# Survival and growth performance of the purple mud crab (*Scylla tranquebarica*) instar fed with commercial formulated feed and minced fish diets

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**Abstract**. The present study aims to evaluate the survival and growth of the instar of purple mud crab (*Scylla tranquebarica*) fed with commercial formulated feed and minced fish (*Decapterus* sp.), referred to as CF and MF, respectively. Crab instar specimens (with initial body weight of 41±3 mg, carapace width of 5.5±0.2 mm) were stocked with a stocking density of 20 instars per tank in triplicates. Instars were fed with CF and MF at 8% body biomass twice daily in the morning and evening. After 3 weeks, the survival value was significantly higher in specimens fed with CF (72.2%) compared to MF (44.4%) (p = 0.034), with a final body weight of 0.5 g in CF fed specimens compared to 0.4 g in MF fed specimens (p = 0.016) and body weight gain of 1129% in CF fed specimens compared to 905% in MF fed specimens (p = 0.001). Similarly, the specific growth rate was significantly greater in CF fed instars than in MF fed instars (17.9 and 16.5 %day$^{-1}$, p = 0.001), with a higher moulting frequency observed in CF instars (24.7) compared to MF instars (13.3) (p = 0.01). However, the total feed intake per crab was significantly lower in CF fed specimens (0.4 g) than in MF fed specimens (1.0 g) (p = 0.001) with a feed conversion ratio of 6.3 in CF specimens compared to 18.8 in MF fed specimens (p = 0.008). Generally, instars fed with CF showed better survival and growth performance compared to instars fed with MF, indicating that the instar specimens accepted and utilized CF for living processes. Thus, this study shows that CF can be used in the purple mud crab instar nursery phase culture.

**Key Words**: natural food, nursery, moulting frequency, purple mud crab instar.

**Introduction**. Purple mud crab (*Scylla tranquebarica*) is one of the most important fishery candidates among ASEAN countries including Malaysia due to high demand and is gradually gaining interest among the aquaculturists (Sharif et al 2016; Quinitio 2017). Due to overfishing and exploitation of the wild population (Sharif et al 2016), seed production of mud crabs from aquaculture is timely needed to alleviate the fishing pressure on the wild population (Quinitio 2017). In mud crab seed production, larvae are reared in the hatchery and the produced miniature crab instar with approximately 3-4 mm carapace width are kept in the nursery for 2-4 weeks before stocking them into the grow-out facilities after reaching the size of a juvenile, which is about 25 to 30 mm in carapace width (Ut et al 2007; Parado-Estepa et al 2015; Quinitio 2017).

Most mud crab aquaculture rely on natural food such as low-cost fish, bivalves like mussels and oysters, squid, and others as the main food source for feeding purposes in all stages, including the crab instar nursery phase, juvenile grow-out, as well as in broodstock management (Genodepa & Failaman 2016; Alava et al 2017; Quinitio 2017). However, the usage of natural food showed disadvantages over seasonal availability, vulnerability to disease transmission, deteriorating water quality, and fluctuating market prices (Catacutan et al 2015; Gong et al 2017). Formulated feed, with a known nutritional profile, easy availability, convenient handling, and storage, is a more sustainable and eco-friendlier alternative to replace natural food (Aaqillah-Amr et al 2021) as it reduces feed cost, labour, and waste output (Gong et al 2017; Huang et al 2017). Formulated feed is well developed and commercially available for aquaculture species such as finfish and shrimp. These feeds contain essential nutrients that can support the nutritional requirement for different life stages of the animal. Nowadays, formulated feed plays a vital role in expanding the aquaculture industry as witnessed through the use of formulated feed in intensive farming of salmon (*Salmo salar*), Nile tilapia (*Oreochromis niloticus*), and shrimp (*Litopenaeus vannamei*) worldwide.

In the last two decades, attempts to develop formulated feed for mud crabs were mostly focused on juveniles, sub-adults, and broodstock (Triño et al 2001; Unnikrishnan & Paulraj 2010; Catacutan et al 2015; Thien & Yong 2017), with the development of feed for mud crab instars only initiated in recent years. A previous study conducted on the *S. serrata* instar using a diet formulation modified from juvenile mud crab diet, had reported lower growth compared to specimens fed with mussel meat (Alava et al 2017). As a result, further works had been focused on dietary crude protein (Zheng et al 2020) and crude lipid (Xu et al 2020) requirements of the mud crab instar.

Information on the nutritional requirements for mud crab instar survival and growth remain under investigation and more works is needed to refine the diet formulation. Hence, due to the lack of availability of commercial formulated feed exclusively for mud crabs, the current mud crab farming feeding management mostly depends solely on the use of natural food such as low-cost fish or bivalves which are available abundantly in the local waters. While works are in progress to obtain the nutritional requirement profiles for feed development for the mud crab instar, there is ongoing demand for alternative commercial feed beside natural food to support the expanding mud crab aquaculture farming. A previous study had reported the use of commercial feed such as finfish and shellfish on the mud crab instar. Milkfish (*Chanos chanos*) and tiger prawn (*Penaeus monodon*) commercial formulated feeds were tested on the giant mud crab, *S. serrata* instar, and the result revealed that the tiger prawn feed was better than the milkfish feed to support the survival and growth of the instar (Genodepa & Failaman 2016). Since both shrimps and crabs are crustaceans, shrimp commercial feed is possible to show better advantage than the finfish feed on the survival and growth of the crabs.

Hence, the present study aims to evaluate the survival and growth of the purple mud crab instar using the commercial shrimp formulated feed and the low-cost forage fish, *Decapterus* sp. which are available abundantly in the local wet market. Currently, *Decapterus* sp. is commonly used in the local mud crab aquaculture farming. Hence, the present finding will provide feasible practical information and feedback to the local farmers to enhance the mud crab aquaculture farming, particularly the instar nursery phase feeding management.

## Material and Method

***Experiment protocol***. This study was conducted in the Crustacean Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah, Malaysia. The purple mud crab instars with an initial body weight of 41±3 mg and carapace width of 5.5±0.2 mm were obtained from the institute hatchery and stocked into the fibreglass tanks (0.61m x 0.40m x 0.61m) with a stocking density of 20 instars in each tank, equivalent to 50 ind $m^{-2}$ in triplicates group (Ut et al 2007). The culture tank was filled with filtered seawater and well aerated. Black garden nets were provided in the culture tank as a shelter for the crab instar (Rodriguez et al 2007). Water exchange of 50% was performed daily in the morning before feeding (Quinitio et al 2001). Water quality was monitored daily. Salinity was maintained at 27.26±0.76 ppt, temperature at 27.69±0.60$^o$C, dissolved oxygen and pH at 4.43±0.21 mg $L^{-1}$ and 7.43±0.14, respectively. The experiment was conducted for 3 weeks in November of 2020.

There were two feed treatments tested using *P. japonicus* commercial formulated feed (Higashimaru, Japan) and raw minced fish (*Decapterus* sp.), referred to as CF and MF, respectively. Due to the miniature size of the instar, raw fish was chopped into minced meat to facilitate feeding of the instar. The instars were fed twice daily in the morning and evening at 8% of total body biomass with half of the ration given at 0800 h and the other half at 1700 h (Rodriguez et al 2003). Uneaten feed was collected daily and siphoned into a mesh cloth pocket (70 µm mesh), rinsed with distilled water, and oven-dried at 55ºC to estimate the total feed intake of the instar throughout the trial (Unnikrishnan & Paulraj 2010).

***Crude protein and lipid analysis***. Crude protein and lipid analysis of the CF and MF were conducted with reference to AOAC (1990). The raw fish was chopped, freeze-dried, and grind into powder before conducting the analysis. The crude protein content of the CF and MF were 52.9% and 79.6%, respectively (Table 1), while crude lipid was 11.6% for CF and 5.2% for MF.

Table 1

Proximate content of the commercial formulated feed and minced fish presented in dried weight basis

| *Analysis* | *Treatments* | |
|---|---|---|
| | *Commercial formulated feed (CF)* | *Minced fish (MF)* |
| Crude protein (%) | 52.9±1.3 | 79.6±1.4 |
| Crude lipid (%) | 11.6±0.02 | 5.2±0.3 |
| Crude fiber (%) | 2.8±0.2 | - |
| Crude ash (%) | 19.2±0.8 | 9.1±0.1 |
| Moisture (%) | 4.7±0.7 | 76.6±0.4 |
| Nitrogen free extract (%) | 13.5±0.5 | 6.1±0.2 |

***Observation and parameters to be determined***. The number of instars moulting in each culture tank was observed daily and recorded as moulting frequency. At the end of the trial, final body weight, body weight gained (BWG), final carapace width, carapace width gained (CWG), specific growth rate (SGR), condition factor (CF), moulting frequency (MGF), survival rate (SR), total feed intake per crab (TFI), and feed conversion ratio (FCR) were determined and calculated using the formulae listed below (Zhao et al 2015; Kaka et al 2019):

BWG(%) = [(Final body weight (g)–Initial body weight (g))/Initial body weight (g)] × 100

CWG(%) = [(Final carapace width (cm)–Initial carapace width (cm))/Initial carapace width (cm)] × 100

SGR(% $day^{-1}$) = [(In (Final body weight) (g)–In (Initial weight) (g))/Duration (days)] × 100

CF(%) = [Crab body weight (g)/crab carapace width exp 3 (cm)] × 100

MF = Number of moulting recorded daily in each replicate tank for 3 weeks period

SR(%) = Final number of survived crabs/initial number of crabs × 100

TFI per crab (g) = Total feed intake of an instar for 3 weeks period (g)

FCR = Dry feed intake (g)/Wet weight gain (g)

***Statistical analysis****.* Statistical analysis was performed by conducting Independent Sample T-Test to compare the mean of the parameters using IBM SPSS statistical software (version 20) for Windows. The mean value was significant when $p < 0.05$. Data are presented as mean±standard deviation.

**Results**. Overall, the instars fed with CF showed better survival, growth performance, and moulting frequency compared to the specimens fed with MF. A similar observation was also exhibited in the feeding performance whereby the specimens fed with CF showed a better trend than those fed with MF.

***Survival, growth performance, and moulting frequency***. Survival value was significantly higher in CF fed instars (72.2%) than MF fed instars (44.4%) (p = 0.034) (Table 2). A similar trend was also observed in the growth performance where significantly higher results observed within the specimens fed with CF than MF in terms of final body weight (0.5 g in CF and 0.4 g in MF, p = 0.016), body weight gained (1129% for CF and 905% for MF, p = 0.001), carapace width gained (169% in CF and 137% for MF, p = 0.045), and specific growth rate (17.9 and 16.5% $day^{-1}$, respectively for CF and MF, p = 0.001). Furthermore, the instar fed with CF showed greater final carapace width (1.5 cm) and condition factor (18.9) value compared to the MF fed instar (1.3 cm and 18.0, respectively) without a significant difference between each factor (p = 0.773).

Additionally, the moulting frequency also exhibited a similar trend as the survival and growth performance where a significantly greater value was noted in the CF (24.7) than in the MF (13.3) group (p = 0.01).

Table 2
Growth performance and survival of the purple mud crab instar fed commercial formulated feed and minced fish after 3 weeks of the culture period

| *Parameters* | *Treatments* | | *P-value* |
|---|---|---|---|
| | *Commercial formulated feed (CF)* | *Minced fish (MF)* | |
| Survival rate (%) | 72.2±11.1[b] | 44.4±9.6[a] | 0.034 |
| Final body weight (g) | 0.5±0.01[b] | 0.4±0.06[a] | 0.016 |
| Body weight gained (%) | 1129±21[b] | 905±30[a] | 0.001 |
| Final carapace width (cm) | 1.5±0.1 | 1.3±0.1 | 0.101 |
| Carapace width gained (%) | 169±15[b] | 137±13[a] | 0.045 |
| Specific growth rate (% $day^{-1}$) | 17.9±0.1[b] | 16.5±0.2[a] | 0.001 |
| Condition factor (%) | 18.9±2.6 | 18.0±2.8 | 0.773 |
| Moulting frequency | 24.7±1.2[b] | 13.3±4.0[a] | 0.010 |

Values represented as mean±SD (n = 3). Different superscripted letters within the same row indicate significant differences (p < 0.05).

***Feeding performance***. Overall CF fed specimens showed better feeding efficiency than the MF group. Feeding performance in terms of total feed intake per crab (0.4 g in CF and 1.0 g in MF, p = 0.001), and feed conversion ratio (6.3 and 18.8 for CF and MF respectively, p = 0.008) were significantly lower in CF than MF (Table 3).

Table 3
Feeding performance of the purple mud crab instar fed commercial formulated feed and minced fish for 3 weeks of the culture period

| *Parameters* | *Treatments* | | *P-value* |
|---|---|---|---|
| | *Commercial formulated feed (CF)* | *Minced fish (MF)* | |
| Total feed intake per crab (g)* | 0.4±0.1[a] | 1.0±0.1[b] | 0.001 |
| Feed conversion ratio (FCR) | 6.3±2.4[a] | 18.8±3.6[b] | 0.008 |

*Feed expressed in dry weight form. Values represented as mean±SD (n = 3). Different superscripted letters within the same row indicate significant differences (p < 0.05).

**Discussion**. The results indicate that the CF diet is more promising to attain higher survival among the purple mud crab instar. The present findings are supported by the previous study on the survival value of the giant mud crab (*S. serrata*) instar, which showed a higher trend when the specimens were fed with formulated diet (66.8%) than mussel meat (60.7%) after being reared in the nursery phase for 3 weeks (Alava et al 2017). Moreover, *S. paramamosain* instar specimens fed with formulated feed and fish meat (*Harpadon nehereus*) for 60 days also reported a higher survival value (48.7%) in formulated feed group compared to the fish meat group (45.8%) (Gong et al 2017). Furthermore, the *S. serrata* juvenile (initial body weight 20.8 g) stocked in the grow-out earthen pond fed with formulated diet attained a 77% survival value after 108 days (final body weight 333.7 g) (Catacutan et al 2015). Interestingly, another study conducted on *S. serrata* instars 2 which were fed with milkfish (*C. chanos*), tiger prawn (*P. monodon*) commercial formulated feed, and natural food (consisted of the mixture of mussel meat and *Acetes* sp.) reported significantly higher survival values in the tiger prawn group (55%) compared to the milkfish (35%) feed; whereas, the natural food reported the highest survival value (73%) against the milkfish (35%) and tiger prawn (55%) feed groups (p < 0.05) (Genodepa & Failaman 2016). In this study, the researchers explained that the mussel and *Acetes* sp. mixture possibly compensated for the nutrients that were lacking in the formulated feed, hence promoting the best growth of the instar among all treatments (Genodepa & Failaman 2016). Ideally, an optimal nursery diet helps to enhance survival and promote fast growth of the mud crab instar under captivity (Alava et al 2017). Hence, the nutritional profile of the formulated diet is crucial for the survival and growth of the crab in conjunction to reduce the reliance on natural food.

In the present study, the growth performance of the instar also exhibited a similar trend as the survival performance, whereby the instars fed with CF showed better growth compared to the MF diet. The present finding is supported by the previous study conducted on green mud crab (*S. paramamosain*) instar 3 fed with formulated feed (crude protein 56% and lipid 17%) and fish meat (*H. nehereus*) (crude protein 68% and lipid 16%), which reported significantly higher SGR (5.49% in formulated feed and 3.77% in fish meat), final body weight (8.92 g in formulated feed and 3.69 g in fish meat) and carapace width increment (2.1 cm in formulated feed and 1.42 cm in fish meat) in the formulated feed than fish meat diet ($p < 0.05$) (Gong et al 2017). The formulated feed was suggested to contain well-balanced nutrients needed by the crab instar for growth (Gong et al 2017). This reflected the advantage of using formulated feed as the feed can be customized and formulated according to the nutrient requirement needed by the crabs for optimum growth and survival.

Optimal growth of the animal is attained when the crude protein and lipid requirements of the animal are fulfilled (Zheng et al 2020). Crude protein and lipid requirements of the mud crab instar had been reported in the range between 43 to 52% (Zheng et al 2020) and 9 to 13% (Xu et al 2020), respectively. The CF used in the present study contained 52.9% of crude protein and 11.6% of crude lipid which is within the recommended level of the crude protein and lipid requirement of the mud crab instar. This is supported by the previous study conducted on *S. serrata* instar fed with commercial formulated feed of milkfish (crude protein 34% and crude lipid 6%) and tiger prawn (crude protein 45% and crude lipid 8%), which showed significantly better SGR in tiger prawn (0.108 g $day^{-1}$) compared to milkfish (0.066 g $day^{-1}$) feed, comparable to the SGR observed within the specimens fed with natural food (0.118 g $day^{-1}$) (Genodepa & Failaman 2016) ($p < 0.05$). The researchers explained that this observation was most likely due to the tiger prawn feed presenting the optimum crude protein and lipid requirement of the crab instar in comparison to the milkfish feed (Genodepa & Failaman 2016). Hence, shrimp feed, which is a crustacean feed, is a better option than the finfish feed in order to fulfil the nutrient requirements needed by crab instar, also a crustacean species. This deduction supports the better instar growth observed in the CF group compared to the MF group in the present study.

The present growth observation is also supported by the feeding data where the purple mud crab instar accepted and successfully utilized the CF diet for survival and growth. The animal must be able to digest and absorb the nutrient from the diet to utilize the nutrient for growth and survival. Previous studies had documented that mud crab juveniles and sub-adults manage to digest animal and plant-based protein, fish oil, starches, and celluloses where these were the common ingredients used in producing formulated feed (Tuan et al 2006; Truong et al 2008, 2009). Hence, these studies supported the present finding that CF was successfully utilized by the instar for survival and growth.

On the other hand, the total feed intake of MF was noted to be significantly higher than CF, although resulting in a lower SGR than CF ($p < 0.05$). In the present study, MF was chopped into minced meat to be fed to the instar. This made the recollection of the uneaten fish meat for uneaten feed estimation difficult due to the dispersion of finer minced fish in the culture water. Hence, this probably caused an error in estimating the MF uneaten feed where the number of eaten feeds could had been overestimated. This could also explain that a higher intake of MF than CF in the present study does not necessarily promote better growth of the instar. Hence, minced fish is not recommended in the crab instar feeding as it deteriorates the quality of the culture water, easily causes nutrients to leach out, and is not eco-friendly. Meanwhile, a formulated diet that lasts longer in the culture water is much eco-friendlier and more desirable as crab instar feed.

In the present study, the feeding performance indicated by total feed intake per crab and FCR were significantly lower in CF than in MF ($p < 0.05$). These parameters indicated that the instar seems to consume less amount of CF than MF, however, utilized the protein source in CF efficiently for growth purposes as reflected by the SGR value. Hence, the feed intake of MF does not correspond to the instar growth and could be explained by the error of the MF intake estimation described above. Besides, there is also a possibility of protein utilization for metabolic purposes rather than the growth of the animal (Lee & Lee 2018).

The present results also reveal that the instar fed with CF showed a higher moulting frequency than MF. Mud crab instar and juvenile grow by moulting activities and crude proteins are reported to be tremendously important for successful moulting in crustaceans (Aaqillah-Amr et al 2021). *S. serrata* juvenile was reported to suffer from mortality due to moult failure or prolonged moulting intermoult duration when protein levels were insufficient to support the animal growth (Unnikrishnan & Paulraj 2010). Hence, the CF with the recommended crude protein level is suitable to promote the moulting and growth of the instar mud crab.

Generally, formulated feed had been formulated to fulfil the nutrients requirement of the animal for survival and growth (Ali et al 2011). Hence, the animal managed to obtain sufficient nutrients from the formulated feed for survival and growth in different life stages; whereas natural food has seasonal variations that causes fluctuations in quality and deteriorates faster in the culture water, thus becomes a challenge to provide an optimum level of nutrients needed by the animal (Gong et al 2017). These factors help to support the benefits observed in CF than MF in the present study to enhance the survival and growth of the instar purple mud crab. Since mud crab aquaculture begins with the instar nursery phase, therefore providing the suitable functional formulated feed for the instar is essential to ensure the sustainability of the mud crab aquaculture farming.

**Conclusions**. The present study revealed that the commercial crustacean formulated feed that met both the crude protein and lipid requirements was better than the local low-cost fish (*Decapterus* sp.) for the purple mud crab instar in promoting better survival and growth. Hence, the current use of the commercial formulated feed in the instar nursery phase is possible to reduce the reliance on forage fish in the local mud crab farming. Furthermore, the formulated feed quality can be further enhanced by adding supplementation such as antioxidants, carotenoids, phospholipids, nucleotides, and others. Development of the functional feed exclusively for the mud crab instar in the future is practical to expand the local mud crab aquaculture farming.

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**Conflict of interest**. The authors declare that there is no conflict of interest.

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