Chemosensitivity and role of swimming legs of mud crab, *Scylla paramamosain*, in feeding activity as determined by electrocardiographic and behavioural observations

Gunzo Kawamura 1, Chi Keong Loke 1, Leong Seng Lim Corresp. 1, Annita Seok Kian Yong 1, Saleem Mustafa 1

1 Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

Corresponding Author: Leong Seng Lim
Email address: leongsen@ums.edu.my

Swimming crabs have a characteristic fifth pair of legs that are flattened into paddles for swimming purposes. The dactyl of these legs bears a thick seta along its edge. The chemoreceptive and feeding properties of the seta are supported with scientific evidence, however, there is no available data on the sensitivity of the setae in portunid crabs. The underlying mechanisms of the chemo- and mechano-sensitivity of appendages and their involvement in feeding activities of the mud crab (*Scylla paramamosain*) were investigated using electrocardiography and behavioural assay, which focused on the responses of the mud crab to chemical and touch stimulus. Electrocardiography revealed the sensory properties of the appendages. The dactyls of swimming legs and the antennules were chemosensitive, but not mechanosensitive and vice versa for the antennae. However, the mouthparts, claws, and walking legs were chemo- and mechanosensitive. Only the chemosensitive appendages, including the swimming legs, were directly involved in feeding. The flattened dactyls of the swimming legs were more efficient than the pointed dactyls of the walking legs in detecting the food organism crawling on the substrate. The structural features enhanced the capacity of the crab in coming into contact with scattered food items. This study revealed that the swimming legs are important appendages for feeding in the mud crab.
Chemosensitivity and role of swimming legs of mud crab, *Scylla paramamosain*, in feeding activity as determined by electrocardiographic and behavioural observations

Gunzo Kawamura¹, Chi Keong Loke¹, Leong-Seng Lim¹*, Annita Seok Kian Yong¹ and Saleem Mustafa¹

¹Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia

E-mail: leongsen@ums.edu.my

ABSTRACT

Swimming crabs have a characteristic fifth pair of legs that are flattened into paddles for swimming purposes. The dactyl of these legs bears a thick seta along its edge. The chemoreceptive and feeding properties of the seta are supported with scientific evidence, however, there is no available data on the sensitivity of the setae in portunid crabs. The underlying mechanisms of the chemo- and mechano-sensitivity of appendages and their involvement in feeding activities of the mud crab (*Scylla paramamosain*) were investigated using electrocardiography and behavioural assay, which focused on the responses of the mud crab to chemical and touch stimulus. Electrocardiography revealed the sensory properties of the appendages. The dactyls of swimming legs and the antennules were chemosensitive, but not mechanosensitive and vice versa for the antennae. However, the mouthparts, claws, and walking legs were chemo- and mechanosensitive. Only the chemosensitive appendages, including the swimming legs, were directly involved in feeding. The flattened dactyls of the swimming legs were more efficient than the pointed dactyls of the walking legs in detecting the food organism crawling on the substrate. The structural features enhanced the
capacity of the crab in coming into contact with scattered food items. This study revealed that the swimming legs are important appendages for feeding in the mud crab.

Keywords
Feeding appendages, ECG, Sensory system, Antenna, Antennule, Mud crab

INTRODUCTION

Mud crabs, *Scylla* spp., are distributed in estuarine, sheltered coastal habitats, and mangroves in the Indo-Pacific region and South Africa (*Ikhwanuddin et al.*, 2011; *Alberts-Hubatsch et al.*, 2016). Four commercially important *Scylla* species (*S. serrata*, *S. olivacea*, *S. paramamosain*, and *S. tranquebarica*) live in the same geographical area in Malaysia (*Alberts-Hubatsh et al.*, 2016) and are often captured in the same traps (*Kawamura et al.*, 2021). In the wild population, inter-species mating has also been reported (*Fazhan et al.*, 2020).

In crustaceans, all the appendages and several body parts are responsible for chemoreception (*Ache*, 1982). The most important chemosensory structures include the antennae, antennules (first antennae), walking legs, and mouthparts (*Schmidt & Mellon*, 2011). Several investigations have been conducted on lobsters (*Nishida et al.*, 1990; *Gomez & Atema*, 1996; *Garm & Høeg*, 2000; *Garm* 2004a; *Garm et al.*, 2005; *Sahlmann, Chan & Chan*, 2011), crayfish (*Dunham & Oh* 1992; *Garm*, 2004b; *Mellon*, 2012), hermit crab (*Garm 2004a; Kamio et al.*, 2005), and blue crab *Callinectes sapidus* (*Gleeson, Adams & Smith*, 1984; *Keller, Powell & Weissburg*, 2003; *Aggio et al.*, 2012). Nevertheless, *S. serrata* has been studied for the chemosensory function of its appendages amongst the *Scylla* spp. (*Wall, Paterson & Mohan*, 2009).

Decapod crustaceans possess setae on many parts of their appendages (*Ache*, 1982). To understand the behaviour of decapod crustaceans in the wild and under culture environments, it is important to study the sensory function of the appendages and their role in feeding and social communication. The setae could either be sensory or non-sensory. The former includes chemoreception or mechanoreception, which are determined using either one or a combination of behavioural, physiological, and morphological analysis. For studies based on behavioural
assessment, an animal may have the capacity to perceive a stimulus, but it does not always lead to a change in response (Nishi & Kawamura, 2005). Physiological-based studies are limited as a result of non-detection of neural response, especially when the receptor’s size is either small or labile (Kawamura, 1981; Jordão, Cronin & Oliveira, 2007), and when receptor potentials are unstable (Kawamura et al., 2020). The availability of different morphological types of setae among the decapod species makes the method uncertain while morphological analysis of setae provides a better understanding of the structures and functions (Garm, 2004b).

Electrocardiography is a reliable method of detecting the sensitivity of an animal to external stimuli (Kawamura, Nakaizumi & Motohiro, 1992; Nishi, Kawamura & Matsumoto, 2004; Nishi & Kawamura, 2005). In the tetrapods, the heart pacemaker is innervated by the excitatory sympathetic nervous system and the inhibitory parasympathetic nerve. However, in the heart of teleosts, the parasympathetic vagus nerve is the sole regulator of heart rate. Activation of the vagus nerve results in the release of acetylcholine – a neurotransmitter substance that acts via muscarinic receptors to influence the force and rate of contraction of the heart muscles. During resting or inactivity, inhibitory vagal stimulation on the heart is absent (Taylor et al., 2007). Fishes and crustaceans show a typical electrocardiogram response, slower heartbeat, or wider interbeat interval, in response to external stimuli. Electrocardiography has been applied to examine the response of crab species to temperature, pressure (Mickel & Childress, 1982), visual stimulus (Grober, 1990; Hermitte & Maldonado, 2006; Burnovicz, Oliva & Hermitte, 2009; Burnovicz & Hermitte, 2010), and chemical stimulus (Ketpadung & Tangkrockolan, 2006; Medesani et al., 2011).

Mud crab (family Portunidae) is distinguished from other crabs by the structural adaptation of their fifth legs, which are transformed into flattened paddles and used for swimming purposes (White & Sprito, 1973). Hence, the structures are known as swimming legs. The swimming legs confer on the crabs a power to dart at high speed through water bodies, while the dactyl bears thick setae along its edge. The chemoreceptive and feeding properties of the setae have been studied by different authors, however, there is no available data on the sensitivity of the setae in portunid crabs. This study investigated the chemo- and mechano-sensitivity of appendages and their involvement in feeding activities of the mud crab (Scylla paramamosain) using
electrocardiography and behavioural assay. Both techniques were focused on the responses of the
mud crab to chemical and touch stimulus.

MATERIALS & METHODS

Crabs and holding condition
A total of 20 female and male specimens of adult mud crab, *S. paramamosain*, (9−11 cm in
carapace width, 80−100 g in body weight) caught from the wild population were obtained from a
local market, Kota Kinabalu, Sabah. They were stocked in a plastic tank (122 cm × 148.5 cm, 55
cm high) filled with 55 L of seawater (30 cm deep) and placed in the roofed Shrimp Hatchery,
Borneo Marine Research Institute, Universiti Malaysia Sabah. Half of the tank bottom was covered
with 5 cm thick coral rubble to function as a biofilter substrate. Five pipes of polyvinyl chloride,
11 cm diameter and 20 cm long, were placed at the tank bottom as shelters for the crab. Tank water
was aerated to ensure adequate oxygen level and the temperature, salinity, pH, and concentration
of dissolved oxygen ranged from 25.8 to 28.9º C, 19.47−20.05 ppt, 6.89−7.29, and 5.6−6.8 mg L⁻¹,
respectively. These parameters were measured using a pH/ORD/EC/DO tester (Hanna
Instruments, HI 9828, Washington, USA). The crabs were fed with fresh squid (*Loligo* sp.) in the
morning and fish (*Decapterus* sp.) in the afternoon.

Electrocardiography
Three crabs (one male and two females) were used for the electrocardiography experiment. The
specimens were excitable in response to the movement of the experimenter, so their eyes were
painted with white nail polish to temporarily block their vision. The crabs were cold-anesthetised
followed by drilling a small hole of about 0.5 mm diameter at each cardiac lobe of the carapace to
expose the pericardial membrane. Two active electrodes (enamel-coated copper wire, 0.05 mm
diameter) were implanted through the holes and glued with cyanoacrylate. Crab specimens with
implanted electrodes were left for a day to recover in separate tanks with well-aerated seawater at
a temperature 28º C, pH 8.1, and salinity 20 ppt.

To record the electrocardiogram (ECG), a test crab with two implanted electrodes was placed
in a plastic test chamber (17 cm × 30 cm × 9.5 cm) filled with filtered seawater to 5 cm depth. The
water level was enough to submerge the carapace. A third electrode was placed in the chamber as a ground reference. The recording wires were of sufficient length to allow the freedom of movement of the crab. However, the test specimens seldom moved during the ECG recordings. The test chamber water was kept at a temperature of 28°C, pH of 8.1, and salinity of 20 ppt. The ECG was linked to an amplifier (MEG-1200, Nihon Kohden, Tokyo, Japan) aligned to a digital converter (FTT3P7PVB, ALS Controls, and Instruments, Nishinomiya, Japan), which converted analogue signals to digital signals. The signals were then processed with EasyLogger software (Easy SYNC Ltd., Glasgow, Scotland) and displayed on a laptop monitor. Stable ECGs were obtained 15–30 min after the crab specimens were placed in the test chamber (Fig. 1). Subsequently, a stimulus (touch or sugarcane juice) was applied to the appendages. The ECGs were recorded during 25 s of stimulation.

**Stimuli**

Each appendage of the crab was stimulated thrice in two ways (touch and chemical). The appendages were touched three times at intervals of 15–30 min with a hand-held micropipette (heat-pulled glass capillary with a 50 μm tip). For each crab, touch stimulation was conducted at the swimming leg dactyls, walking leg dactyls, claws, antennae (tip and middle part), antennules (lateral and medial flagella), and mouthpart (third maxilliped). Although the lateral and medial flagella are close to each other and frequently in motion, it was possible to touch them with the micropipette separately.

For the chemical stimulation, fresh sugarcane juice was selected because it contains 9.6–10.9% sucrose and 0.22–0.33% glucose and fructose, which are more effective feeding stimulants than amino acids for the fiddler crab *Uca pugilator* (*Robertson, Fudge & Vermeer, 1981*). The sugarcane juice measuring 0.1 mL (diluted to 50% or 10% with test chamber water, and filtered through paper Whatman No.1) was applied to the appendages by a hand-held Teflon pipette. Pipetting is one of the common methods to deliver a chemical solution to test crustaceans (*Cericola & Daniel, 2010; Aggio et al., 2012*). During the chemical stimulation, the threshold concentration of the sugarcane juice at the mouthparts and walking legs were maintained to comply with previous reports. According to Liew et al. (2020), the threshold concentration of the sugarcane juice at the mouthparts and walking legs was between 1% and 10% in *S. tranquebarica*. The movement and
dispersion of the sugarcane juice around the appendages were conspicuous due to its greenish
colouration. Thereafter, the crab was removed and the test chamber was emptied. A control test
was performed by replacing the sugarcane juice with the tank water (0.1 mL) before the application
of the sugarcane juice, which was presented in triplicates.

To prevent the diffusion of the sugarcane juice from the antennule to the antennae, the
antennules were cauterised using an electric cauterizer (Gemini BC00314, Cellpoin Scientific,
Gaithersburg, Germany) after applying the sugarcane juice. Thereafter, the sugarcane juice was
applied to the antennae. For the cauterization, the crab was taken out of the test chamber, cold-
anesthetised, and gently held with a wet towel, and the tip of the cauterizer was touched on the
distal segment bearing the outer and inner flagellum. After the cauterisation, the crab was placed
back into the chamber and left for 20 to 30 min to recover to its stable and uniform ECG. The
antennae were stimulated with 10% and 50% sugarcane juice. Each stimulation was delivered at
an interval of 20–30 min to minimise residual responses to a previous stimulus and to avoid
desensitization.

**Recording and measuring electrocardiograms**

Fifty-four stable ECGs were obtained from three test crabs: constant interbeat intervals (IBIs) of
0.5–0.8 s and constant amplitude of 0.3–0.4 mV. During the stimulus application, the ECGs were
examined for the IBIs, measured to 0.1 s. The first post-stimulus IBI was considered as the relevant
response. The IBI values were normalised by logarithmic transformation (Nishi et al. 2004):
\[
\text{normalised interval} = \log_{10}(T+1), \quad \text{where } T \text{ is the raw value of the IBI (s).}
\]
Each test IBI was then compared with the mean pre-test IBI (the test-beat is out of the 95% confidence interval calculated
using 10 pre-test IBIs).

**Behavioural bioassay**

During the stimulation at each appendage, the motion of other appendages was observed visually
and combined with video recordings using CCD cameras with temporal resolutions of 0.017 s,
corresponding to 60 frames per second (Olympus Tough TG-3, Olympus Corporation, Tokyo,
Japan). A total of 63 video recordings were analyzed, which were played back to examine the
feeding behaviour on a computer screen.
After the electrocardiography experiment, the feeding behaviour of each crab was observed in the test chamber. The antennule was stimulated with a tweezered small piece of salted fish flesh (Decapterus sp.) (about 10 mm × 5 mm, 2mm thick) obtained from a local market. To stimulate the walking legs or swimming legs, a small piece of salted fish flesh was placed close to their dactyl. As described by Liew et al. (2020), the feeding behaviour in response to the stimulation was evaluated based on three appendage movements: (i) increase in antennular flicking rate, (ii) remarkable movement of the third maxillipeds, and (iii) probing the test chamber bottom with the claws. These behaviours were visually observed, video-recorded, and a total of 30 video recordings were analyzed.

RESULTS

Electrocardiography

The electrocardiogram showed typical heartbeat response, slower heartbeat, or wider IBI of the crab, after the 10% or 50% sugarcane juice stimulation was presented to the swimming leg dactyl (Fig. 2A). However, no response was observed by mechanical touch stimulation (Fig. 2B). The crab with the antennules showed the heartbeat response, while those with the cauterised antennules showed no response to the stimulation by the sugarcane juice application given to the antennae. A statistically significant ($P < 0.05$) cardiac response to the sugarcane juice was recorded for the swimming legs, walking legs, claws, antennules (both the lateral and medial flagella), and mouthparts (Table 1). Regarding the touch test, statistically significant responses ($P < 0.05$) were observed for the walking legs, claws, antennae, and mouthparts of three specimens (Table 2). Both the lateral and medial flagella were not touch-sensitive. In the control test, the crab exhibited neither the cardiac response nor the feeding behaviour in response to tank water that was applied to all appendages.

Behaviour change in response to stimuli

Stimulation with the sugarcane juice elicited both the cardiac and feeding response of the crab. During electrocardiography, the mud crab exhibited an increase in the antennule flicking rate, claw probing, and movement of the third maxillipeds. These responses occurred following the
stimulation of the antennules and dactyls (both swimming and walking) with sugarcane juice. Such feeding behaviour was not observed since the antennae were touch-sensitive (Table 2), but not chemosensitive (Table 1). The touch stimulus alone did not elicit the feeding behaviour at all.

In response to a small piece of salted fish flesh placed on the swimming leg dactyl, the mud crab immediately exhibited movement of mouthpart appendages and kicked the flesh to the claw under its body (Fig. 3). Other exhibited features include pushing the flesh to the walking leg dactyl and grabbing the piece with the claw to bring it to the mouth. There was a clear involvement of the swimming legs in feeding.

**DISCUSSION**

The present study reinstated the importance of swimming legs of the mud crab for swimming and its vital role in food detection. The dactyl of swimming legs was found to be chemosensitive and when the dactyl touched a piece of food, the food was quickly kicked forward under the body by the motion of swimming legs, grabbed by the claw, and ingested. The kicking motion was done without touch sensitivity. Among the legs in the mud crab, the swimming legs are farthest from the mouth, nevertheless, the motion of the swimming leg secures food grabbing by the claw.

Mud crab is a benthic predator, an opportunistic scavenger, and feeds on sessile or slow-moving benthic macroinvertebrates, mainly gastropods, crustaceans, and molluscs (Alberts-Hubatsch et al., 2016). It also consumes fresh and decaying flesh of all kinds, hence, regarded as a detritivorous animal (Mamun et al., 2008; Nesakumari & Thirunavukkarasu, 2014; Viswanathan & Raffi, 2015; Paul et al., 2018). Previous studies have shown that S. serrata uses the dactyls of the walking legs by chemoreception to locate food (Alberts-Hubatsch et al., 2016). Besides, the flattened dactyls of the swimming legs might be more efficient than the pointed dactyls of the walking legs during food location. When crawling on the substrate, the dactyls of the swimming legs sweep the surface, which increases the likelihood of contacting scattered food items compared with the walking legs. Thus, the swimming legs of the mud crab are the most suited more appendage in locating food.
The swimming legs are also used in burying behaviour (*Parkes, Quinitio & Vay, 2011*). In aquaria, a pair of swimming legs are applied to flick sediment over the anterior of the carapace in *S. serrata* (*Parkes, Quinitio & Vay, 2011*). To achieve this burying motion, the mud crab requires information about the quality of bottom sediment. Since the dactyl of the swimming legs is not touch-sensitive, the cue of the sediment quality might be mediated by the other mechanosensitive appendages such as the walking legs. According to *Fedotov* (2009), the hair receptors on chelipeds, antennae, and antennules in crayfish and other decapods are innervated by mechano- and chemoreceptor neurons and bimodal sensillae. In the present study, it was evident that the short antenna of the mud crab, unexpectedly, was touch-sensitive but not chemosensitive to food-related organic substances. In contrast, the antennae have been reported to be used mechanically in feed deposit and suspension in decapod crustaceans (*Boxshall & Jaume, 2013*). This event suggests that the antennae are not directly involved in food detection. However, the potential role of the antennae in detecting the source of food odour is not ruled out. *Weissburg* (1994) reported that the blue crab could not orient the source of food odour in still water, but proceeded directly upstream toward the food odour source. Their findings indicated the importance of both rheotactic and chemical information for successful orientation.

Mechanoreceptors mediate remote perception of hydrodynamic signals such as disturbances in flow field caused by particles suspended in water columns or by the motions of live prey (*Boxshall & Jaume, 2013*). Assuming that the touch-sensitive antennae mediate hydrodynamic signals, the mud crab would be able to orient food odour source by olfaction aided by the perception of hydrodynamic signals by the antennae. The orientation may also be enhanced by the monomodal touch-sensitive sensillae reportedly present on the bristle patches on the carapace of *Scylla* spp. (*Kawamura et al., In Press*). The bristle patches are located behind the eyes in both males and females of four species of the mud crabs, and a touch stimulus given to the bristles is a mechanical signal for their courtship behaviour.

The antennule plays an important role in many behaviours of the mud crab, including locomotion, feeding, and mating (*Boxshall & Jaume, 2013*). The lateral flagellum of the antennule bears bimodal sensilla, which are innervated by mechanoreceptor and chemoreceptor neurons in decapod crustaceans (*Schmidt & Mellon, 2011*). The bimodal sensitivity of the antennule has been
reported in several species of lobsters and shrimps. However, in the present study, the lateral flagellum of the antennule of the mud crab is a unimodal chemosensor, distinctly chemosensitive, but not touch-sensitive. van Weel & Christofferson (1966) conducted an electrophysiological study of appendages of two portunid crabs: Podpphthalmus vigil and Portunus sanguinolentus. In their experiment, touch stimulation of walking legs, antennae, and mouthparts all caused the electroactivity in the antennule. This is possibly due to the uncommon arrangement of a reference electrode, which was inserted into the carapace and was not grounded. A reference electrode is typically connected to a grounded electrode, which is essential for most electrophysiological recordings. Therefore, the results of van Weel & Christofferson (1966) are questionable so far as this aspect is concerned. The present study revealed the bimodal sensitivity of the mouthparts, claws, and walking legs of the mud crab, which seemed to be inconsistent with those of other decapod crustaceans based on available literature findings.

CONCLUSIONS

Electrocardiography revealed that the thick setae along the edge of the swimming leg dactyl of the mud crab, Scylla paramamosain, were chemosensitive but not mechanosensitive, whereas the antennae were mechanosensitive but not chemosensitive. Behavioural observations showed the involvement of swimming legs in feeding. A combination of electrocardiographic and behavioural patterns of appendages indicated that the body parts directly involved in feeding include the mouthparts, claws, walking legs, swimming legs, and antennules.

Ethical approval

All experimental animals were cared for and handled following the guidelines suggested 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.

Author Contributions

Investigation and writing of original draft, Gunzo Kawamura; Investigation, Loke C. Keong; investigation, writing-review, editing, and correspondence, Leong-Seng Lim; funding acquisition
and project administration, Annita S. Yong; language editing, Saleem Mustafa. All authors have
read and agreed to the published version of the manuscript.

REFERENCES

Ache BW. 1982. Chemoreception and thermoreception. In: Atwood HL & Sandeman DC, eds. The biology of crustacean 3; Neurobiology: Structure and function. New York: Academic Press, 369–398.

Aggio JF, Tieu R, Wei A, Derby D. 2012. Oesophageal chemoreceptors of blue crab, Callinectes sapidus, sense chemical deterrents and can block ingestion of food. Journal of Experimental Biology 215:1700–1710 DOI: 10.1242/jeb.065854.

Alberts-Hubatsch H, Lee SY, Meynecke JO, Diele K, Nordhaus I, Wolff M. 2016. Life-history, movement, and habitat use of Scylla serrata (Decapoda: Portunidae): current knowledge and future challenges. Hydrobiologia 763:5–21.

Boxshall G, Jaume D. 2013. Antennules and antennae in the crustacea. In: Walting L & Thiel M, eds. Oxford: Oxford University Press, 199–236.

Burnovicz A, Oliva D, Hermitte G. 2009. The cardiac response of the crab Chasmagnathus granulatus as an index of sensory perception. Journal of Experimental Biology 212:313–324 DOI: 10.1242/jeb.022459.

Burnovicz A, Hermitte G. 2010 Conditioning of an autonomic response in Crustacea. Physiology & Behavior 101:168–175 DOI: 10.1016/j.physbeh.2010.04.034.

Cericola VJ, Daniel PC. 2010. Chemically-mediated antennular grooming behavior and associated asymmetric setae: toward a hypothesis on their evolution in reptantian decapods. Journal of Crustacean Biology 30:557–570 DOI: 10.1651/09-3204.1.

Dunham DW, Oh JW. 1992. Chemical sex discrimination in the crayfish Procambarus clarkii: role of antennules. Journal of Chemical Ecology 18:2363–2372.

Fazhan H, Waiho K, Quinitio E, Baylon JC, Fujaya Y, Rukminasari N, Azri MFD, Shahreza MS, Ma H, Ilkwanuddin M. 2020. Morphological descriptions and morphometric discriminant
function analysis reveal and additional four groups of *Scylla* spp. *PeerJ* 8:e8066 DOI: org/10.7717/peerj.8066.

Fedetov VP. 2009. Systems of chemoreception in decapod crayfish. *Journal of Evolutional Biochemistry and Physiology* 45:1–26.

Garm A. 2004a. Mechanical functions of setae from the mouth apparatus of seven species of decapod crustaceans. *Journal of Morphology* 260:85–100 DOI: 10.1002/jmor.10213.

Garm A. 2004b. Revising the definition of the crustacean seta an setal classification systems based on examinations of the mouthpart setae of seven species of decapods. *Zoological Journal of the Linnean Society* 142:233–252 DOI: 10.1111/j.1096-3642.2004.00132.x

Garm A, Høeg JT. 2000. Functional mouthpart morphology of the squat lobster *Munida sarsi*, with comparison to other anomalans. *Marine Biology* 137:123–138.

Garm A, Shabani S, Hoeg JT, Derby CD. 2005. Chemosensory neurons in the mouthparts of the spiny lobsters *Panulirus argus* and *Panulirus interruptus* (Crustacea: Decapoda). *Journal of Experimental Marine Biology and Ecology* 314:75–186 DOI: 10.1016/jembe.2004.08.016.

Gleeson RA, Adams MA, Smith AB. 1984. Characterization of a sex pheromone in the blue crab, *Callinectes sapidus*: crustecdysone studies. *Journal of Chemical Ecology* 10:913–921.

Gomez G, Atema J. 1996. Temporal resolution in olfaction: stimulus integration time of lobster chemoreceptor cells. *Journal of Experimental Biology* 199:1771–1779.

Grobel MS. 1990. Luminescent flash avoidance in the nocturnal crab *Portunus xantusii*. *Journal of Experimental Biology* 148:415–427.

Hermitte G, Maldonado H. 2006. Cardiovascular component of the context signal memory in the crab *Chasmagnathus*. *Journal of Comparative Physiology* 192:69–83 DOI: 10.1007/s00359-005-0052-y.

Alberts-Hubatsch H, Lee S.Y., Meynecke JO, Diele K. 2016. Life-history, movement, and habitat use of *Scylla serrata* (Decapoda, Portunidae): current knowledge and future challenges. *Hydrobiologia* 763:5–21.

Ikhwanuddin M, Azmie G, Juariah HM, Zakaria MZ, Ambak MA. 2011. Biological information and population features of mud crab, *Scylla* from mangrove areas of Sarawak, Malaysia. *Fisheries Research* 108:299–306 DOI: 10.1016/j.fishres.2011.01.001

Jordão JM, Cronin TW, Oliveira RF. 2007. Spectral sensitivity of four species of fiddler crabs (*Uca pugnax, Uca pugilator, Uca vomeris* and *Uca tangeri*) measured by *in situ*
microspectrophotometry. *Journal of Experimental Biology A* 210:447–453 DOI: 10.1242/jeb.12658.

Kamio M, Araki M, Nagayama T, Matsunaga S, Fusetani N. 2005. Behavioral and electrophysiological experiments suggest that the antennular outer flagellum is the site of pheromone reception in the male helmet crab *Telmessus cheiragonus*. *Biological Bulletin* 208:12–19.

Kawamura G. 1981. Recording of C-type of S potential from the retina of Sparidae. *Bulletin of Japanese Society of Scientific Fisheries* 47:825.

Kawamura G, Nakaizumi H, Motohiro T. 1992. Chemical perception and response of the Nile tilapia to geosmin. *Water Science Technology* 25:277–282 DOI: 10.2166/wst.1992.0062.

Kawamura G, Bagarinao TU, Cheah HS, Saito H, Yong ASK, Lim LS. 2020. Behavioural evidence for colour vision determined by conditioning in the purple mud crab *Scylla tranquebarica*. *Fisheries Science* 86: 299-305 DOI: 10.1007/s12562-019-01395-z.

Kawamura G, Bagarinao TU, Loke CK, Au HL, Lim LS, Yong ASK. 2021. Touch-sensitive bristles on the carapace of the mud crab *Scylla paramamosain* may be receptors for courtship signals. *Fisheries Science* 87:65–70 DOI: 10.1007/s12562-020-01478-2.

Keller TA, Powell I, Weissburg MJ. 2003. Role of olfactory appendages in chemically mediated orientation of blue crabs. *Marine Ecology Progress Series* 261:217–231.

Ketpadung R, Tangkrockolan NT. 2006. Changes in oxygen consumption and heart rate of the blue swimming crab, *Portunus pelagicus* (Linnaeus, 1766) following exposure to sublethal concentrations of copper. *Journal of Environmental Biology* 27:7–12.

Liew KS, Yong ASK, Lim L-S, Kawamura G. 2020. Dietary sugarcane juice as a feeding stimulant for the purple mud crab *Scylla tranquebarica*. *Aquaculture Research* 51:2164–2167 DOI: 10.1111/are.14545.

Mamun AA, Begum M, Mia MY, Alam MJ. 2008. Food and feeding habits of the mud crab *Scylla serrata* (Forskal) in Bangladesh. *Journal of Bangladesh Society of Agriculture Science and Technology* 5:141–144.

Medesani DA, Cervino CO, Ansaldo M, Rodriguez EM. 2011. Effect of parathion on cardiac rate, ventilatory frequency and hemolymphatic gas levels in the estuarine crab *Neohelica granulata* (Decapoda, Brachyura). *Journal of Toxicology and Environmental Health Science* 3:74–79 DOI: 10.1183/20734735.009817.
Mellon DJr. 2012. Smelling, feeling, tasting and touching: behavioral and neural integration of antennular chemosensory and mechanosensory inputs in the crayfish. *Journal of Experimental Biology* 215:2163–2172 DOI: 10.1242/jeb.069492.

Mickel TJ, Childress JJ. 1982. Effects of pressure and temperature on the EKD and heart rate of the hydrothermal vent crab *Bythograea thermydron* (Brachyura). *Biological Bulletin* 162:70–82.

Nesakumari CSA, Thirunavukkarasu N. 2014. Food and feeding behaviour of mud crab *Scylla tranquebarica* (Fabricius, 1798). *Indian Journal of Veterinary & Animal Science Research* 43:229–235.

Nishi T, Kawamura G, Matsumoto K. 2004. Magnetic sense in the Japanese eel, *Anguilla japonica*, as determined by conditioning and electrocardiography. *Journal of Experimental Biology* 207:2965–2970 DOI: 10.242/jeb.01131.

Nishi T, Kawamura G. 2005. *Anguilla japonica* is already magnetosensitive at the glass eel phase. *Journal of Fish Biology* 67:1213–1224 DOI: 10.1111/j.1095-849.2005.00817.x.

Nishida S, Booth JD, Nemoto T, Kittaka J. 1990. Comparative morphology of the mouthparts and foregut of the final-stage phyllosoma, puerulus, and postpuerulus of the rock lobster *Jasus edwardsii* (Decapoda: Palinuridae). *Journal of Crustacean Biology* 10:293–305 DOI: 10.1163/193724090X00104.

Parkes L, Quinitio ET, Vay LL. 2011. Phenotypic differences between hatchery-reared and wild mud crabs, *Scylla serrata*, and the effects of conditioning. *Aquaculture International* 19:361–380.

Paul AK, Alam MM, Islam MS, Hussain M, Das SK. 2018. Feeding behaviour of mud crab *Scylla serrata* in north of Sundarbans, Bangladesh. AACL Bioflux 11:701–708.

Pearson WH, Olla BL. 1977. Chemoreception in the blue crab, *Callinectes sapidus*. *Biological Bulletin* 153:346–354.

Robertson JR, Fudge JA, Vermeer GK. 1981. Chemical and live feeding stimulants of the sand fiddler crab, *Uca pugilator* (Bosc). *Journal of Experimental Marine Biology and Ecology* 53:47–64 DOI: 10.1016/0022-0981(81)90083-6.

Sahlmann C, Chan TY, Chan BKK. 2011. Feeding models of deep-sea lobsters (Crustacea: Decapoda: Nephropidae and Palinuridae) in Northwest Pacific waters: Functional
morphology of mouthparts, feeding behaviour and gut contents analysis. *Zoologischer Anzeiger* 250:55–66.

Schmidt M, Mellon DJr. 2011. Neural processing of chemical information in crustaceans. In: Breithaupt T, Thiel M, eds. *Chemical communication in crustaceans*. New York: Springer, 123–147.

Taylor EW, Leite CL, Cambell H, Intanai I, Wang T. 2007. Control of the heart in fish. In: Fernandes MN, Rantin FT, Glass ML, eds. *Fish respiration and environment*. Plymouth: Science Publisher, 341–375.

van Weel PB, Christofferson JP. 1966. Electrophysiological studies on perception in the antennulae of certain crabs. *Physiological Zoology* 39:317–325.

Viswanathan C, Raffi SM. 2015. The natural diet of the mud crab *Scylla olivacea* (Herbst, 1896) in Pichavaram mangroves, India. *Saudi Journal of Biological Sciences* 22:698–705.

Wall D, Paterson B, Mohan R. 2009. Behaviour of juvenile mud crabs *Scylla serrata* in aquaculture: Response to odours of moulting or injures crabs. *Applied Animal Behaviour Science* 121: 63–73. DOI: 10.1016/j.applanim.2009.08.005

Weissburg MJ. 1994. Odour plumes and how blue crabs use them in finding prey. *Journal of Experimental Biology* 197:349–375.

White AQ, Sprito CP. 1973. Anatomy and physiology of the swimming leg musculature in the blue crab, *Callinectes sapidus*. *Marine Behaviour and Physiology* 2:141–153 DOI: 10.1080/10236247309386921.
Figure 1

Representation of the experimental set up used for recording the heart beat during the application of the different stimuli.
Figure 2

Typical electrocardiograms showing the heart beat response of *Scylla paramamosain* after the presentation of 50% sugarcane juice (A) and no change in heart beat interval in response to a single touch stimulus (B) delivered to the swimming leg dactyl.

Arrows represent the application of the stimulus.
Figure 3

Sequential images of a food capture process with the swimming leg of *Scylla paramamosain*.

A, touching the dactyl of the swimming legs by a piece of fish flesh (arrow head); B, kicking the fish flesh under the body toward the mouth; C, grabbing the fish flesh with a claw and conveying it to the mouth.
Table 1 (on next page)

Change in interbeat intervals (IBI) in *Scylla paramamosain* in response to sugarcane juice delivered to appendages.

The stimulation was triplicated but cardiac responses were shown only for the first single touch. Mean pre-test IBI was calculated for 10 pre-test IBI. * denotes significantly larger test-beat interval than 95% confidence interval calculated using 10 pre-test IBIs.
| Appendage   | Crab | Sugarcane juice concentration (%) | Pre-test IBI: log10-transformed mean IBI (s) | Pre-test IBI: log10-transformed IBI 95% confidence interval (s) | Test-beat: log10-transformed IBI (s) |
|------------|------|-----------------------------------|-------------------------------------------|--------------------------------------------------|----------------------------------|
| Swimming leg | A (male) | 10 | 0.248 | 0.171–0.325 | 0.646* |
|             |       | 50 | 0.237 | 0.169–0.305 | 0.898* |
|             | B (female) | 10 | 0.290 | 0.249–0.331 | 0.643* |
|             |       | 50 | 0.266 | 0.233–0.299 | 0.981* |
|             | C (female) | 10 | 0.237 | 0.207–0.267 | 0.620* |
|             |       | 50 | 0.247 | 0.202–0.292 | 0.929* |
| Walking leg | A | 10 | 0.404 | 0.350–0.458 | 0.737* |
|             |       | 50 | 0.261 | 0.206–0.316 | 0.729* |
|             | B | 10 | 0.331 | 0.261–0.401 | 0.659* |
|             |       | 50 | 0.246 | 0.178–0.314 | 0.676* |
|             | C | 10 | 0.400 | 0.354–0.446 | 0.898* |
|             |       | 50 | 0.203 | 0.168–0.238 | 0.484* |
| Claw | A | 10 | 0.273 | 0.223–0.313 | 0.979* |
|             |       | 50 | 0.243 | 0.196–0.290 | 0.587* |
|             | B | 10 | 0.250 | 0.204–0.296 | 0.754* |
|             |       | 50 | 0.276 | 0.234–0.318 | 1.157* |
|             | C | 10 | 0.261 | 0.194–0.328 | 0.618* |
|             |       | 50 | 0.285 | 0.109–0.461 | 0.771* |
| Antenna | A | 10 | 0.216 | 0.188–0.244 | 0.236 |
|             |       | 50 | 0.137 | 0.066–0.208 | 0.090 |
|             | B | 10 | 0.180 | 0.150–0.210 | 0.207 |
|             |       | 50 | 0.089 | 0.020–0.158 | 0.117 |
|       | C    | 10  | 0.246 | 0.206–0.286 | 0.217 |
|-------|------|-----|-------|-------------|-------|
|       | 50   | 0.198| 0.127–0.269 | 0.196 |
| Antennule | A    | 10  | 0.223 | 0.169–0.277 | 0.633*|
|       | 50   | 0.284| 0.241–0.327 | 0.844*|
|       | B    | 10  | 0.187 | 0.136–0.238 | 0.375*|
|       | 50   | 0.253| 0.201–0.305 | 0.832*|
|       | C    | 10  | 0.255 | 0.173–0.337 | 0.862*|
|       | 50   | 0.180| 0.144–0.216 | 1.097*|
| Mouthparts | A    | 10  | 0.308 | 0.261–0.355 | 0.671*|
|       | 50   | 0.339| 0.307–0.371 | 0.969*|
|       | B    | 10  | 0.301 | 0.265–0.337 | 0.746*|
|       | 50   | 0.306| 0.285–0.327 | 0.859*|
|       | C    | 10  | 0.277 | 0.224–0.310 | 0.633*|
|       | 50   | 0.339| 0.280–0.398 | 0.754*|
Change in interbeat intervals (IBI) in *Scylla paramamosain* in response to touch stimulus delivered to appendages.

The stimulation was triplicated but cardiac responses were shown only for the first single touch. Mean pre-test IBI was calculated for 10 pre-test IBI. * denotes significantly larger test-beat interval than 95% confidence interval calculated using 10 pre-test IBIs.

**Table 2**

Change in interbeat intervals (IBI) in *Scylla paramamosain* in response to touch stimulus delivered to appendages.
| Appendage     | Crab  | Pre-test IBI: log10-transformed mean IBI (s) | Pre-test beat: log10-transformed IBI 95% confidence interval (s) | Test-beat: log10-transformed IBI (s) |
|---------------|-------|---------------------------------------------|------------------------------------------------------------------|-------------------------------------|
| Swimming leg  | A (male) | 0.139                                      | 0.063-0.215                                                        | 0.097                               |
|               | B (female) | 0.170                                      | 0.125-0.215                                                        | 0.146                               |
|               | C (female) | 0.210                                      | 0.181-0.239                                                        | 0.193                               |
| Walking leg   | A       | 0.337                                      | 0.287-0.387                                                        | 0.782*                              |
|               | B       | 0.307                                      | 0.237-0.377                                                        | 0.646*                              |
|               | C       | 0.329                                      | 0.287-0.371                                                        | 0.868*                              |
| Claw          | A       | 0.323                                      | 0.264-0.382                                                        | 0.627*                              |
|               | B       | 0.330                                      | 0.316-0.344                                                        | 0.658*                              |
|               | C       | 0.359                                      | 0.326-0.392                                                        | 0.718*                              |
| Antenna       | A       | 0.226                                      | 0.163-0.289                                                        | 0.679*                              |
|               | B       | 0.244                                      | 0.219-0.269                                                        | 0.668*                              |
|               | C       | 0.245                                      | 0.213-0.277                                                        | 0.765*                              |
| Antennule     | A       | 0.139                                      | 0.063-0.215                                                        | 0.124                               |
|               | B       | 0.244                                      | 0.197-0.251                                                        | 0.220                               |
|               | C       | 0.184                                      | 0.115-0.253                                                        | 0.225                               |
| Mouthparts    | A       | 0.251                                      | 0.205-0.297                                                        | 0.684*                              |
|               | B       | 0.319                                      | 0.289-0.349                                                        | 0.647*                              |
|               | C       | 0.154                                      | 0.126-0.182                                                        | 0.563*                              |