### Feeding preference of juvenile mud crab (*Scylla olivacea*, Herbst, 1896) in captivity

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#### Abstract

Mud crab (Scylla olivacea, Herbst 1896) is a valuable organism which is highly priced in the seafood industry. In Malaysia, the aquaculture industry of mud crabs is still in its infancy stage. In order to develop a feeding strategy for the aquaculture of mud crabs, their feeding preference and behaviour should be well understood. This study was conducted to identify if there is any preference to food for juvenile mud crabs kept in captivity within laboratory conditions. Prior to behavioural observations, crabs were starved for 5 days. Crabs were observed for a duration of 30 minutes and tested with natural food (fish muscle and chicken intestine), pelleted feed (fish meal pellets, PF; poultry offal meal pellets, PP) and agar-based feed (fish meal agar, AF; poultry offal meal agar, AP). The experiment was conducted by observation for a period of 30 minutes and time taken to approach and consume each given feed was recorded. The frequency of the type of food chosen was expressed as attraction to feed score index. When presented with two types of natural diets, the mud crabs had a significant inclination towards the chicken intestines displaying higher attraction to feed score index. Whilst given the choice between fish meal-based pellets (PF) and poultry offal meal-based pellets (PP), no significant difference was displayed in the time taken to approach and consume the feed, but significantly higher attraction to feed scores for PP. Chicken intestine was presented with the four types of formulated feed respectively, but revealed no significant differences in attraction to feed score index. Fish meal-based agar diets (AF) showed significant attraction to feed index score and faster approach time compared to PP. Overall results revealed that juvenile mud crabs accepted all types of tested feed, and showed significant preference towards AF when compared to PP. The findings indicate potential for the utilization of agar based diets for aquaculture of mud crabs.

Keywords: Mud crab, Crustacea, Aquaculture, Feeding behaviour

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### Introduction

Scylla olivacea or generally known as mud crab is an important species cultured to provide for the seafood demands. Mud industry crab aquaculture industry has several critical gaps which needs to be addressed, namely one being the development of suitable type of feed with valuable nutritional components. The life cycle of the mud crab involves planktonic and iuvenile crabs adult zoea. broodstock stages (Suprayudi et al., 2002). For each of these stages, different types of feed need to be offered. In the hatchery stage, the planktonic zoea required live feed such as rotifers and artemia (Baylon et al., 2004; Waiho et al., 2018). Mud crabs in the wild are exposed to various types of resources their food in natural environment. They consume mollusc, fish, crustacean, detritus from the mangrove floor and sometimes mud (Shelley and Lovatelli, 2011).

For mud crab aquaculture, the common practice is that crabs are usually separated in individual containers and reared in an outdoor pond rearing system due to their territorial and cannibalistic behaviour. In general, mud crabs are fed pieces of trash fish or general wastes from the poultry industry such as chicken intestines or offal. To move aquaculture of mud crabs into a more viable and sustainable industry for the future, several improvements should be considered. Instead of clearing mangroves and setting up cultures of mud crab in outdoor ponds, indoor systems should be the way forward (Syafaat et al., 2019; Paterson and Mann, 2011). A suitable formulated diets that addresses the nutritional needs of mud crabs for best growth and value for expenditure cost should be developed. This study was conducted as a baseline to understand if juvenile mud crabs had preference towards natural feeds and formulated feeds with different textures. Their food choice selection was observed and compared to better facilitate future production of aquafeeds with potential replacement of the fish meal component as a protein source with the cheaper option of poultry offal meal.

### Materials and methods

## Organism procurement and maintenance

Juvenile mud crabs for this research was obtained from local fishermen. The mud crabs were housed in individual glass aquaria supplied with filtered artificial sea water (29ppt). A total of 27 mud crabs (100-200g) were used in this study.

### Experimental diet preparation

Several feed were prepared prior to the feeding observation experiments. Three categories of diets were prepared, namely, natural food, pellet food and agar-based food. Natural food prepared were fresh fish and chicken intestine, cut into 3x1 cm pieces. Four types of formulated feed (Table 1) were also prepared whereby the main protein

sources were fish meal and poultry offal meal. The formulated feed were divided into two main textures: pelleted feed (cylindrical, hard pellet form) and agarbased feed (soft cube form). A preliminary integrity test of the formulated feed were conducted by soaking them in 29ppt seawater for 24 hours and measuring the swell rate of the diets.

Table 1: Diet formulation for feeding preference behaviour of juvenile mud crab ( <i>Scylla olivacea</i> ).
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Component	Pellet Feed (g/100g)		Agar Feed (g/100g)	
	Diet PF	Diet PP	Diet AF	Diet AP
Fish meal	47	0	47	0
Poultry offal meal	0	47	0	47
Algae powder	23	23	23	23
Corn starch	16	16	6	6
Corn oil	5	5	5	5
Vitamin mix	3	3	3	3
Mineral mix	3	3	3	3
Binder	3	3	0	0
Agar	0	0	10	10

# Feeding preference behaviour observation

The observation test arena (Fig. 1) was prepared by using a glass aquarium with 3 dark painted sides, and 1 transparent side. The testing arena was separated into 2 main sections, one for crab acclimation (Fig. 1A) and the other section is for feeding behaviour observation. Before the feeding observations were conducted, the mud crabs were starved for a period of 5 days.

Filtered sea water was filled and crab placed into the acclimation area of testing arena for 30 minutes before the feeding test was conducted. The food tested will be placed in a far corner of the testing arena (Fig. 1C) and the separator of the tank removed once food is placed. Timer was started and the crab's behaviour was observed and time taken for approach or consumption of food was noted down. Each feeding observation was conducted for a period of 30 minutes using the focal point observation method. Feeding observations were conducted with diet combination trials as in Table 2.

# Food preference and attraction to feed scores

From the observation trials, mud crabs response towards the food given was noted. To indicate preference of food given, the first approach and consumption of food was taken into consideration. The time to begin food consumption was divided by the total trial time (1800 seconds) and displayed as a percentage score. The attraction to feed was scored by giving a value to the selection of food by the mud crabs, whereby score of 1 was given to the first type of food approached and consumed, score of 0.5 given if the second type of food was approached and consumed, and a score of 0 was

given if none of the food given was consumed. The total score was divided by the number of replicate animals tested and expressed as a percentage. The total scores calculation method was modified based on a similar study done on prawns (Bardera *et al.*, 2019). Attraction to feed scores was used to determine the food combination trials selection.

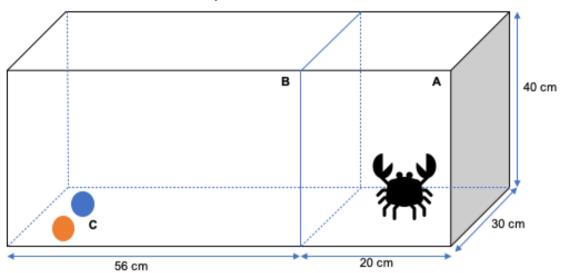


Figure 1: Testing arena setup for feeding behaviour observations of juvenile mud crab. Section A: acclimation of crab prior to experiment, B: feeding area with removable separator, C: food placement area.

 Table 2: Diet combinations for feeding preference behaviour observation trials of juvenile mud crabs.

	Feed category	Description
Trial 1	Natural feed	Fish muscle
Trial 2	Natural feed	Fish muscle Chicken intestine
Trial 3	Pellet feed	Diet PF Diet PP
Trial 4	Agar feed	Diet AF Diet AP
Trial 5	Natural feed vs Pellet feed	Trial 2: preferred natural feed Diet PF Diet PP
Trial 6	Natural feed vs Agar feed	Trial 2: preferred natural feed Diet AF Diet AP
Trial 7	Pellet feed vs Agar feed	Trial 3: preferred pellet feed Trial 4: preferred agar feed

#### Statistical analysis

Statistical analysis was performed using SPSS ver. 20 software and paired t-test was utilized to determine significance of results obtained.

#### Results

# Characterization of formulated diet integrity in water

The prepared pellets (PF and PP) and agar-based (AF and AP) diets were submerged in 29ppt seawater to understand their integrity in water. The agar-based diets withheld their shape for the entire 24 hours of integrity monitoring. Contrastingly, the pellet type diets increased in size and showed swelling each hour of observation and completely disintegrated at the 5-hour mark.

# Feeding preference behaviour observation

Response towards food given in the feeding arena was expressed as a score (%) of the time at first attempt of the mud crab to consume the feed given in the testing arena. The crabs were observed visually once the tank The was removed. crab separator behaviour of locomotion, feeding and inactivity were observed. The feeding behaviour of juvenile mud crabs in terms of time taken for the first approach and consumption of feed is presented in Figure 2.

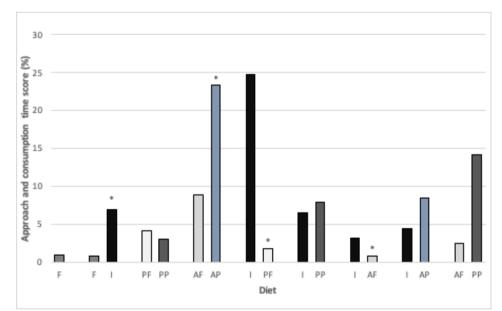


Figure 2: Time taken for juvenile mud crab first approach and consumption of diets expressed as the approach score (%) (F: fish, I: chicken intestine, PF: fish meal-based pellet, PP: poultry meal-based pellet, AF: fish meal-based agar diet, AP: poultry meal-based agar diet). Asterisk (\*) indicates significant difference at p<0.05 analysed with paired t-test.

For crabs fed only one type of diet, which is the fish muscle, only 50% attempted to consume the feed, with an average time of 17.4 seconds. When they were presented with two types of natural feed, fish and intestine, both foods were accepted but it took them significantly faster time to approach the fish muscle comparative to the chicken intestine. Interestingly, in terms of frequency, chicken intestine scored a higher attraction to feed index (Fig. 3) as opposed to the fish muscle.

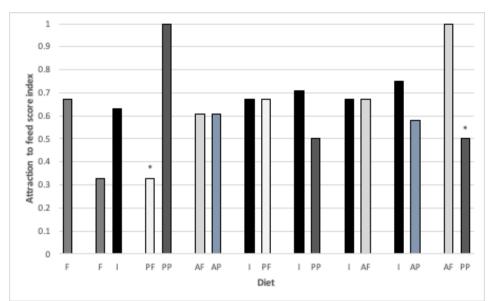


Figure 3: Frequency for juvenile mud crab to first approach and consume diets expressed as attraction to feed score index. (F: fish, I: chicken intestine, PF: fish meal-based pellet, PP: poultry meal-based pellet, AF: fish meal-based agar diet, AP: poultry meal-based agar diet). Asterisk (\*) indicates significant difference at p<0.05 analysed with paired t-test.

Both fish meal and poultry offal meal pellets were presented at the same time to the mud crabs and although insignificantly different, they seem to approach the poultry offal meal-based diet (PP) a fraction of time faster than the fish meal-based pellets (PF). They also indicated a high attraction to feed index score for PP compared to PF significantly. Fish meal-based agar diet (AF) fared better in terms of faster approach time compared to poultry offal meal-based agar diet (AP) but showed equal attraction index scores. This was also reflected when AF was compared to the chicken intestine, but had the higher index score significantly when compared to PP. The attraction frequency index scores were higher for intestines when presented with PP and AP. This was also reflected in a faster time to approach and consume the chicken intestines compared to PP and AP.

#### Discussion

Mud crabs feeding behaviour was observed in this study to contribute towards the development of formulated diets in aquaculture of this species. Two types of natural feeds were tested, and fish muscle was quickly consumed to chicken intestines. comparative However, in terms of frequency, the intestines had a higher attraction to feed score index compared to fish and was selected for the subsequent experimental food choice trials.

We chose to compare natural feed with two different forms of diets, namely conventionally prepared pellet type diets and agar-based cube diets. Mud crabs are equipped with chelipeds used to capture prey and at the same time used to scavenge and pick up food items from their environment (Keenan and Blackshaw, 1997). In the wild, Scylla olivacea were reported to predominantly consume other crustaceans, fish, detritus and mud (Viswanathan and Raffi, 2015). The formulated test pellets and agar cubes presented to the crabs are in between 1-2cm in length. From the observation, mud crabs did not find it difficult to pick up the pellets or the agar cubes for feeding. PP pellets had significantly higher attraction to feed score index compared to PF, and faster approach time. Based on their choice of natural feed, being more inclined towards chicken intestine compared to fish in terms of frequency of attraction, this was also reflected in their choice of pellets presented. Pellets in water tend to diffuse scent molecules and capture the attention of crabs and attract them to it. Chemical changes in the water when food is given will trigger the chemosensory systems of crabs, and draws them closer (Waldrop et al., 2016). This observation indicates preference towards poultry offal meal fish meal compared to pellets. Contrastingly, when agar-based AF and AP diets were presented, equal preference was shown in terms of frequency of attraction to feed.

However. there were significant differences (p < 0.05) in the approach time where fish meal-based AF diet was consumed at a faster rate compared to AP diet. Agar cubes most likely had a slower diffusion rate in water compared to pellets and there is a possibility that the fish meal used in the diet preparation had a stronger scent compared to the poultry offal meal and attracted the crabs towards them faster comparatively.

The poultry offal meal was prepared in the lab by cleaning with water and drying chicken intestines and innards in the oven and ground into fine powder. There is a possibility of the loss in some scent due to the processing of the ingredient compared to the fresh chicken intestine used in the natural feed feeding trials. This finding is reflected in the attraction to feed score index when intestine was compared with the formulated diets. Even if not significant statistically, the patterns obtained supports this statement. When intestine was presented with PF or AF, the attraction to feed score was equal, but the approach time for PF and AF were significantly faster, potentially indicating preference towards fish meal-based pellets and agar cubes compared to fresh intestine. Their choice on fish meal also may be attributed to the familiarity of a marine based scent compared to an unfamiliar terrestrial based scent. Opposingly, when both choices involved poultry items, namely when intestines were presented with PP or AP, similar

patterns was observed where fresh intestines had higher attraction to feed scores and faster approach time.

To date, most studies tried to elucidate the nutritional requirements of mud utilizing conventional crabs by ingredients such as fish meal and soybean meal as the main protein source (Catacutan, 2002; Zhao et al., 2015). The main objective of this study was to shift the mud crab aquaculture industry towards using formulated feeds and finding the ingredients which are preferred by the mud crabs. Eventually the goal would be to provide formulated feeds which are suitable and well accepted by the crabs. Based on the preference trials conducted, chicken intestines seem to be well accepted by mud crabs. The utilization of poultry offal meal in aquafeeds is gaining popularity and also shows that it is able to be replace fish meal completely in the diet of juvenile mahseer with no adverse effect of growth and survival (Ismail Kamarudin. 2013). and Alternative protein sources using poultry waste, like chicken feather are utilized in juvenile gilthead also seabream formulated feed to increase the attractiveness and palatability (Al-Souti et al., 2019). They are more attracted to intestines compared to poultry-based pellets or agar cubes; however, when intestine was presented with fish-based pellets or agar cubes they chose PF or AF significantly faster.

The final combination of food presented was the most preferred pellet (PP) compared to the most preferred

agar diet (AF). The results indicated that mud crabs were more attracted to the AF diet and consumed them faster than the poultry-based PP diet. When fish meal-based diets were presented, they were approached and consumed faster compared to poultry-based feeds. Testing the textures of different type of formulated feed was essential as commercially produced pellets specifically for mud crabs are scarcely available. Mud crabs also have the capacity to turn cannibalistic and the right type of housing and food should be provided (Mirera and Moksnes, 2013). Their natural behavior is to remain hidden in the muddy environment of the mangrove areas and emerge past sunset to commence feeding (Mamun et al., 2008). As nocturnal feeders, it is recommended that feed given to cultured crabs should be able to withstand longer periods of submersion in water. If a culture farm is operating on normal hours, the crabs may not consume the diets provided during the day-time immediately. Agarbased diets have high potential to be utilized in the mud crab aquaculture industry as they remained intact in the water for longer period of time compared to the pellet.

### Conclusion

As a conclusion, comparison made with fish meal-based formulated feed were faster approached and consumed by mud crabs. In terms of the preferred formulated feed, we recommend using agar-based diet, as it indicated higher attraction to feed score index and faster approach and consumption time compared to PP diet. Agar based diets is also more stable, holds its shape and integrity better compared to pellet diets which disintegrate faster in the water.

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