle-like exopods of the third maxillipeds 3m holding a pellet at the mouth and the pincer of 1w holding another.

◀ Anterior

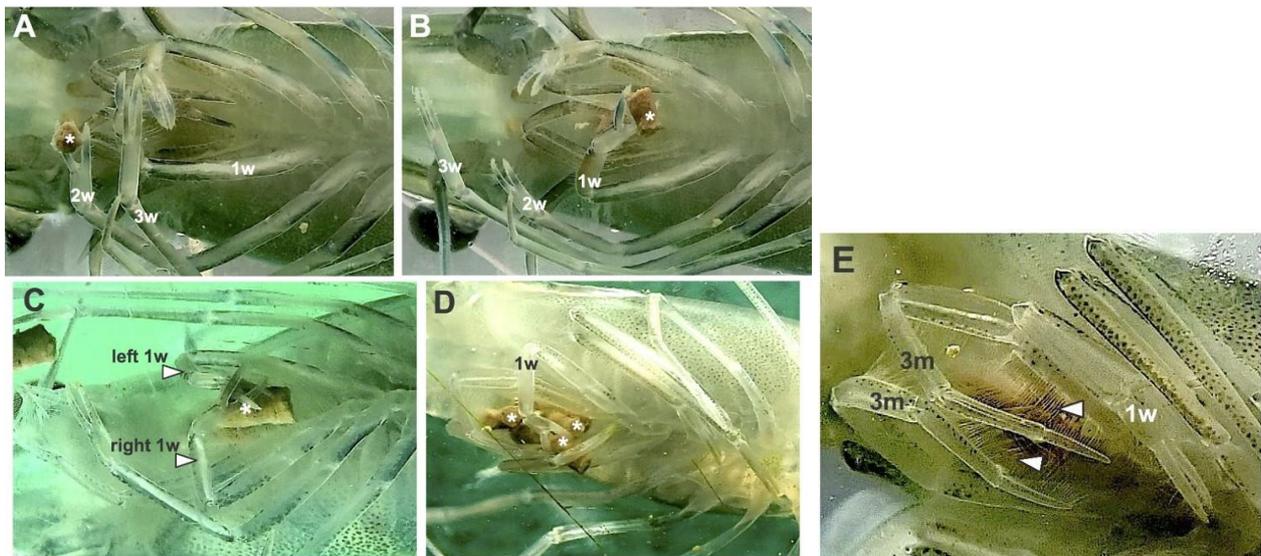


Figure 10. *Litopenaeus vannamei* handling pellets. A and B, Pincers of walking legs 1w and 2w picking up pellets (indicated with *). C, Pincers of 1w (arrowheads) holding a pellet (*) at the mouth. D, 8 pellets held at the mouth by the pad-like endopods of the 1st maxillipeds and the pincers of 1w. E, Pellets held in place by the feather-like setae (arrowheads) of the long endopods of the 3rd maxillipeds 3m.

Table 3. *Macrobrachium rosenbergii*. Grabbing pellet frequencies by the right and left pincers.

Stage	Frequency				χ^2 -test
	Right 1st pincer	Right 2nd pincer	Left 1st pincer	Left 2nd pincer	
Adult	73	12	37	16	$\chi^2 = 7.949$ $0.005 < P < 0.010$
Total	85		53		
Juvenile	15	29	8	8	$\chi^2 = 13.067$ $0.005 < P < 0.010$
Total	44		16		

Table 4. *Litopenaeus vannamei*. Grabbing pellet frequencies by the right and left pincers.

Stage	Frequency						χ^2 -test
	Right 1st pincer	Right 2nd pincer	Right 3rd pincer	Left 1st pincer	Left 2nd pincer	Left 3rd pincer	
Adult	56	28	9	32	12	1	$\chi^2 = 16.696$ $P < 0.005$
Total	93			45			
Juvenile	35	16	3	27	3	0	$\chi^2 = 66.499$ $P < 0.005$
Total	54			30			

Shrimp eggs

A female *M. rosenbergii* ate her own eggs scattered on the tank bottom and accidentally video recorded for 86 s. Discarding and eating undeveloped eggs by berried females are commonly observed in decapod crabs and shrimps in tanks (Annita Yong Seok Kian, personal observation). The female *M. rosenbergii* picked up eggs with a pair of the 1st pincer and quickly conveyed to the mouth alternately (Figure 11) at a frequency of 2.3/s. When the 1st pincer picked up an egg clump, more than two eggs were grabbed with the pincer at one time. The 2nd and 3rd pincers and the maxilliped endopods were not employed in eating eggs.

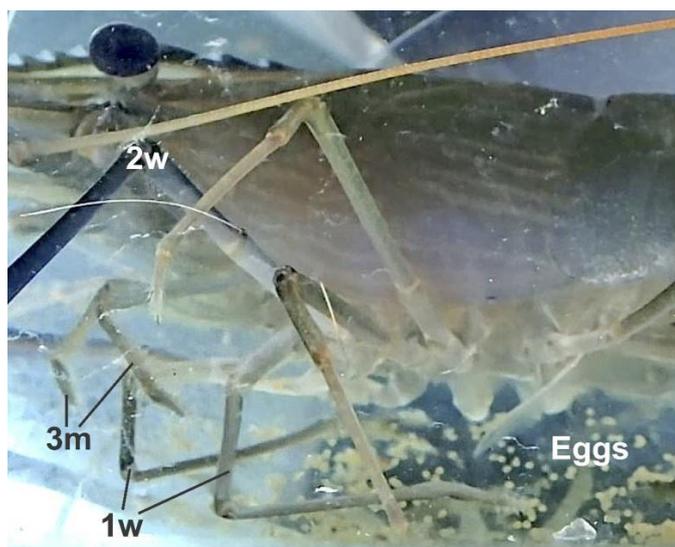


Figure 11. *Macrobrachium rosenbergii*. Berried female picking up her eggs scattered on the aquarium bottom with the walking legs 1w. 2w, 2nd walking leg; 3m, 3rd maxilliped endopod

Elastic squid and fish foods

During the video recordings, pieces of squid mantle were given to adult specimens of *M. rosenbergii* and *L. vannamei* and pieces of fish flesh were given to *L. vannamei* adults and juveniles. Both the species accepted and ingested these food items without any hesitation on the first day of the experiment.

M. rosenbergii

While *M. rosenbergii* clamped an end of a piece of squid with the mandibles, the maxilliped endopods held it and pulled the main mass downward repeatedly until a morsel was tore in the mouth (Figure 12). The 2nd maxillipeds did not indulge in this sort of tearing. In a video recording, a series of this tearing motion was continued for 5 min 30 s until the whole squid piece was ingested. In other two videos, half of a main squid mass was discarded without consuming all. *M. rosenbergii* touched with the 1st and 2nd pincers but did not eat it in the 9 videos prepared, and totally disregarded in 2 videos out of 21 videos recorded with squid food. *M. rosenbergii* ingested squid pieces of 1st pincer size without tearing them in three videos.

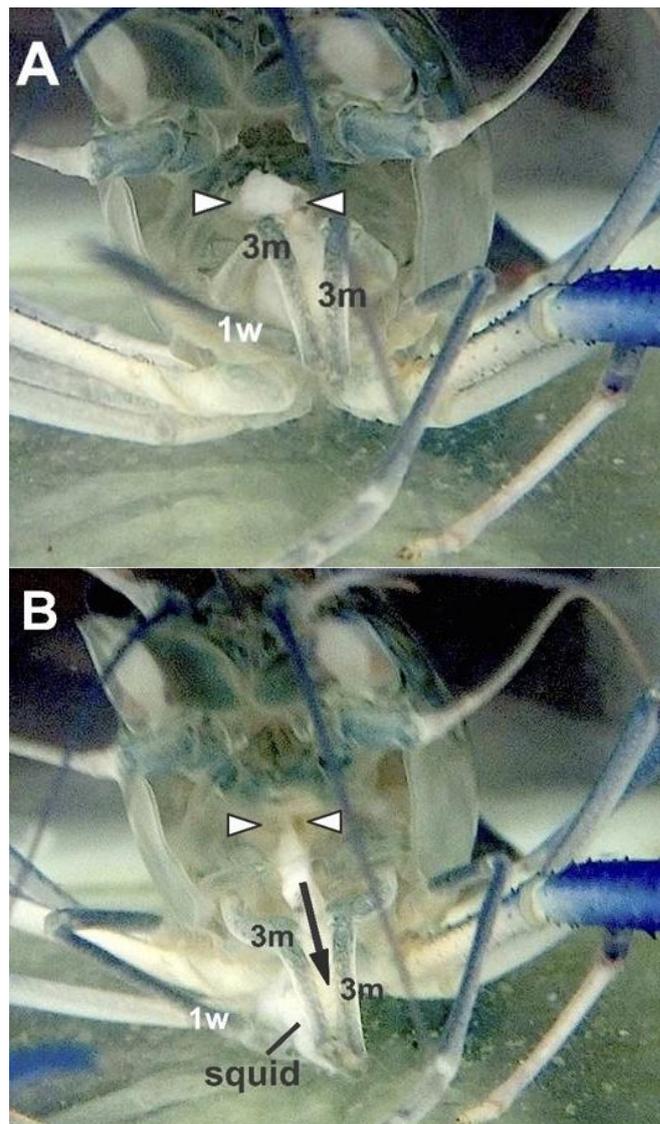


Figure 12. *Macrobrachium rosenbergii*. Screenshots showing motion of the 3rd maxilliped endopods (3m) and first walking legs (1w) to tear a piece of squid muscle (white object). A, Mandibles (white arrow heads) and pincer of 1w holding squid piece at the mouth. B, Endopods of 3m pulling down (arrow) the squid piece

L. vannamei

L. vannamei exhibited similar motion of the appendages during the tearing squid muscle and fish flesh shown by *M. rosenbergii*, but ingested all pieces of the squid and fish flesh (Figure 13).

Food tearing time

Figure 14 compares the time expended for tearing a morsel between squid and fish food for the adults of *L. vannamei* and *L. vannamei*. The time was significantly much shorter for fish pieces than for squid pieces at $\alpha = 0.05$, showing that the fish was much easier to be torn than the squid for *L. vannamei*. *L. vannamei*: squid, $n = 81$, median = 19 s, 95% confidence interval = 18.04–35.81 s; fish pieces, $n = 86$, median = 5 s, 95% confidence interval = 7.35–17.71 s. *M. rosenbergii*: squid pieces, $n = 25$, median 15, 95% confidence

interval = 11.60–24.98 s. While statistically insignificant, *M. rosenbergii* tore the squid pieces in shorter time than *L. vannamei* indicating tearing squid is easier for *M. rosenbergii* than for *L. vannamei*. *L. vannamei* tore the fish pieces in much more shorter time than *M. rosenbergii*.

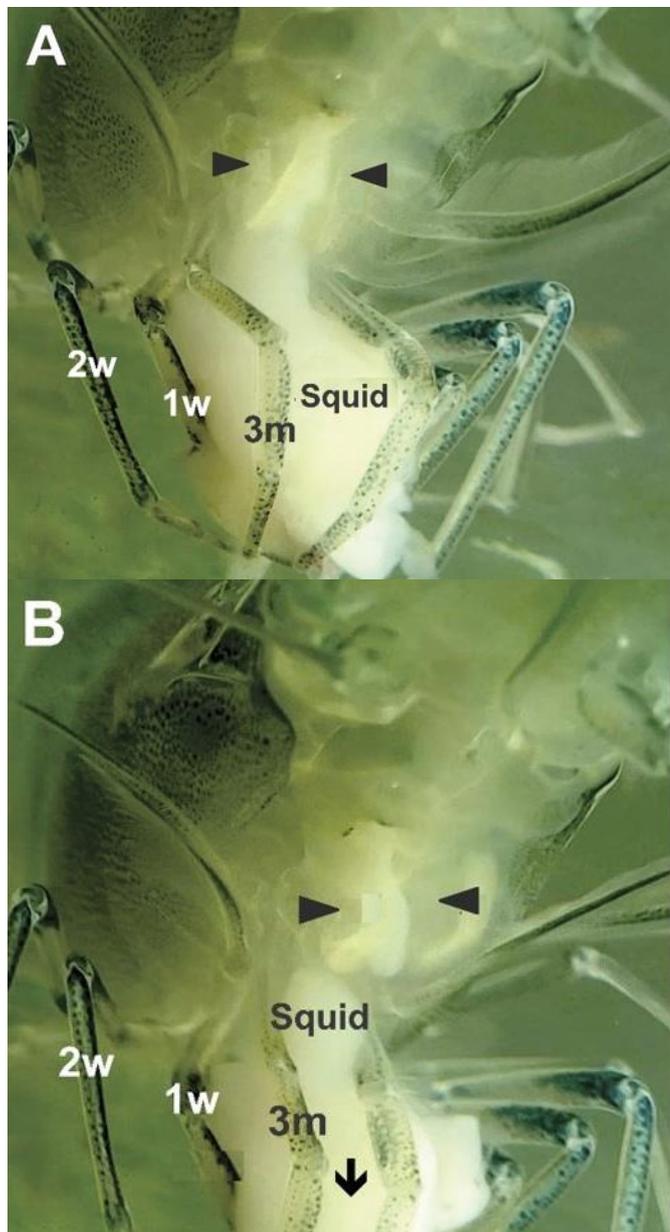


Figure 13. *Litopenaeus vannamei*. Screenshots showing motion of the 3rd maxilliped endopods (3m) and first walking legs (1w) to tear a piece of squid muscle (white object). A, 1w and 3m holding squid piece at the mouth. B, Mandibles (black arrowheads) clamping one end of the squid.

Fanning motion of exopods and current produced

Both species extended the fan-like exopods of the 2nd and 3rd maxillipeds and exhibited rigorous fanning motion regardless of the presence or absence of food in the tanks (Figure 15). The fanning motion was more prominent in *L. vannamei* than in *M. rosenbergii* due to the larger exopods of the former. The water flow produced by the fanning motion

was visualized with milk and shown in Figure 16 for *L. vannamei*. The epipod of 1st maxilliped exhibited a continuous slow waving motion which might be involved in ventilation. The movement of the milk plume showed unidirectional water flow entering the anterior part of the gill chamber, flowing through inside the gill chamber (flow speed at 0.7 carapace length/s) and exiting posteriorly. We did not observe the frontward reversal current.

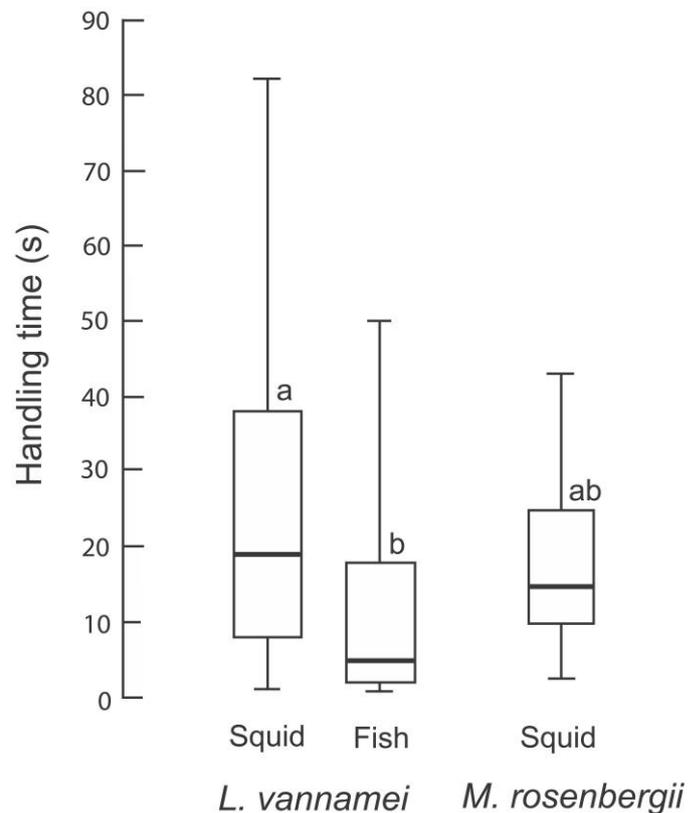


Figure 14. *Litopenaeus vannamei* and *Macrobrachium rosenbergii* handling times (expended for tearing pieces of squid and fish before ingestion).

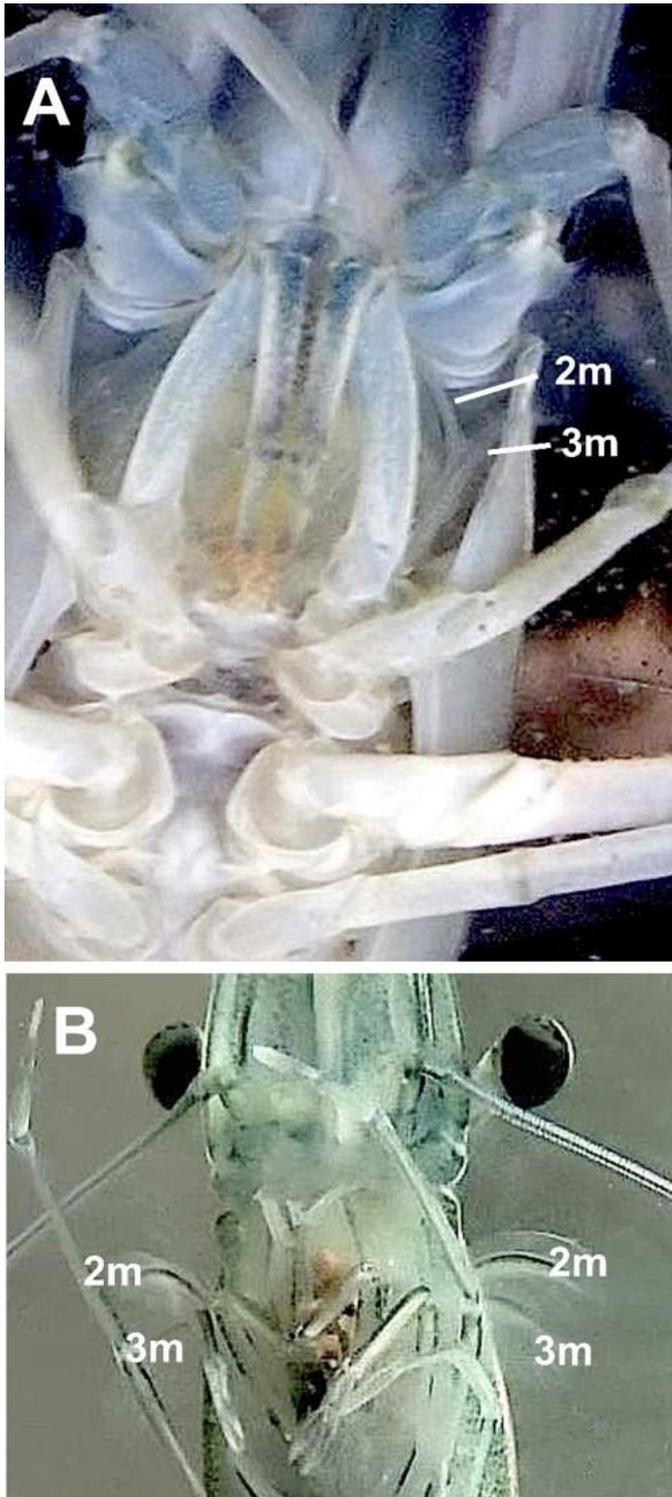


Figure 15. *Macrobrachium rosenbergii* (A) and *Litopenaeus vannamei* (B). Screenshots showing fanning motion of the exopods of maxillipeds 2m and 3m.

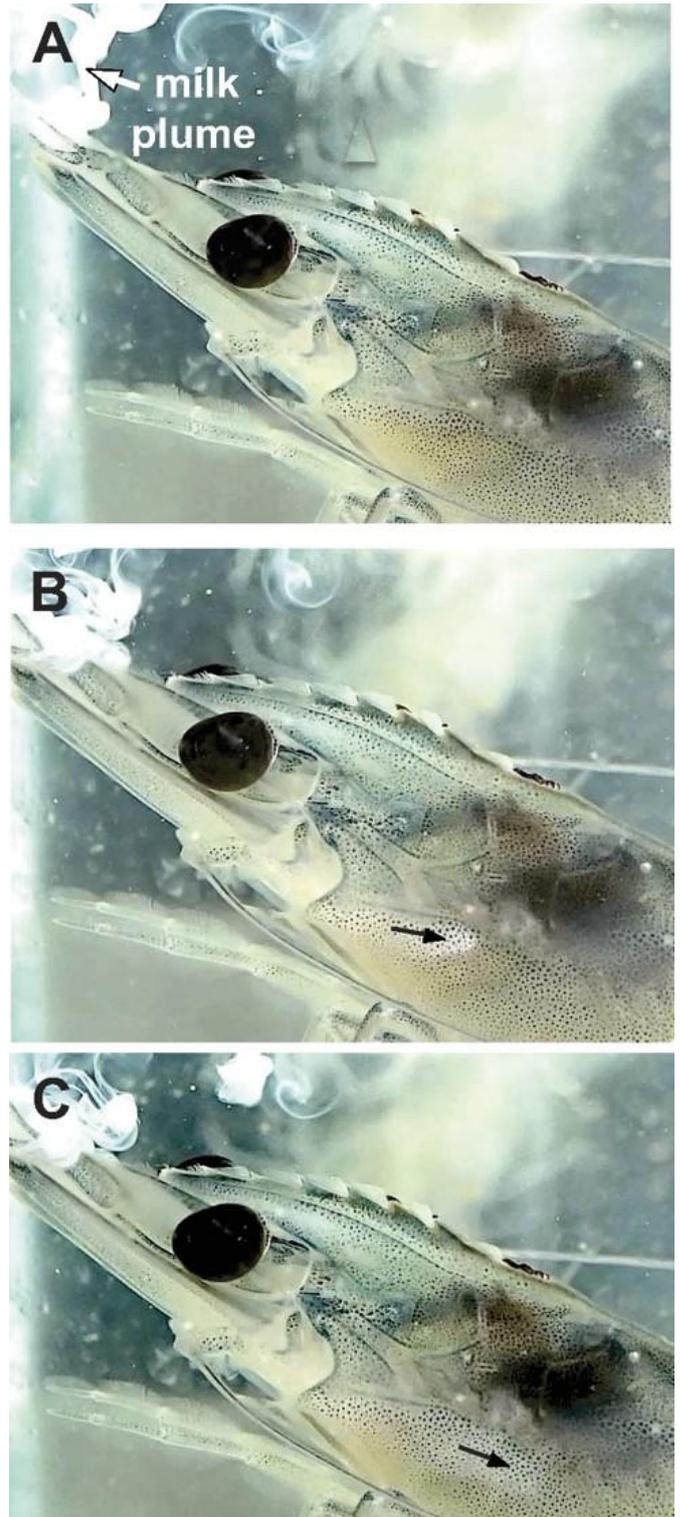


Figure 16. *Litopenaeus vannamei*. Successive screenshots showing the movement of milk plume from the front (A) to the back passing through inside the gill chamber (B and C, shown by black arrows) of the shrimp. Movement speed of the milk plume was 0.7 carapace length/s in the carapace due to the fanning motion of the exopods of the 2nd and 3rd maxillipeds.

Discussion

Function of walking legs in feeding

Both species searched for food only with chelate walking legs by probing the bottom and then picked up the items. The food picking up by the first three pair of walking legs was also reported for the banana prawn (*Penaeus merguensis*) (Hindley and Alexander, 1978). The walking legs without a chela were not involved in food searching. Though the dactyls of the walking legs without chela bear thick (*M. rosenbergii*) or thin setae (*L. vannamei*) the setae seems not to be chemosensitive to food organisms.

The foods picked up and conveyed to the mouth were held with a pair of the 3rd maxilliped endopods and a pair of the 1st walking legs. The pincers of the 1st walking legs were used also for gill cleaning in *L. vannamei*.

In both species, the walking legs were not equally used in picking up foods. *M. rosenbergii* adults used predominantly the 1st pincers and juveniles used the 2nd pincers. *L. vannamei* adults and juveniles used predominantly the 1st pincers in picking up foods. Both species were 'right-handed' in picking up foods. The biological significance of right-handed pincers is unknown. In *L. vannamei*, among the three paired walking legs, the 3rd walking legs were longest and heaviest and seemed to be adapted to aggressive actions rather than the feeding behaviour. *L. vannamei* is a strong aggressor. Chavanich *et al.* (2016) examined feeding behaviour of the invasive *L. vannamei* and five Thai native shrimp species and reported that *L. vannamei* was much more aggressive in competing for food than the native shrimps.

The role of pincers seems different among shrimp species. The chelate walking legs were reported to play a role in cutting large food items in the eastern king prawn (*Penaeus plebejus*) (Suthers, 1984). However, in the present study, we did not observe cutting of elastic foods with pincers in *M. rosenbergii* and *L. vannamei*.

Function of maxillipeds in feeding

The two species picked up pellets and elastic foods only with chelate walking legs and the maxilliped endopods were not involved in this feeding behaviour. Nicol (1932) reported that when Galathea was feeding on finely divided material the 3rd maxillipeds were used for collecting food. Suthers (1984) stated that the eastern king prawn gripped food items with 3rd maxillipeds. Garm (2004) examined mechanical functions of mouth apparatus of seven species of decapod crustaceans such as tiger prawn (*Penaeus monodon*), Baltic prawn (*Palaemon adspersus*), banded coral shrimp (*Stenopus hispidus*), red claw crayfish (*Cherax quadricarinatus*), Caribbean spiny lobster (*Panulirus argus*), hermit crab (*Pagurus bernhardus*), and shore crab (*Carcinus maenas*) and reported that the 3rd maxilliped endopods pick up prey items either directly from the substratum or from one of the walking legs. Kalpana and Meena (2016) reported that the freshwater prawn (*Macrobrachium kistnensis*) picked up food particles with the chelate walking legs and the 3rd maxillipeds and conveyed them to the mouth.

Kawamura *et al.* (2017) reported that *L. vannamei* with pincers cauterized changed food intake manner and picked up pellets with the 3rd maxilliped. However, in the present study, we did not observe picking up the foods with the 3rd maxilliped endopods regardless of food size. A female *M. rosenbergii* used a pair of the 1st pincers to pick up her discarded eggs. The mean size of *M. rosenbergii* eggs was reported as small as 0.67 mm by Habashy *et al.* (2012).

Function of mandibles in feeding

The major morphological difference in the mouthparts between *M. vannamei* and *L. vannamei* is in the mandibles. Both the species did not snip foods with the pincers and were not able to process the food exteriorly. All foods were masticated only with the mandibles prior to ingestion. During feeding, the incisor process has been believed to be mostly used in cutting and slicing of food particles into more manageable portions while the molar process is usually thought to have a grinding function (Bauer, 2004). Lavalli and Factor (1992) stated that, as the molar process in the juveniles of the lobster (*Homarus americanus*) is reduced or recessed from the incisor process, the mandibles do not grind food prior to ingestion, but mastication is relegated to the gastric mill. Although the mandibles lack the molar process, *L. vannamei* adults in this study, tore squid food into small pieces by the action of the mandibles and 3rd maxilliped endopods, masticated and ingested it, showing that *L. vannamei* is able to masticate food by mandibles without the molar process. The mastication with the mandibles was evident during eating the elastic and hard foods.

The role of the mandibles seems to be species specific. Barker and Gibson (1977) examined the tearing process of the European lobster eating fish flesh and reported that the mandibles did not possess a masticatory function, merely serving to grip food morsels during the tearing process. This action was affected by the pulling action of the third maxillipeds. Felgenhauer and Abele (1985) found that the mandible of two shrimps *Atya innocuous* and *Potimirim glabra* was not used for crushing the food. Ashelby *et al.* (2015) stated that the form of the mandible is much more complex than previously thought and the arrangement and form of the teeth in *Gnathophyllum elegans* and *Hymenocera picta* suggest that it does not grind food at all.

The mandible of *L. vannamei* lacked the molar and was not hard enough to masticate the pellets indicating the ability of *L. vannamei* was limited to prey of a particular hardness. The mandible of *M. rosenbergii* was hard and effectively masticated a range of food items of different hardness in the present study. Ogle and Beaugez (1991) conducted food preference test using 14 food items in the laboratory and reported that *L. vannamei* preferred fresh food such as *Artemia*, krill, bloodworms, oyster, sandworm, anchovy and squid over 5 different types artificial pellet foods including three maturation pellets. This preference might indicate that *L. vannamei* showed preference for elastic foods over hard artificial pellets.

In the wild, *L. vannamei* is carnivorous and consumes a wide variety of invertebrates such as polychaetes, molluscs, and crustaceans (Panutrakul *et al.*, 2010). From an evolutionary point of view, *L. vannamei* did not need to develop hard mandibles for masticating hard prey organisms. On the other hand, *M. rosenbergii* depends on a larger variety of food items such as aquatic worms, insects, insect larvae, small molluscs, crustaceans, fish, and materials of plant origin (D'Abramo and New, 2000). The hard and robust mandibles enable *M. rosenbergii* to pursue voracious feeding habits. Even though the mandible of *M. rosenbergii* was hard and strong enough to crunch pellets, they were not sharp enough to gnaw off fibrous squid food in one bite.

Food acceptability and palatability

Measuring the amount of consumed food in a certain time commonly helps in the assessment of food acceptability (Lim *et al.*, 2016; Lim *et al.*, 2017; Teoh *et al.*, 2018). Holland and Borski (1993) stated that, unlike food test which incorporate stimuli into pellet food, assay of agar discs infused with test stimuli is independent of confounding factors such as pellet size, texture, or hardness, and the results are not influenced by other compounds extant in the pellets. However, the food acceptability assessment is usually done based on several parameters such as the chemical and physical characteristics of food (Quintero and Roy, 2010).

The final step in the feeding process is usually affected by taste in fish (Harpaz, 1997). However, in decapod crustaceans, since a feeding process inevitably includes mastication and chewing, the physical characters of food such as size and hardness are important for total palatability assessment. Harvey and Epifanio (1997) stated that, in farming environment, size and hardness are more important than the taste and odour of artificial food for the common mud crab (*Panopeus herbstii*). *M. rosenbergii* adults, in this study, accepted well the fibrous squid food given in the beginning of the experiment but often rejected or disregarded later, indicating that *M. rosenbergii* negatively responded to the squid food after the feeding experience. The jetting squid mantle is chiefly composed of a mass of circular muscle collagen fibres and thick sheets of connective fibres that reinforce the muscle fibres (Macgillivray *et al.*, 1999). Fish somatic muscle is striated and consisted of myotomes (muscle fibres) and myocommata (connective tissue makes up 2 to 5% of the muscle) (Kießling *et al.*, 2006). Due to this difference in musculature of squid mantle and fish flesh, latter can be much readily torn. Thus the masticability of elastic food is important for the crustaceans. The total palatability assessment suggested that the fish flesh was more palatable than squid for *M. rosenbergii* and *L. vannamei*.

The size of pellet, in this study, did not significantly affect the feeding manner of *M. rosenbergii* juveniles but frequent spitting out larger pellets (length 4.67 mm) indicated easier mastication for smaller pellets (2.35 mm). Quintero and Roy (2010) stated that parameters that should be evaluated for shrimp culture include physical

characteristics such as colour, pellet size and moisture in feed evaluation and acceptance. However, Obaldo and Masuda (2006) reported that the variation in pellet size (0.7, 1.2 and 3.0 mm) did not affect the growth, food conversion ratio and survival of *L. vannamei* and noted that the shrimp fed with larger pellet were significantly more aggressive than those fed with smaller pellet during feeding, and recommended video observations of feeding behaviour. *L. vannamei* juveniles spat out 55% of the larger pellet and 7 % of the smaller pellets without consuming all in this study, indicating the preference of the juveniles for the smaller pellet.

Function of fanning motion of exopod in ventilation

L. vannamei and *M. rosenbergii* have fan-shaped exopods of the 2nd and 3rd maxillipeds, which are very different from those of the banana prawn (*P. merguensis*) reported by Alexander *et al.* (1980). *L. vannamei* and *M. rosenbergii* exhibited continuous horizontal fanning motion of the exopods regardless of the presence or absence of food at the mouth, and this fanning motion produced an unidirectional water flow moving backwards in the gill chamber (visualized with milk) in this study. This means that the fanning motion of the exopods is involved not in feeding but in ventilation. Hunt *et al.* (1992) noted the irrigation of the gill chamber by the rapid beating action of the 2nd maxillae of *P. merguensis* and opined that this action to not related to ventilation but to the filter feeding by the comb setae of the maxillae. Garm and Høeg (2001) reported that, in decapods, the 2nd maxilla is responsible for ventilating the gills *via* the scaphognathite. There is a lateral flap on the 2nd maxilla and, by beating a narrow pump chamber, draws a respiratory stream of water through the bronchial chamber (Warner, 1977). Nicol (1932) showed the direction of the respiratory current around the mouthparts when the flagella of the exopods of both maxillipeds were beating; the current was complex and not unidirectional. Barker and Gibson (1977) stated the possibility of involvement of wafting action of the exopods in the exhalant current which swept small particles away from the mouth in the European lobster. However, water flow visualization had never been done in those studies.

It is important to visualize the current in the gill chamber and in the vicinity of the mouth for understanding ventilation. The visualization was done for the first time in the present study and the continuous unidirectional current was confirmed in video observations. The continuous slow waving motion of the epipods, lateral extensions from the base of the 1st maxilliped, was also video observed through the translucent carapace even when the fanning motion of the exopods stopped. The waving motion of the 1st maxillipeds seemed to be involved in ventilation.

Recommendations

The hardness of foods has an important effect on the behaviour of the shrimp in its intake. In this study, *L. rosenbergii* could not masticate the pellet well and repeatedly spat out them because the pellets were hard to

crunch. If *L. rosenbergii* were fed with softer pellet they might crunch it in much the same way as *M. rosenbergii* did. Therefore, the hardness of the present formulated pellets should be improved to acceptable levels for captive shrimp stocks.

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