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# LOCOMOTOR, ESCAPING ACTIVITIES AND FATTY ACID COMPOSITION OF MUD CRAB, SCYLLA OLIVACEA AT DIFFERENT WATER VELOCITIES

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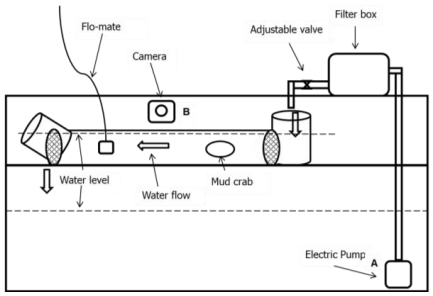
## Graphical abstract




## Abstract

This study was designed to determine the locomotor, escaping capability and fatty acids (FAs) composition of muscle *Scylla olivacea* mud crabs at different water velocities. Male and female immature *S. olivacea* were cultured at 0, 20, 40 and 60 cm/s in a recirculating marine aquaculture system. Increase in flow velocity increased the mean locomotor activity and escaping capability of the crabs. Significant differences were observed between sexes for both activities in all velocities tested ($p < 0.05$). Male and female crabs reared at the lowest flow velocity (0 cm/s) exhibited a mean of 13 and 55 locomotion per 15 minutes tested, respectively. Meanwhile, in the highest flow velocity (60 cm/s), male and female crabs exhibited 61 and 70 locomotion's per 15 minutes. A total of 3111 locomotion were recorded during the entire experiment. An increase in the water velocities increased the mean escaping capability of the crabs. A total of 32 crabs attempted to escape during the flow velocity tests (0 cm/s; $n = 3$, 20 cm/s; $n = 9$, 40 cm/s; $n = 11$, and 60 cm/s; $n = 9$). The locomotor of crabs increased gradually and peaked at 40 cm/s (seeking for shelter). By contrast ≥ 40 cm/s the locomotor of crab decreased (defensive mode). For FAs analysis, total fatty acids (TFAs) content was found highest at 20 cm/s compared to other velocities. This study clearly show that the locomotor activities, escaping capabilities and FAs composition of *S. olivacea* were affected by water velocities under laboratory conditions.

*Keywords*: Aquaculture, behaviour, dislocation, movement, biochemical change

## Abstrak

Kajian ini direka untuk menentukan pergerakan, kebolehan melepaskan diri dan komposisi asid lemak otot oleh ketam bakau *Scylla olivacea* pada kelajuan air

yang berbeza. Induk jantan dan betina yang belum matang diternak pada 0, 20, 40, dan 60 cm/s di dalam sistem air laut yang berpusing. Meningkatnya kelajuan arus menyebabkan peningkatan purata aktiviti pergerakan dan keupayaan ketam melepaskan diri. Terdapat perbezaan yang jelas di antara jantina terhadap kedua-dua ujikaji yang diuji ($p < 0.05$). Ketam jantan dan betina yang diternak pada kelajuan arus yang terendah (0 cm/s) menunjukkan purata 13 hingga 55 pergerakan dalam 15 minit ujian. Manakala, pada kelajuan arus yang tertinggi (60 cm/s), ketam jantan dan betina menunjukkan 61 hingga 70 pergerakan dalam 15 minit. Sebanyak 3111 pergerakan direkodkan sepanjang keseluruhan eksperimen. Semakin meningkat kelajuan air, semakin tinggi keupayaan ketam untuk melepaskan diri. Sebanyak 32 ekor ketam membuat percubaan untuk melepaskan diri dari ujian kelajuan arus (0 cm/s; $n = 3$, 20 cm/s; $n = 9$, 40 cm/s; $n = 11$, dan 60 cm/s; $n = 9$). Pergerakan ketam meningkat apabila ≤ 40 cm/s (mencari perlindungan). Sebaliknya ≥ 40 cm/s pergerakan ketam berkurangan (keadaan bertahan). Untuk analisis asid lemak, kandungan asid lemak yang tertinggi terdapat pada 20 cm/s jika dibandingkan dengan kelajuan yang lain. Kajian ini menunjukkan aktiviti pergerakan, kebolehan melepaskan diri dan komposis asid lemak *S. olivacea* dipengaruhi oleh kelajuan air yang terdapat pada hutan paya bakau.

*Kata kunci*: Akuakultur, kelakuan, beralih tempat, pergerakkan, fisiologi



## 1.0 INTRODUCTION

In estuaries, there is a significant amount of water flow, but it is unknown how aquatic animals in an estuary encounter this situation, whether by passive transport or direct swimming to shallow water, as well as how (or if) once within vegetated areas, they can maintain their positions until molting [1]. Determining the ability of aquatic animals to tolerate, maneuver, and swim in different flow conditions is required for evaluating their capability for actively encountering and remaining in desirable recruitment habitats.

Researches on flow velocities in the closed, recirculating aquaculture of mud crabs remains in an early stage of development, and progress is limited compared with other fish species. In contrast with the conventional flowing aquaculture model, the advantages of manipulating flow velocities in recirculating aquaculture include a high degree of intensiveness, low labor cost, a stable and controllable water environment, improved water quality, and fulfilling the purposes of resource conservation as well as ecological and environmental protection [2, 3]. Based on mud crab's physiological and ecological characteristics, the environmental factors of the recirculating aquaculture system are suitable for the optimal control of growth, survival, and reproduction.

Flow velocity is a critical and complex ecological factor in the aquatic environment; it can stimulate the sensory organs of aquatic organisms for the generation of corresponding movement and response mechanisms. It has multiple ecological effects that directly or indirectly affect the behavior and physiological state of aquatic organisms [4]. However, to the best of the authors' knowledge, the effects of flow velocity on the survival, growth, and molting duration of mud crab, *Scylla olivacea* in a closed, recirculating aquaculture system have not yet been reported.

Historically, work has questioned the ability of several taxa to actively swim or walk between potential settlements habitats because swimming speeds are slow relative to boundary-layer flow velocities [1, 5, 6]. Thus, the encounter rates of grow-out stages with specific habitats are presumed to be set by hydrodynamic processes coupled with vertical swimming, with little or no effect of directed horizontal swimming. Some studies on locomotor activities have been done on brachyuran crabs such as blue swimming crab, *Portunus pelagicus* [6-9]. In addition, study by Peter [10] showed that flow velocities up to 30 mm/s were recorded during the crab burrows experimental study. Orange mud crabs (*Scylla olivacea*) broodstock are involved in estuarine and benthic habitat selection [11-12]. They are commonly found in intertidal zones of mangrove forests and estuaries, and they are usually associated with mangrove forests during the monsoon season [13-14].

In the group of crustacean's food, crabs ranks third after prawns and lobsters by quality of their muscle (flesh taste) and have good price in fishery [15]. The exact dietary muscle profile of mud crabs regarding to differences in water velocities between sexes have not been investigated properly. The effect water velocities on biochemical composition and nutritional value should be precise to assist the fattening, handling, operation, and selling of upgrade crab products for human demand. Therefore the current

work was initiated with the aim of determining the fatty acid profile of body muscle of *S. olivacea* with respect to sexes and water velocities manipulation.

In addition, fatty acid assessment were used to investigate how aquatic organism flesh, varied with different environmental conditions specifically omega-3, which helping in various physiological and immunological function [16-26]. According to Gil [19], docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), linoleic acid (LA, 18:2n-6), and Linolenic acid (LNA, 18:3n-3), are importance indicators of the dietary value of lipid. Thus, dietary value in muscle of the different sexes of orange mud crab cultures at different water velocities were tested using FAs analysis approach.

## 2.0 METHODOLOGY

### 2.1 Animals Sampling and Preparation

The study was conducted at the hatchery and laboratories of the Institute of Tropical Aquaculture and Fisheries Research, Universiti Malaysia Terengganu (UMT). Immature male and female mud crab were locally bought from fisherman, Terengganu, Malaysia. The mud crabs were brought back to UMT alive in a basket. In the present study, the following equipment was used for culturing: 30 units of PVC pipe prototype, two holding tanks (3000 L), and one treated brackish water (20 ppt) stoking tank (3000 L). A study by Ikhwanuddin *et al*. [27] on the size of maturity (Carapace Width with 50% maturity size - $CW_{50}$) for males at maturity was 8.97 cm CW for *S. olivacea*; hence immature male crabs with a carapace width less than 8.97 cm were selected for this study. In addition, only immature female *S. olivacea* were selected. According to Ikhwanuddin *et al*. [28], the size at maturity ($CW_{50}$) recorded for female *S. olivacea* was 9.06 cm. Thus, immature female crabs with an external carapace width of less than 9.06 cm and a small, pale abdominal flap, were sampled for this study.

The crabs' body weight (BW) and carapace width (CW) were recorded before the experiment started. The size of the crabs was measured as the external CW, which was the distance between the tip of the 9$^{th}$ anterolateral spines of the carapace. For each crab, the CW was measured with a Vernier caliper (accuracy, 0.01 mm), while BW was measured using a digital balance (accuracy, 0.01 g). The crab was then held in the holding tank under ambient light and temperature conditions for 1–2 days before being placed into the PVC pipe prototype canal. During the holding stage and canal experiments, the crabs were kept in brackish water at 20 ppt. When measured, the water temperature averaged ±26°C, but the temperature was not controlled during the experiment. Cleaning took place by siphoning the feces, excess food sediment, and metabolic waste at the bottom. These activities were carried out in the morning before introducing a new fish fillet (*Decepterus* sp.). Fifty percent of the water was changed every two days.

### 2.2 Experimental Design

An experiment was carried out with the PVC pipe prototype. Four different water flow rates were brought about by controlling the valve of the system, resulting in Treatments 1, 2, 3, and 4 with rates of 0, 20, 40, and 60 cm/s, respectively. Figure 1 shows the 1-unit arrangement of the PVC pipe prototype, where Figure 1A illustrates the side view and 1B shows the upper view.

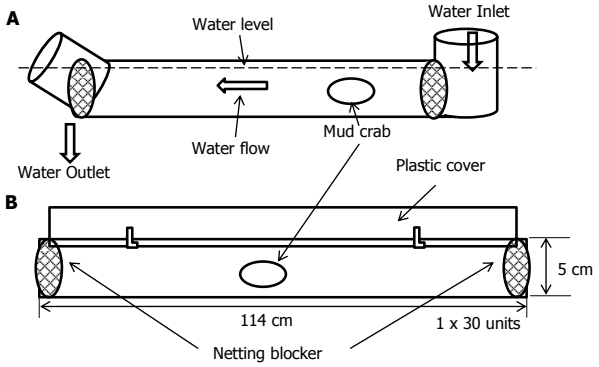



**Figure 1** A: Side view; B: upper view of the one unit arrangement of the mud in PVC Pipe Prototype of different water flow rates experiment by individual *Scylla olivacea* crab

Experiments were conducted in Re-circulating Marine Aquaculture System (RMAS) with video camera installed above the culture system (Figure 2). The flow channel of the PVC pipe was constructed and measured as 114 cm long × 5 cm wide; for all the trials, the channel was filled with filtered brackish water to a depth of 4 cm. Flow was generated in the channel by an electric pump, with a power of 6000 L/h. Observations of eye movements in the channel revealed the water flow along the channel. In a mangrove swamp, the mean magnitude of the water velocity is around 10 cm/s [29-30]. Therefore, four different downstream current velocities, namely, 0, 20, 40, and 60 cm/s were applied as free stream velocities mimicking typical natural flow conditions.

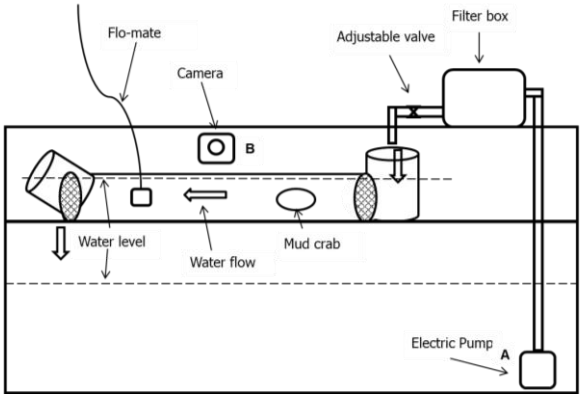



**Figure 2** diagram showing (A) Re-circulating Marine Aquaculture System (RMAS) with (B) video camera installed above the system

A Marsh–McBirney Model 2000 Flo-Mate made in the US was the portable flow-meter used in this study. Velocity measurements (cm/s) were quantified using the electromagnetic method, with an accuracy of ± 5 cm/s. The Flo-Mate measures flow using the Faraday Principle, whereby movement in a conductor perpendicular to the magnetic field produces voltage that is recorded by the apparatus. The magnitude of the generated voltage is directly proportional to the velocity at which the conductor moves through the magnetic field. A pair of carbon electrodes is used to measure the generated voltage, which is processed by the electronically components and is output as a linear measurement of velocity.

For the current investigation, 30 crabs were individually placed in the flume for 24 h prior to the experiment. All experimental trials were run at night because crabs are nocturnal organisms. When conducting the trials, the flow velocity was set to the desired level, ambient lights were extinguished, and the system was allowed to run for 15 min. The light was then switched on and crabs were recorded for 15 min as they walked in the center of the channel before turning the lights off again. The flow speed was adjusted to the next level, and the sequence described above was repeated. Flow speeds were initially increased from 0 cm/s to 60 cm/s, after which they were decreased back to 0 cm/s. A video camera was mounted on a tripod approximately 1.5 m from the channel and was set to focus through the sidewall approximately midway along the channel length. In preliminary trials, the camera was mounted above the channel to provide a plan view of the channel [Figure 3(A-C)].

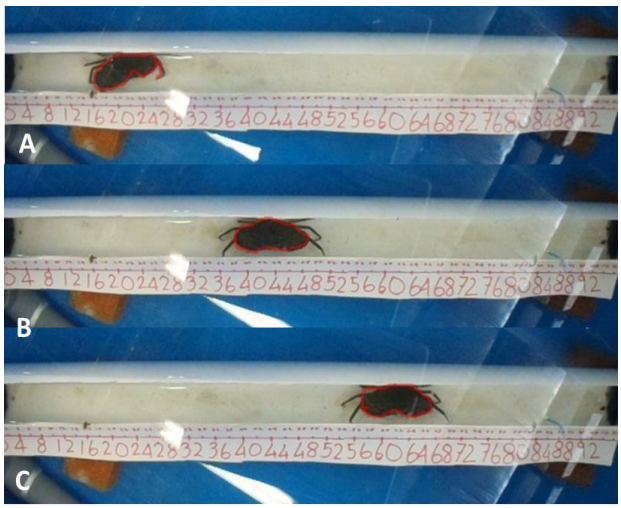



**Figure 3** Figure (A-C) showed the crab movement from left to right in the PVC pipe used in this study

Walking frequency and directions were subsequently extracted from the video records for at least six crabs in each trial for the 15-min period during which the lights were turned on, and the process was repeated until movements of 48 crabs had been recorded. The recorded video (MPEG-TS Video File) was analysed using behavioural coding software Solomon Coder [31] [Figure 4 (A-B)]. A 114 cm PVC pipe prototype was used as an experimental interval for all flow velocities tested.

## 2.3 One Step Method Procedure

Three active mud crabs were sampled at various intervals (0, 30 and 60 days) from each treatment. The mean crab CW was scaled with a mean weight of 200-300 g in the range of 100-130 mm. The crabs were dissected and muscle tissues were expelled from the shells. Samples were kept at -80 °C for two days and then freeze dry for 48 hours before the FA analysis was ready to be performed [32]. This study used an internal standard, nonadecanoic acid (19:0). The concentration of FAs was calculated using the following formula:

$$C_{FA} = A_S/A_{IS} \times C_{IS}/W_S$$

Where $A_S$ is the peak area of fatty acid in the sample in chromatogram, $A_{IS}$ is peak area of internal standard in chromatogram, $C_{IS}$ is concentration of internal standard (mg) and $W_S$ is the weight of sample (g).

One-step technique process comprising esterification and extraction procedure was applied to a 50 ml individual tube. Each sample (200-300 mg) with three replicate were extracted using 4 ml of hexane and 1 ml of Nonadecanoid acid in each tube. For esterification purpose, 2 ml of 14% $BF_3$ in methanol and a magnetic stirring bar were added into tube, the balance air in the tube were replaced by $N_2$ about 30

second and closed tightly with screw-cap. The capped tube was incubated on a hot plate at 100°C for two hour in mild stirring.

Subsequently cooled at ambient temperature, 1 ml of hexane and 2 ml of distilled water were added into tube and shaken strongly about 60 second and blended for 3 minutes at 2500 rpm (650×g). Two layers appeared; the top layer comprising the FAMEs. Lastly, ~1–2 ml of FAMEs was injected into the GCMS for FAME quantification. FAMEs were isolated and measured by gas chromatograph equipped with mass spectrometer (GCMS-QP2010 Ultra). Isolation was done with column ppms-5, with pressure 50.0 kPa, used column flow 0.96 ml/minute, linear velocity 35.5 cm/second, and helium was applied as carrier gas. 1 µl of FAMEs samples in hexane were inserted at 50°C and allowed to stand for 1 min then oven temperature was increased to 300°C at a level of 5°C/minute, and then lastly stabilized at 5 minutes.

### 2.4 Statistical Analyses

Differences between mean walking frequencies (irrespective of motion direction) of male and female crabs were tested using one-way ANOVA, followed by a SNK multiple comparisons test. Compliance with the assumptions of data distribution normality and homoscedasticity was assessed using standard normal plots and Cochran's C test. The differences in the frequency distributions of locomotion activity between ascending and descending velocity trials within a flow treatment were tested using Kolmogorov-Smirnov test. Data pertaining to locomotion activity within a flow treatment were grouped into *post hoc* categories of 20 cm/s intervals and the hypothesis stipulating equal frequency distributions among all flow treatments was tested via Tukey HSD test of significance ($p < 0.05$).

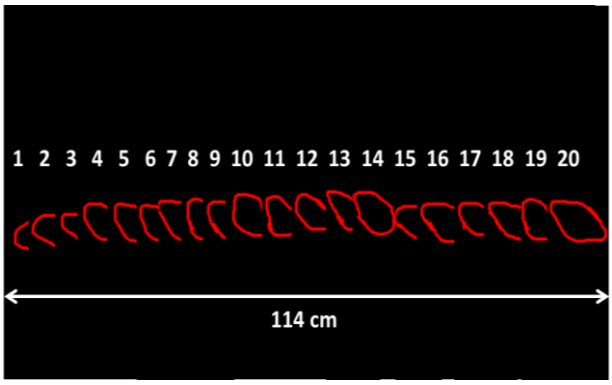



**Figure 4** (A) The example of crab movement from left to right recorded 20 movements per 15 min by using Solomon coder beta 17.03.22

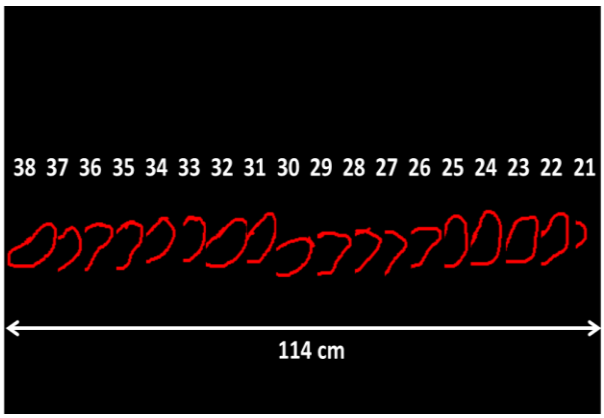



**Figure 4** (B) The example of crab movement from right to left recorded 18 movements per 15 min by using Solomon coder beta 17.03.22

## 3.0 RESULTS AND DISCUSSION

### 3.1 Crab Movement

Significant differences in the movement of male crabs were observed when water velocity was modified in the 0−60 cm/s range ($p<0.05$) (Figure 5).

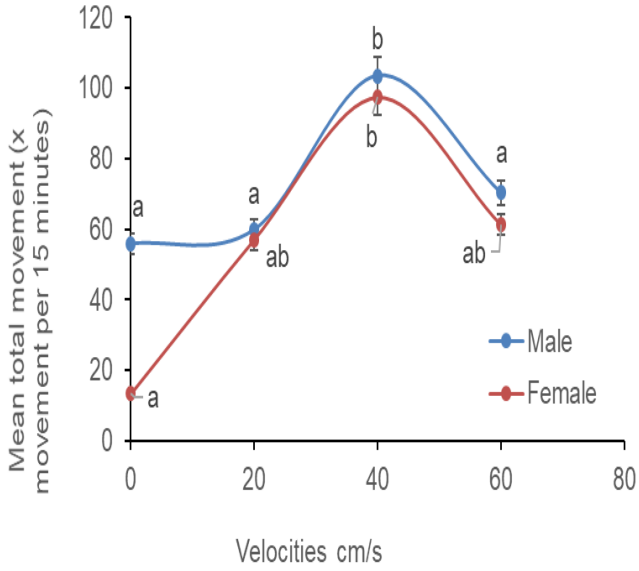



**Figure 5** Mean locomotors activities of male and female mud crab in recirculate aquaculture system

The crabs cultured in static water (i.e., moving with 0 cm/s velocity) produced 56 movements on average, whereas at the highest velocity of 60 cm/s the crabs exhibited 70 movements per 15 min. As can be seen from Figure 5, the greatest number of movements (104) was recorded at the flow velocity of 40 cm/s. During the entire experiment, 1,737 crab movements were recorded, with 56 to 104 movements per 15 min light-on interval. Unlike males, female crabs produced significantly different movements at

different water velocities ($p<0.05$). While 13–97 movements were captured per 15-min interval, 1,374 movements were recorded during the entire experiment.

Male *S. olivacea* are better walkers than their female counterparts during the growth phases. The maximum and average horizontal walking frequencies of male crabs exceeded those reported for other marine invertebrates (103.50 and 72.38, respectively, vis-à-vis female crabs' 97.33 and 57.25) [33]. This difference is expected since the crab is larger by several orders of magnitude than the larvae, for which swimming speeds have been reported in previous studies. Nevertheless, these findings provide the first data on the walking rates of *S. olivacea* in flowing water, establishing the crab's capacity for active exploration and retention within habitat sites via horizontal walking. The crabs were able to walk upstream at flow velocities less than approximately 40 cm/s. At greater flow rates, the crabs were unable to walk upstream and to maintain a consistent orientation at the highest flow setting (60 cm/s) and managed to walk only weakly.

While our findings provide useful information for establishing the role horizontal walking might play in habitat selection by mud crabs, scaling considerations suggest that caution should be exercised in extrapolating to natural environments. Light intensity and frequency spectrum were not adjusted to simulate natural conditions. Depth and width constraints of the flume severely restricted the scale of both water and crab movement.

The spatial scale over which we performed walking frequency measurements (114 cm) is at the lower end of the range of scales of interest in field environments. The ability of the crabs to maintain these walking frequencies over greater distances is unknown. Short, narrow flumes, such as the one used in the current study, have inherent problems with respect to dynamic scaling. Consequently, while this system is practical for making behavioural observations, the results regarding the flow dynamics are best viewed as first-order estimates. Finally, we stress that these results are derived from a single experiment.

While replication at the level of individual crab is appropriate for the tests performed here, repeating the experiment with different batches of crab and similar design would permit estimates of other sources of variation. Despite the shortcomings of this study, our findings do reveal several ecologically important aspects regarding walking of *S. olivacea*. First, crab are present throughout the water column over the range of flow rates used here. Second, these crabs can tolerate a wide range of current, hence, able to walk upstream at low to moderate flow velocities. Third, at moderate to high flow rates *S. olivacea* are unable to maintain their horizontal orientation and are transported largely at the mercy of water currents. Given that a correlation has been reported between molt stage of *C. sapidus* megalopae and settlement intensity [34] and that there may be ontogenetic changes in phototaxis, interactions between physical factors (light and current speed) and developmental changes in walking behavior [1] and ability need to be examined. In shallow-water estuarine habitats, tidally-driven currents can be expected to fall below the maximum speed against which crab can swim for a significant portion of most tidal cycles. This may be particularly true in vegetated habitats in which flow velocities are significantly reduced [1]. Our data suggest that during those times crab possess the ability to maneuver and actively affect their encounter rates with suitable habitat sites.

### 3.2 Escaping Capabilities ($F_{escp}$)

The escaping capabilities ($F_{escp}$) of male crabs at different water velocities were not significantly different ($p>0.05$) (Figure 6).

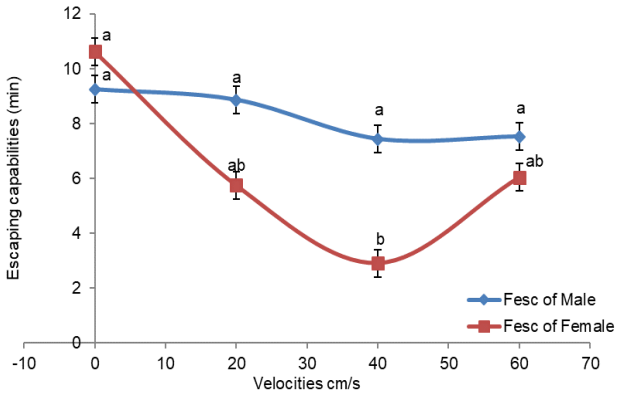



**Figure 6** Mean time escapes of male and female crab in recirculate aquaculture system

The crabs cultured in static water tended to escape within 9.24 min on average, whereas those subjected to 60 cm/s water flow exhibited required 7.52 min. While $F_{escp}$ ranged from 7.43 to 9.24 minutes within the 15-min interval, the shortest escape time (7.43 min) for male crabs was observed under 40 cm/s flow velocity. On the other hand, $F_{escp}$ of female crabs at different flow velocities was significantly different ($p<0.05$), ranging from 10.62 min at 0 cm/s to, 6.04 min at 60 cm/s. During a 15-min period, $F_{escp}$ ranged from 2.91 to 10.62, with the shortest $F_{escp}$ recorded at 40 cm/s.

The behavior (escaping flow) of organisms can represent a direct or indirect response to unfavorable conditions. Individuals of *S. olivacea* demonstrated numerous behavioral changes when subjected to changes in flow velocities. For both male and female crab, an increased rate of flow velocities (>40 $cms^{-1}$) implies stress in terms of increased locomotor activity to escape from stress conditions. The use of behaviors to maintain homeostasis in decapod crustaceans has been explored [35]. Shumway [36] found that exposing hermit crabs to low salinities resulted in increased in their activity, most likely in an attempt to move away from the unfavorable conditions, and

they only withdrew into their shell when escape was impossible. This may explain the increase in the percentage of time spent attempting to escape from the dental wax which restrained movement of the shell when subjected to all stress factors, as the greater flow velocities motivate the crab to increase energy spent endeavoring to keep itself within its optimal distribution. In this study, the shortest observed flow escape time for female crab was 2.91 min for 40 cm/s compared to the time for male crab to escape which was 7.43 min for 40 cm/s.

### 3.3 Fatty Acids Change of Muscle

The average muscle total fatty acid (TFAs) profiles of *S. olivacea* among different velocities water are shown in Figure 7 (A-D). In velocities 20 cm/s, omega-3 and omega-6 was highest recorded in day 30 compared to other velocities Figure 8 (A-D). The impacts of water velocities on muscle fatty acid profiles of *S. olivacea* are shown in Table 1-4. The outcomes noted that lower TFAs documented in both male and female in 60 cm/s. Amazingly, TFAs profiles were increased dramatically in 20 cm/s compared to other velocities (0, 40 and 60 cm/s) (Figure 4).

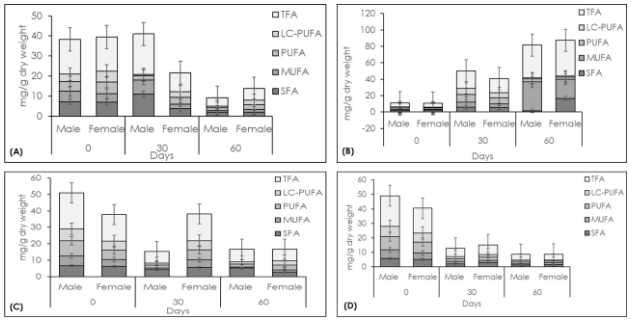



**Figure 7** Fatty acid concentrations of muscle *S. olivacea* in different water velocities (A - 0 cm/s; B – 20 cm/s; C – 40 cm/s; D – 60 cm/s). * Saturated Fatty Acids (SFA), Monounsaturated Fatty Acids (MUFA), Polyunsaturated Fatty Acids (PUFA), Long-Chain Polyunsaturated Fatty Acids (LC-PUFA), Total Fatty Acid (TFA)

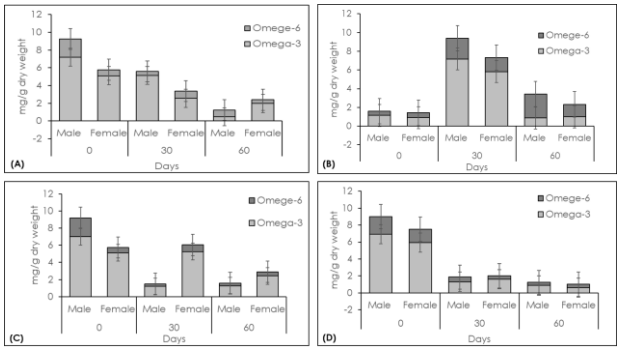



**Figure 8** Omega-3 and omega-6 concentrations of muscle *S. olivacea* in different water velocities (A - 0 cm/s; B – 20 cm/s; C – 40 cm/s; D – 60 cm/s)

The good quality of crabs are mostly related to lipids and particularly omega-3 (PUFA) [37]. The Table 1-4 represented fatty acid profiles of muscles male and female *S. olivacea* with various water velocities. The maximum total fatty acid composition (TFA) for male was (40.17 mg/g) and for female was (43.7 mg/g). Relationship between omega-3 to omega-6 PUFA were applied as an indicator for fatty acid index and a greater value normally represent the best dietary indexes [38].

In the present study, omega-3 to omega-6 ratio valued from 0.62 to 11.25 in muscle of male and for female ranged 1.74 to 8.49 of *S. olivacea*.

**Table 1** Fatty acids profiles (mg/g dry weight) in hatchery sampled muscle of *S. olivacea* broodstock reared at different water velocities (0 cm/s). Values are mean±SD, n=30

| Days | 0 | | 30 | | 60 | |
|---|---|---|---|---|---|---|
| Fatty acids | Male | Female | Male | Female | Male | Female |
| C4:0 | 0.21±0.11 | 0.28±0.03 | 0.32±0.05 | 0.62±0.03 | 0.15±0.02 | 0.14±0.04 |
| C6:0 | 0.77±0.03 | 0.68±0.03 | 0.23±0.04 | 0.36±0.03 | 0.08±0.04 | 0.14±0.07 |
| C8:0 | 0.06±0.03 | 0.11±0.03 | 0.05±0.03 | 0.34±0.04 | 0.05±0.02 | 0.07±0.05 |
| C10:0 | 0.16±0.12 | 0.14±0.06 | 0.11±0.05 | 0.07±0.04 | 0.06±0.04 | 0.04±0.03 |
| C11:0 | 0.09±0.04 | 0.07±0.02 | 0.14±0.05 | 0.34±0.06 | 0.07±0.02 | 0.08±0.04 |
| C14:0 | 0.07±0.02 | 0.06±0.02 | 0.04±0.03 | 0.03±0.02 | 0.04±0.02 | 0.09±0.03 |
| C15:0 | 0.07±0.03 | 0.09±0.03 | 0.03±0.02 | 0.05±0.03 | 0.04±0.02 | 0.07±0.06 |
| C16:0 | 2.12±0.15 | 1.36±0.03 | 0.40±0.09 | 0.46±0.03 | 0.32±0.01 | 0.23±0.05 |
| C17:0 | 0.22±0.05 | 0.29±0.01 | 0.06±0.02 | 0.04±0.03 | 0.04±0.01 | 0.06±0.03 |
| C18:0 | 1.38±0.02 | 1.15±0.04 | 0.34±0.04 | 0.46±0.08 | 0.17±0.05 | 0.24±0.03 |
| C20:0 | 0.26±0.06 | 0.17±0.03 | 0.25±0.02 | 0.06±0.03 | 0.08±0.04 | 0.04±0.03 |
| C21:0 | 0.10±0.02 | 0.25±0.04 | 0.23±0.06 | 0.08±0.03 | 0.12±0.01 | 0.12±0.03 |
| C22:0 | 0.33±0.08 | 0.33±0.02 | 0.26±0.03 | 0.22±0.09 | 0.17±0.02 | 0.11±0.03 |
| C23:0 | 0.04±0.03 | 0.26±0.03 | 0.05±0.02 | 0.04±0.03 | 0.03±0.01 | 0.08±0.05 |
| C14:1 | 0.05±0.04 | 0.06±0.02 | 0.10±0.06 | 0.04±0.03 | 0.04±0.01 | 0.14±0.03 |
| C15:1 | 0.75±0.12 | 0.86±0.06 | 0.19±0.02 | 0.22±0.05 | 0.11±0.02 | 0.16±0.03 |
| C16:1 | 0.39±0.09 | 0.34±0.04 | 0.11±0.05 | 0.06±0.02 | 0.07±0.02 | 0.09±0.04 |
| C17:1 | 0.30±0.02 | 0.09±0.02 | 0.04±0.03 | 0.05±0.04 | 0.05±0.04 | 0.04±0.03 |
| C20:1 | 0.15±0.07 | 0.06±0.05 | 0.07±0.02 | 0.05±0.02 | 0.03±0.02 | 0.10±0.03 |
| C24:1 | 0.55±0.09 | 0.51±0.19 | 0.61±0.03 | 0.63±0.07 | 0.45±0.05 | 0.35±0.03 |
| C18:1N9T | 3.60±0.12 | 2.29±0.57 | 0.55±0.07 | 0.51±0.07 | 0.36±0.03 | 0.29±0.04 |
| C18:1N9C | 0.05±0.01 | 0.07±0.04 | 0.04±0.04 | 0.03±0.02 | 0.04±0.03 | 0.08±0.03 |
| C22:1N9 | 0.12±0.04 | 0.12±0.04 | 0.09±0.02 | 0.11±0.03 | 0.04±0.02 | 0.17±0.05 |
| C18:2N6T | 0.12±0.04 | 0.81±0.03 | 0.10±0.04 | 0.04±0.02 | 0.08±0.04 | 0.04±0.03 |
| C18:2N6C | 1.63±0.23 | 0.17±0.03 | 0.10±0.05 | 0.09±0.04 | 0.07±0.03 | 0.05±0.03 |
| C18:3N6 | 0.12±0.05 | 0.10±0.02 | 0.30±0.03 | 0.14±0.01 | 0.20±0.02 | 0.05±0.02 |
| C20:3N6 | 0.05±0.01 | 0.09±0.04 | 0.06±0.03 | 0.07±0.02 | 0.05±0.03 | 0.07±0.04 |
| C20:4N6 | 0.30±0.04 | 0.40±0.03 | 0.10±0.02 | 0.05±0.03 | 0.03±0.02 | 0.16±0.06 |
| C18:3N3 | 0.08±0.04 | 0.14±0.05 | 0.04±0.01 | 0.08±0.04 | 0.13±0.01 | 0.09±0.05 |
| C20:3N3 | 0.05±0.04 | 0.07±0.04 | 0.04±0.02 | 0.07±0.03 | 0.04±0.02 | 0.11±0.02 |
| C20:5N3 | 0.52±0.06 | 0.52±0.05 | 0.63±0.05 | 0.05±0.04 | 0.39±0.05 | 0.10±0.02 |
| C22:6N3 | 6.37±0.50 | 5.14±0.14 | 0.65±0.04 | 1.37±0.15 | 0.45±0.04 | 0.25±0.04 |
| C20:2 | 0.03±0.02 | 0.05±0.03 | 0.04±0.03 | 0.08±0.04 | 0.05±0.03 | 0.07±0.03 |
| C22:2 | 0.05±0.01 | 0.06±0.04 | 0.05±0.02 | 0.15±0.03 | 0.05±0.02 | 0.04±0.03 |

**Table 2** Fatty acids profiles (mg/g dry weight) in hatchery sampled muscle of *S. olivacea* broodstock reared at different water velocities (20 cm/s). Values are mean±SD, n=30

| Days | 0 | | 30 | | 60 | |
|---|---|---|---|---|---|---|
| Fatty acids | Male | Female | Male | Female | Male | Female |
| C4:0 | 0.40±0.04 | 0.40±0.03 | 0.21±0.11 | 0.28±0.03 | 0.12±0.03 | 6.48±0.03 |
| C6:0 | 0.16±0.01 | 0.26±0.06 | 0.77±0.03 | 0.68±0.03 | 0.05±0.03 | 5.06±0.13 |
| C8:0 | 0.35±0.04 | 0.26±0.03 | 0.06±0.03 | 0.11±0.03 | 0.22±0.04 | 1.57±0.02 |
| C10:0 | 0.04±0.01 | 0.03±0.02 | 0.19±0.12 | 0.14±0.06 | 0.17±0.04 | 0.06±0.02 |
| C14:0 | 0.01±0.01 | 0.02±0.01 | 0.07±0.02 | 0.13±0.15 | 0.05±0.03 | 0.04±0.02 |
| C15:0 | 0.03±0.02 | 0.03±0.02 | 0.07±0.03 | 0.09±0.03 | 0.06±0.04 | 0.17±0.13 |
| C16:0 | 0.25±0.04 | 0.36±0.03 | 2.12±0.15 | 1.36±0.03 | 0.28±0.04 | 1.22±0.09 |
| C17:0 | 0.04±0.01 | 0.04±0.01 | 0.22±0.05 | 0.29±0.01 | 0.14±0.05 | 0.16±0.03 |
| C18:0 | 0.25±0.04 | 0.32±0.11 | 1.38±0.02 | 1.15±0.04 | 0.09±0.05 | 0.07±0.04 |
| C20:0 | 0.12±0.04 | 0.22±0.03 | 0.26±0.06 | 0.17±0.03 | 0.05±0.03 | 0.16±0.04 |
| C21:0 | 0.16±0.03 | 0.06±0.03 | 0.09±0.02 | 0.25±0.04 | 0.06±0.02 | 0.13±0.02 |
| C22:0 | 0.03±0.01 | 0.03±0.01 | 0.33±0.08 | 0.33±0.02 | 0.16±0.03 | 1.49±0.09 |
| C23:0 | 0.04±0.01 | 0.03±0.01 | 0.04±0.03 | 0.26±0.03 | 0.06±0.03 | 0.04±0.03 |
| C14:1 | 0.03±0.02 | 0.03±0.02 | 0.05±0.04 | 0.06±0.02 | 0.08±0.07 | 0.01±0.01 |
| C15:1 | 0.18±0.02 | 0.13±0.03 | 0.75±0.12 | 0.86±0.06 | 0.73±0.05 | 1.24±0.26 |
| C16:1 | 0.06±0.02 | 0.07±0.02 | 0.39±0.09 | 0.34±0.04 | 0.12±0.06 | 0.25±0.04 |
| C17:1 | 0.06±0.03 | 0.04±0.02 | 0.30±0.02 | 0.09±0.02 | 0.13±0.02 | 0.14±0.03 |
| C20:1 | 0.04±0.02 | 0.08±0.02 | 0.15±0.07 | 0.06±0.05 | 0.59±0.09 | 0.21±0.02 |
| C24:1 | 0.31±0.03 | 0.28±0.02 | 0.55±0.09 | 0.51±0.19 | 32.52±0.26 | 33.1±0.95 |
| C18:1N9T | 0.06±0.02 | 0.40±0.03 | 3.60±0.12 | 2.29±0.57 | 0.54±0.05 | 0.72±0.04 |
| C18:1N9C | 0.18±0.04 | 0.09±0.03 | 0.11±0.06 | 0.34±0.49 | 0.08±0.02 | 0.06±0.03 |
| C22:1N9 | 0.24±0.03 | 0.03±0.02 | 3.60±0.14 | 0.14±0.02 | 0.29±0.03 | 0.28±0.04 |
| C18:2N6T | 0.04±0.03 | 0.06±0.03 | 0.15±0.03 | 0.08±0.03 | 0.23±0.03 | 0.12±0.08 |
| C18:2N6C | 0.05±0.03 | 0.06±0.02 | 1.63±0.23 | 0.81±0.03 | 0.59±0.06 | 0.66±0.03 |
| C18:3N6 | 0.23±0.03 | 0.26±0.02 | 0.12±0.05 | 0.10±0.02 | 1.30±0.40 | 0.18±0.03 |
| C20:3N6 | 0.07±0.03 | 0.03±0.01 | 0.05±0.01 | 0.09±0.04 | 0.05±0.02 | 0.18±0.04 |
| C20:4N6 | 0.07±0.02 | 0.11±0.03 | 0.30±0.04 | 0.40±0.03 | 0.39±0.17 | 0.29±0.02 |
| C18:3N3 | 0.03±0.02 | 0.08±0.05 | 0.25±0.02 | 0.09±0.03 | 0.05±0.02 | 0.05±0.03 |
| C20:3N3 | 0.12±0.05 | 0.07±0.03 | 0.05±0.04 | 0.07±0.04 | 0.17±0.04 | 0.11±0.02 |
| C20:5N3 | 0.16±0.05 | 0.02±0.01 | 0.52±0.06 | 0.52±0.05 | 0.45±0.01 | 0.39±0.04 |
| C22:6N3 | 0.85±0.04 | 0.72±0.04 | 6.37±0.50 | 5.14±0.14 | 0.21±0.04 | 0.45±0.03 |
| C20:2 | 0.07±0.04 | 0.09±0.03 | 0.03±0.02 | 0.04±0.02 | 0.07±0.02 | 0.08±0.03 |

**Table 3** Fatty acids profiles (mg/g dry weight) in hatchery sampled muscle of *S. olivacea* broodstock reared at different water velocities (40 cm/s). Values are mean±SD, n=30.

| Days | 0 | | 45 | | 60 | |
|---|---|---|---|---|---|---|
| Fatty acids | Male | Female | Male | Female | Male | Female |
| C4:0 | 0.21±0.11 | 0.28±0.04 | 0.25±0.04 | 0.27±0.03 | 0.53±0.02 | 0.08±0.05 |
| C6:0 | 0.77±0.03 | 1.16±0.30 | 1.07±0.07 | 1.19±0.27 | 1.25±0.04 | 0.46±0.08 |
| C8:0 | 0.06±0.03 | 0.10±0.05 | 0.05±0.02 | 0.07±0.02 | 0.06±0.03 | 0.07±0.04 |
| C10:0 | 0.19±0.12 | 0.08±0.04 | 0.08±0.04 | 0.05±0.04 | 0.06±0.02 | 0.04±0.01 |
| C11:0 | 0.18±0.05 | 0.10±0.06 | 0.34±0.03 | 0.06±0.04 | 0.23±0.02 | 0.07±0.04 |
| C12:0 | 0.31±0.03 | 0.17±0.05 | 0.27±0.03 | 0.16±0.04 | 0.25±0.02 | 0.11±0.08 |
| C13:0 | 0.15±0.04 | 0.19±0.08 | 0.14±0.02 | 0.25±0.03 | 0.07±0.03 | 0.16±0.03 |
| C14:0 | 0.07±0.02 | 0.05±0.02 | 0.06±0.03 | 0.06±0.03 | 0.09±0.02 | 0.05±0.02 |
| C15:0 | 0.07±0.03 | 0.05±0.03 | 0.04±0.02 | 0.03±0.02 | 0.06±0.02 | 0.08±0.04 |
| C16:0 | 2.12±0.15 | 1.51±0.38 | 0.08±0.03 | 1.17±0.04 | 0.05±0.02 | 0.54±0.04 |
| C17:0 | 0.22±0.05 | 0.26±0.14 | 0.15±0.04 | 0.16±0.03 | 0.08±0.03 | 0.15±0.04 |
| C18:0 | 1.38±0.02 | 1.43±0.32 | 0.06±0.02 | 1.23±0.05 | 0.07±0.02 | 0.49±0.06 |
| C20:0 | 0.26±0.06 | 0.45±0.04 | 0.05±0.03 | 0.46±0.07 | 0.06±0.03 | 0.10±0.03 |
| C21:0 | 0.26±0.03 | 0.12±0.03 | 0.06±0.03 | 0.15±0.02 | 0.08±0.06 | 0.05±0.03 |
| C22:0 | 0.33±0.08 | 0.24±0.05 | 1.41±0.22 | 0.26±0.03 | 2.17±0.04 | 0.11±0.02 |
| C23:0 | 0.04±0.03 | 0.07±0.04 | 0.07±0.02 | 0.10±0.02 | 0.07±0.02 | 0.09±0.03 |
| C14:1 | 0.05±0.04 | 0.07±0.03 | 0.05±0.03 | 0.09±0.03 | 0.07±0.02 | 0.06±0.03 |
| C15:1 | 0.75±0.12 | 0.56±0.04 | 0.43±0.04 | 0.55±0.03 | 0.11±0.02 | 0.09±0.02 |
| C16:1 | 0.39±0.09 | 0.28±0.02 | 0.09±0.04 | 0.32±0.06 | 0.07±0.02 | 0.13±0.05 |
| C17:1 | 0.30±0.02 | 0.08±0.03 | 0.08±0.04 | 0.12±0.02 | 0.04±0.02 | 0.09±0.03 |
| C20:1 | 0.15±0.07 | 0.11±0.04 | 0.07±0.02 | 0.13±0.06 | 0.07±0.01 | 0.06±0.03 |
| C24:1 | 0.55±0.09 | 1.18±0.16 | 0.14±0.02 | 1.37±0.15 | 0.12±0.01 | 0.25±0.03 |
| C18:1N9T | 3.60±0.12 | 1.67±0.15 | 0.07±0.02 | 1.77±0.05 | 0.07±0.02 | 0.67±0.02 |
| C18:1N9C | 0.07±0.02 | 0.09±0.02 | 0.08±0.02 | 0.14±0.01 | 0.05±0.02 | 0.07±0.05 |
| C22:1N9 | 0.06±0.02 | 0.05±0.04 | 0.06±0.03 | 0.05±0.04 | 0.10±0.02 | 0.08±0.05 |
| C18:2N6C | 1.63±0.23 | 0.18±0.14 | 0.05±0.03 | 0.38±0.06 | 0.06±0.02 | 0.19±0.05 |
| C18:2N6T | 0.13±0.03 | 0.04±0.03 | 0.08±0.04 | 0.05±0.03 | 0.05±0.02 | 0.09±0.04 |
| C18:3N6 | 0.12±0.05 | 0.09±0.01 | 0.06±0.03 | 0.10±0.03 | 0.05±0.03 | 0.08±0.04 |
| C20:3N6 | 0.05±0.01 | 0.09±0.05 | 0.07±0.04 | | 0.07±0.02 | 0.05±0.02 |
| C20:4N6 | 0.30±0.04 | 0.22±0.03 | 0.05±0.03 | 0.27±0.05 | 0.09±0.02 | 0.07±0.04 |
| C18:3N3 | 0.06±0.02 | 0.05±0.03 | 0.05±0.02 | 0.05±0.04 | 0.07±0.02 | 0.07±0.02 |
| C20:3N3 | 0.05±0.04 | 0.07±0.03 | 0.05±0.03 | 0.10±0.02 | 0.08±0.03 | 0.06±0.03 |
| C20:5N3 | 0.52±0.06 | 0.31±0.04 | 0.06±0.03 | 0.34±0.08 | 0.06±0.04 | 0.13±0.03 |
| C22:6N3 | 6.37±0.50 | 4.70±0.20 | 1.04±0.02 | 4.77±0.05 | 1.06±0.03 | 2.16±0.04 |
| C22:2 | 0.05±0.01 | 0.08±004 | 0.08±0.04 | 0.06±003 | 0.07±0.03 | 0.06±0.03 |

**Table 4** Fatty acids profiles (mg/g dry weight) in hatchery sampled muscle of *S. olivacea* broodstock reared at different water velocities (60 cm/s). Values are mean±SD, n=30

| Duration | 0 | | 30 | | 60 | |
|---|---|---|---|---|---|---|
| Fatty acids | Male | Female | Male | Female | Male | Female |
| C4:0 | 0.21±0.11 | 0.28±0.04 | 0.28±0.02 | 0.17±0.06 | 0.10±0.02 | 0.08±0.01 |
| C6:0 | 0.77±0.03 | 1.16±0.30 | 1.15±0.14 | 0.49±0.06 | 0.08±0.05 | 0.27±0.02 |
| C8:0 | 0.06±0.03 | 0.10±0.05 | 0.15±0.03 | 0.05±0.03 | 0.12±0.03 | 0.05±0.02 |
| C10:0 | 0.19±0.12 | 0.08±0.04 | 0.07±0.02 | 0.13±0.05 | 0.14±0.06 | 0.07±0.02 |
| C11:0 | 0.19±0.06 | 0.27±0.06 | 0.04±0.02 | 0.12±0.05 | 0.08±0.04 | 0.08±0.03 |
| C12:0 | 0.39±0.05 | 0.26±0.03 | 0.17±0.06 | 0.20±0.02 | 0.13±0.04 | 0.11±0.02 |
| C13:0 | 0.24±0.03 | 0.32±0.03 | 0.29±0.01 | 0.19±0.08 | 0.20±0.08 | 0.09±0.01 |
| C14:0 | 0.07±0.02 | 0.05±0.02 | 0.05±0.02 | 0.05±0.04 | 0.07±0.04 | 0.05±0.02 |
| C15:0 | 0.07±0.03 | 0.05±0.03 | 0.06±0.03 | 0.11±0.10 | 0.15±0.04 | 0.04±0.02 |
| C16:0 | 2.12±0.15 | 1.51±0.38 | 1.14±0.02 | 0.59±0.04 | 0.13±0.04 | 0.44±0.04 |
| C17:0 | 0.22±0.05 | 0.26±0.14 | 0.20±0.02 | 0.15±0.07 | 0.09±0.06 | 0.05±0.02 |
| C18:0 | 1.38±0.02 | 1.43±0.32 | 1.59±0.35 | 0.57±0.05 | 0.18±0.05 | 0.34±0.04 |
| C20:0 | 0.26±0.06 | 0.45±0.04 | 0.24±0.07 | 0.18±0.05 | 0.16±0.04 | 0.07±0.02 |
| C21:0 | 0.17±0.01 | 0.29±0.03 | 0.26±0.03 | 0.19±0.05 | 0.14±0.06 | 0.05±0.01 |
| C22:0 | 0.33±0.08 | 0.24±0.05 | 0.35±0.01 | 0.20±0.07 | 0.15±0.08 | 0.10±0.01 |
| C23:0 | 0.04±0.03 | 0.07±0.04 | 0.07±0.02 | 0.16±0.07 | 0.06±0.03 | 0.07±0.02 |
| C24:0 | 0.14±0.05 | 0.23±0.06 | 0.05±0.04 | 0.11±0.05 | 0.06±0.02 | 0.04±0.02 |
| C14:1 | 0.05±0.04 | 0.07±0.03 | 0.18±0.03 | 0.05±0.04 | 0.12±0.05 | 0.06±0.03 |
| C15:1 | 0.75±0.12 | 0.56±0.04 | 0.40±0.01 | 0.28±0.05 | 0.06±0.02 | 0.13±0.01 |
| C16:1 | 0.39±0.09 | 0.28±0.02 | 0.38±0.02 | 0.12±0.08 | 0.08±0.03 | 0.03±0.01 |
| C17:1 | 0.30±0.02 | 0.08±0.03 | 0.33±0.03 | 0.06±0.03 | 0.09±0.04 | 0.05±0.02 |
| C20:1 | 0.15±0.07 | 0.11±0.04 | 0.20±0.03 | 0.08±0.03 | 0.06±0.02 | 0.05±0.03 |
| C24:1 | 0.55±0.09 | 1.18±0.16 | 1.27±0.04 | 0.54±0.07 | 0.09±0.04 | 0.33±0.04 |
| C18:1N9T | 3.60±0.12 | 1.67±0.15 | 1.80±0.01 | 0.85±0.09 | 0.09±0.02 | 0.59±0.12 |
| C18:1N9C | 0.17±0.04 | 0.14±0.08 | 0.26±0.03 | 0.17±0.05 | 0.11±0.04 | 0.05±0.04 |
| C22:1N9 | 0.24±0.04 | 0.05±0.04 | 0.15±0.03 | 0.12±0.03 | 0.10±0.04 | 0.05±0.02 |
| C18:2N6C | 1.63±0.23 | 0.18±0.14 | 0.20±0.05 | 0.27±0.07 | 0.09±0.03 | 0.13±0.03 |
| C18:2N6T | 0.26±0.03 | 0.04±0.03 | 0.03±0.01 | 0.24±0.04 | 0.13±0.04 | 0.11±0.02 |
| C18:3N6 | 0.12±0.05 | 0.09±0.01 | 0.05±0.01 | 0.10±0.04 | 0.22±0.04 | 0.06±0.02 |
| C20:3N6 | 0.05±0.01 | 0.14±0.02 | 0.05±0.01 | 0.04±0.02 | 0.13±0.04 | 0.06±0.03 |
| C20:4N6 | 0.30±0.04 | 0.22±0.03 | 0.15±0.03 | 0.15±0.04 | 0.18±0.03 | 0.06±0.02 |
| C20:3N3 | 0.05±0.04 | 0.10±0.04 | 0.07±0.02 | 0.13±0.05 | 0.13±0.05 | 0.06±0.02 |
| C20:5N3 | 0.52±0.06 | 0.31±0.04 | 0.14±0.01 | 0.18±0.04 | 0.11±0.03 | 0.10±0.03 |
| C22:6N3 | 6.37±0.50 | 4.70±0.20 | 4.84±0.06 | 2.26±0.15 | 0.08±0.04 | 1.81±0.31 |
| C22:2 | 0.05±0.01 | 0.05±0.03 | 0.06±0.02 | 0.18±0.05 | 0.12±0.03 | 0.06±0.02 |

FAO/WHO has recommended dietary omega-3 to omega-6 PUFA ratio to be about 0.1-0.2 and by means greater index (>0.2), greater beneficial to human wellbeing [39], mud crab has been recommended as a nutritional seafood. This study discovered that there is proof of difference in the biochemical changes of mud crabs regarding to their water velocities, gender and duration (Days of culture). The greater index of omega-3 to omega-6 PUFA indicates the high nutritional value and healthy seafood.

Wild investigation have also displays that distribution of mud crab species seems to be highly affected by high water velocities and especially flood seasons [40-41]. At velocities 20 cm/s, higher of TFAs in crab muscle indicated optimum velocities reserved energy for locomotion, while 60 cm/s a lot of energy were used to counter the flow. These studies were supported by [42-45], stated that water velocities in more than 30-50 cm/s have the highly impact on energy distribution. High hydrodynamics combined with increased water flow, however, increase effectiveness and assist them search the prey resources [46] and help the crab for mating purpose. Wild investigation had stressed the importance of water velocities and substrates as key factors affecting crayfishes in streams [40-41, 47].

## 4.0 CONCLUSION

This study showed that locomotor activities of male *S. olivacea* were not significantly different with water flow velocities. Meanwhile, female *S. olivacea* were significantly different with water flow velocities. In addition at flow 40 cm/s, both male and female *S. olivacea* recorded highest movement with 104 and 97 movement. Also time for crab escape for male *S. olivacea* were not significantly different with water flow velocities. Meanwhile, female *S. olivacea* were significantly different with water flow velocities. Furthermore, the shortest time for $F_{esc}$ of male *S. olivacea* were observed in 40 cm/s with 7.43 min. Meanwhile, the shortest time for $F_{esc}$ of female *S. olivacea* were observed in 40 cm/s with 2.91 min. The differences of locomotor activities and time for crab escape between the two genders were mainly due to their physiology, bio-behavior and biochemical differences. Further study were then conducted on FAs analysis, lipid analysis, energy consumption and stress level.

Assessment of fatty acid parameters indicates that the TFA concentration in muscle of *S. olivacea* have relationship with water velocities. Optimum velocities, 20 cm/s give high TFA concentration compared to the other velocities tested. We can simplify that there is a relationship between energy for locomotion in muscle and velocities involved during the cultured. Excessive flow will attributed to loss of TFA which useful for locomotion, searching for food, growth and mating.

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