Time spent in post-feeding activities including feed preference by different weight groups of marron (*Cherax cainii*, Austin 2002) under laboratory conditions.

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Abstract

The current study examined time spent on different post-feeding activities and food preference of five weight groups (<15g, 15-29.9g, 30-44.9g, 45-59.9g and 60-100g) of marron (*Cherax cainii*, Austin 2002) fed two formulated feed pellets and frozen copepods under controlled laboratory conditions. The experimental design consisted of housing an individual marron representing a weight group per 20-L glass aquarium. Each weight group was replicated five times, thus using 25 aquaria. All marron were fed with three different food types; fishmeal (FM) and black soldier fly (BSF) (*Hermetia illucens*) based formulated feed at the rate of 2% of respective body weight and frozen copepods at the rate of approximately 300 individuals per aquarium. Time spent in six selected post-feeding activities was measured in seconds. These activities included walking, resting, searching for food, handling and ingestion of copepods, handling and ingestion of formulated feed, and rejection of food. Results showed that the least amount of time was spent on handling and ingestion of FM by all the weight groups. The handling and ingestion of food in weight group >60 g marron were stopped after half an hour of post-feeding. Where, <15 g and 15-30 g marron spent significantly longer time consuming frozen copepods. Weight groups >45 g marron spent the longest time resting. FM and frozen copepods were consumed by all weight groups, however, weight groups <15 g and 15-30 g rejected the BSF. The number of frozen copepods consumed by marron were significantly higher for <30 g marron and lowest in 60-100 g marron weight group. In conclusion, the post feeding activities and feed preference of marron were weight dependent.

Keywords

Black soldier fly meal, feeding behaviour, food preference, food rejection, frozen copepod, marron.

1. Introduction

Freshwater crayfish are widely used for behavioural studies as they offer advantages over many other invertebrates due to their high level of social interactions in both field and laboratory settings (Gherardi, 2002). Feeding behaviour is an important aspect of animal production as it provides the link between food being provided, the time required to consume the food and what is consumed. Measuring feeding behaviour can be used to understand how animals perceive the food provided (Nielsen et al., 2016). In crayfish, aesthetascs located on the antennules are used for food detection, food particles are picked up with pereopods and are then transferred to the mouthparts where they are ingested. Mandibles are used for gripping, tearing, crushing and biting food before entry into the oesophagus. While searching for food pereopod one and two are constantly probing the substrate (Holdich, 2002). Food preference is an important parameter for determining the growth and survival of a cultured species and its intake is regulated by the hormones and neurotransmitters that induce the feeding and terminate food ingestion upon satiation (Tierney et al., 2020).

Marron (*Cherax cainii*, Austin 2002) are native to Western Australia (WA) and are a commercially important freshwater crayfish species for aquaculture practices and a recreational fishery, and are farmed in extensive and semi-intensive systems. Marron are known to feed on detritus and zooplankton (Beatty, 2006; Meakin et al., 2009), however the feeding biology of *Cherax* species is poorly understood and studies on zooplankton consumption by *Cherax* species under controlled conditions can provide more insight into understanding the trophic role and nutrient partitioning of freshwater crayfish (Meakin et al., 2008).

Although in commercial marron farming in semi-intensive ponds the use of formulated feed is a common practice to increase the marron production, the feed quantity is dependent on environmental factors and marron weight (Fotedar et al., 2015; Tulsankar et al., 2020).

Understanding the cultured species' weight dependent food preference under culture conditions is of vital importance as the efficient feeding can be crucial for profitable aquaculture (Luna et al., 2019); for example, knowing the feed preference of cultured animals at different weight groups may help to enhance their growth rate, improve the feed conversion ratio and reduce the amount of residual feed pellets which may cause significant water quality issues in aquaculture systems (Glencross et al., 2007).

A study by (Meakin et al., 2009) has shown that the juvenile marron weight groups of less than 15 g (1.0-2.9 g, 3.0-7.9 g, and 8.0-15.0 g) are avid feeders of *Daphnia*. However, the feeding behaviour and feed preference of different weight groups of marron is to date unexplored. In this study we used three different foods: two formulated feed pellets with fishmeal and black soldier fly based proteins, and frozen copepods to investigate whether the time spent on post-feeding activities, feeding behaviour and food preference of marron is weight dependent. To test the hypothesis, an experiment was conducted under controlled indoor laboratory conditions with five different weight groups of marron ranging from <15 to 100 g. The study will provide novel information on the post-feeding activities, feeding behaviour and weight dependent feed preference of marron, allowing the comparison of activities displayed by other species.

2. Materials and methods

2.1. Experimental design

Five different weight groups of marron ranging from <15 g to 100 g were used to evaluate their post-feeding activities, including feeding behaviour and their food preference between two formulated feed pellets using fishmeal (FM) and black soldier fly (BSF) meal, and frozen copepods. Each weight group had five replicates and marron were stocked individually.

2.1.1. Experimental animal collection

Marron were collected from an extensive culture commercial farm in Dwellingup (32.7143°S, 116.0665°S), Western Australia (WA). In the dam these marron were fed with a mixture of commercial formulated feed (Western Premium Marron Pellets™ with 22% crude protein, ingredients being fishmeal, edible oil, salt, cereal grains, vegetable protein meals, vitamins and minerals), crushed lupin and leftovers from the local sardine processing plant (Communication with Mickel Mitchell, Aquanat, Dwellingup). Marron were transported in a thermacol box with a wet hessian bag to the Curtin Aquatic Research Laboratory (CARL) within 90 minutes of sampling. On reaching CARL, marron were divided into five weight groups <15 g (10.1±0.78); 15-29.9 g (19.5±0.83); 30-44.9 g (37.9±0.69), 45-59.9 g (55.0±0.84) and 60-100 g (80.9±1.60) and were stocked individually with one marron per tank. Twenty five glass aquaria (36cm x 22cm x 26 cm) with a 20 L water capacity were filled with 8 L of freshwater to acclimatise the marrons to laboratory conditions for three weeks. The treatments were applied in a randomized complete block design. Customised mesh screen was used as aquaria lids to prevent marron from escaping. Continuous aeration was provided to each tank and 25% water exchange were conducted once a week. Twelve hours of photoperiod was provided to entire set up and constant water temperature was maintained at 21 °C by using automatic submersible glass aquaria heaters (Aqua One, Australia).

2.1.2. Copepod collection and culture

Zooplanktons were collected from Blue Gum Lake (32.0374° S, 115.8482° E), Booragoon, WA using a 60-µm mesh by dragging on the lakes' surface water. Collected zooplanktons were screened and cleaned by using 2-mm mesh to achieve >2 mm size zooplanktons especially copepods and to remove unwanted smaller zooplanktons. Separated zooplanktons were stocked in 20 L glass tank and grown to achieve a pure copepod culture which had *Calanoid* copepods under laboratory conditions in CARL. Copepod cultures were started at the density of approximately 289 individuals L^{-1} three weeks prior to the commencement of the experiment to achieve the required abundance, as 7500 individuals per day were needed to feed the marrons. The continuous culture system was used to culture copepods. Green algae *Chlorella* spp. was cultured to feed copepods and to maintain their growth and density. *Chlorella* spp. were grown under the laboratory conditions with continuous aeration and 24 hours light conditions in CARL.

2.1.3. Feed preparation and zooplankton availability

The formulated feed pellets were prepared in CARL. The fishmeal formulated feed pellet had fishmeal as a main animal protein source and BSF formulated feed had BSF meal as a main animal protein source, the dry ingredients were acquired from Speciality Feeds Company, Glen Forrest, WA. To acclimatise marron to the formulated feed pellets, marron were fed with pellets at 2% of their body weight and frozen copepod at a density of approximately 300 individuals per tank per day in the evening for three weeks. An hour following the introduction of the food in the tank, the uneaten feed and zooplankton were removed from the tank, using 25-micron mesh.

In order to maintain similar copepod density in each tank, copepods were counted using Sedgwick rafter by following the procedure described by (Meakin et al., 2008) with some modifications. Copepods were counted by using Sedgwick rafter and placed into twenty five 500 mL containers filled with 250 ml of distilled water. A sub-sample of 10 mL were taken from 500 mL container, to recount the copepods, and the counting was repeated 10 times to calculate the average numbers of copepod in 500 mL container. After counting, the copepods were screened and frozen until used for feeding.

2.2. Water quality

The water parameters including temperature, dissolved oxygen (DO) and pH were checked daily. An Oxyguard® digital DO meter (Handy Polaris 2, Norway) was used for DO and temperature measurements, and an Ecoscan pH 5 meter (Eutech instruments, Singapore) was used to record pH. All water parameters were maintained in an optimum range for the growth of marron (Morrissy, 1990).

2.3. Post-feeding activities and food preference observations

On the completion of the acclimation period all marron were starved for 48 hours before the post-feeding observations. FM and BSF pellets at the rate of 2 % of respective marron weight and an average of 300.2 ± 0.02 frozen copepods were introduced into the front right corner of the aquarium at the start of post-feeding observations. All feeds were introduced into the tank water at the same time. Feeding observations were conducted for one hour immediate post-feeding. The observations were made visually and were categorised as: walking- including on the tank surface, climbing on tank walls and backward walking; resting- staying still at same place for more than a 30 seconds; searching for food- continuously moving antenna, first pereopod and maxillipeds; handling and ingestion of copepods- picking up copepods with the 2nd pair of pereopods and pushing in mouth through maxillipeds; handling and ingestion of formulated feed pellets- picking up the feed pellets and pushing in mouth through maxillipeds; Rejection of feed- picking up the feed, pushing it towards the maxillipeds and dropping down without ingestion. The time invested on each activity was recorded in seconds. At the end of the feeding observations, the leftover feed and zooplankton were filtered with fine mesh and stocked in separate bottles to count the numbers of leftover copepods.

2.4. Post-feeding activities including food handling and their characteristics

The description of post-feeding activities and their significant characteristics observed during the post-feeding observations are explained in Table 1.

Table 1. The activities conducted by different weight groups of marron during one hour of post-feeding observations.

Resting	Marron staying still at same place for more than a 30 seconds.
Walking	Moving across the substrate on walking legs including on tank surface, climbing on tank walls and backward walking.
Searching for food	Continuously moving antenna, first pereopods and maxillipeds.
Handling and consumption of food	Probing the ground with walking leg, picking up and conveying the food particles with the 2nd pair of pereopods in mouth through maxillipeds.
Rejection	Attracted and picking up BSF feed pellet, pushed towards maxilliped and dropped down multiple times without ingestion.

2.5. Copepod consumption

Copepod consumption (CC) was analysed by using the following formula;

$$CC = Number\ of\ copepods\ added\ to\ the\ tank - Number\ of\ copepods\ collected\ from\ the\ tank\ after\ feeding$$

2.6. Statistical analyses

All the numerical data were analysed using SPSS version 26 (IBM®) and the results are presented as mean ± S. E. A one way ANOVA with LSD post hoc test was used to find the significance between the treatments. A Kruskal-Wallis test was used when data lacked homogeneity. All tests were considered statistically significant at $p<0.05$.

2.7. Animal ethics

The animal ethics approval was not required as the marron are invertebrate aquatic crustaceans.

3. Results

3.1. Water quality

Temperature, dissolved oxygen and pH were maintained at an optimum level for marron growth throughout the experimental time as shown in Table 2.

Table 2. Water quality parameters dissolved oxygen (DO; mg L^{-1}), temperature (°C) and pH ranges maintained in marron tanks during the experimental days (One way ANOVA with Duncan test; mean ± S. E.; n=5).

Water parameters/weight groups	<15 g	15-29.9 g	30-44.9 g	45-59.9 g	60-100 g	Min./Max
DO	7.23 ± 0.01	7.25 ± 0.02	7.29 ± 0.02	7.27 ± 0.01	7.26 ± 0.02	7.21-7.32
Temperature	21.5 ± 0.06	21.3 ± 0.15	21.4 ± 0.12	21.2 ± 0.10	21.5 ± 0.09	20.9-21.7
pH	7.95 ± 0.06	7.78 ± 0.10	7.85 ± 0.14	7.72 ± 0.11	7.76 ± 0.10	7.60-7.79

3.2. Post-feeding time spent on different activities by marron

Out of the total time spent on different activities, consumption of fishmeal formulated feed pellet was conducted in the least amount of time by all weight groups of marron. The longest time was spent on eating frozen copepods by weight group <15 g ($p < 0.05$). Resting activity time (%) were significantly longest in >45 g weight groups than other weight groups (Fig. 1A, B, C, D, E, F.). Weight groups <15 g and 15 - 30 g spent a significantly longer amount of time on investigating BSF pellets. The feeding activity in weight group 60-100 g stopped after 30 minutes.

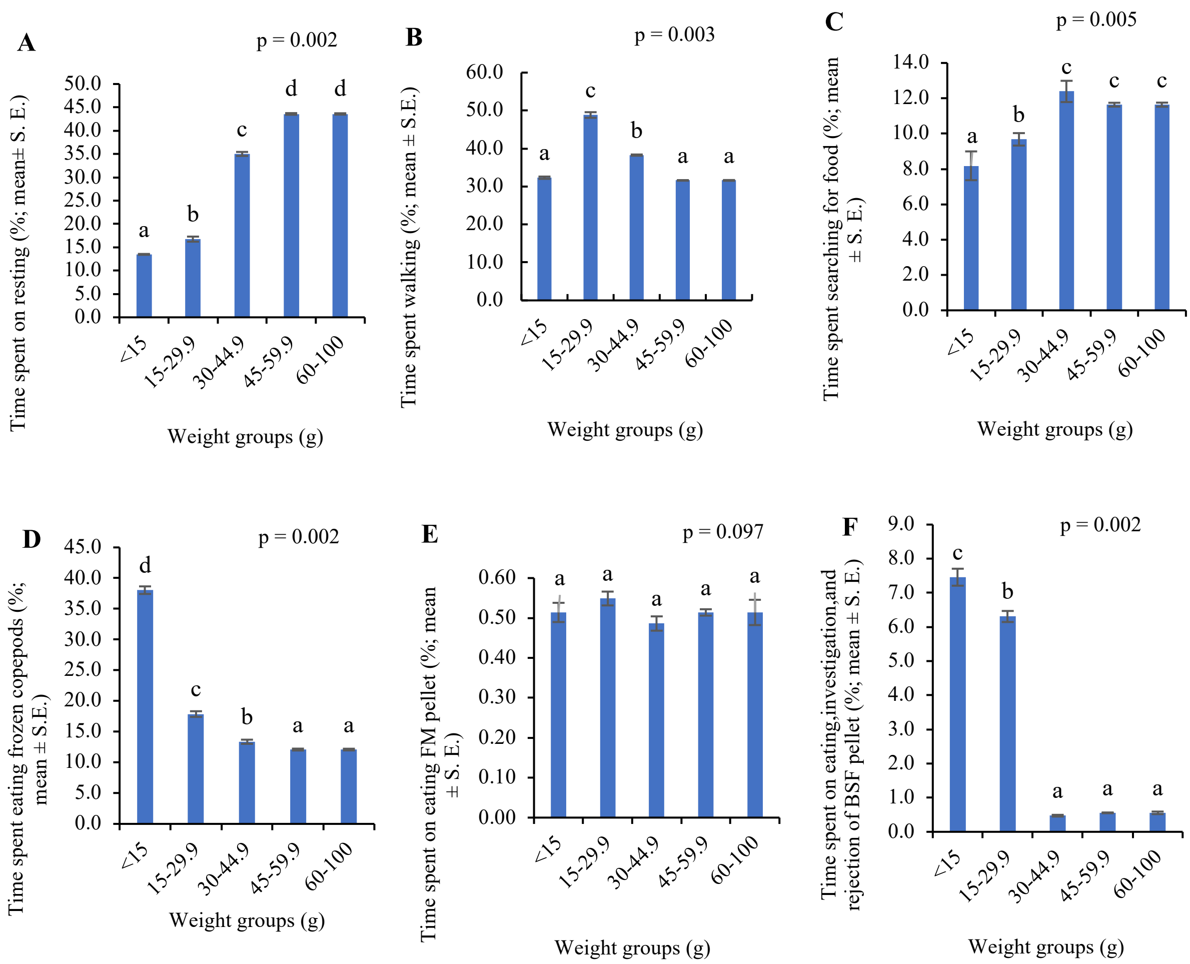

Fig. 1. The total time spent (%, mean ± S.E.) on post-feeding activities by five different weight groups (n = 5 per group) of marron ranging from <15 g to 100g: A) time spent on resting, B) the time spent on walking, C) time spent on searching for food, D) the time spent on eating frozen copepods, E) time spent eating FM pellets and F) time spent eating, investigation and rejection of BSF pellets. One way ANOVA was used for the Fig. 1A, C, D and E and Kruskal-Wallis test was used for Fig. 1B and F. No significant differences ($p<0.05$) were observed between the weight groups for time spent on feeding on the FM pellet. Letters a, b, c and d represents the significant differences between the weight groups. **Abbreviations:** FM: Fishmeal formulated feed pellet, BSF: Black soldier fly formulated feed pellet.

3.3. Marron preference for the provided feeds

Feed preference of different weight groups of marron were recorded and were chronologically ranked based on the marron response towards the provided feed and frozen copepods (Table 3). FM was the first preference for the <45 g marron and second for the >45 g marron. BSF was the first preference for the >45 g marron and second for 30-45 g marron. Frozen copepod was the second preference for <45 g marron and third for >45 g marron.

Table 3. Marron feed preference based on the feeding behaviour observations (n = 5 per goup).

Marron weight groups	FC	FM	BSF
<15 g	2^{nd} preference (5/5 R)	1^{st} preference (5/5 R)	Rejected (5/5 R)
Qualification	Searching and feeding on copepods after consuming FM pellet.	Picking up and consuming FM pellet immediate post-feeding within least amount of time of feed addition.	Attracted and picking up BSF feed pellet, pushed towards maxilliped and dropped down multiple times without ingestion.
15-29.9 g	2^{nd} preference (5/5 R)	1^{st} preference (4/5 R)	Rejected (5/5 R)
Qualification	Searching and feeding on copepod after consuming FM pellet.	Picking up and consuming FM pellet within least amount of time of feed addition.	Attracted and picking up BSF feed pellet, pushed towards maxilliped and dropped down multiple times without ingestion.
30-44.9 g	3^{rd} preference (4/5 R)	1^{st} preference (5/5 R)	2^{nd} preference (4/5 R)
Qualification	Feeding on frozen copepods after consuming FM and BSF pellets.	Picking up and consuming FM pellet within least amount of time of feed addition.	Feeding on BSF pellet after consuming FM pellet.
45-59.9 g	3^{rd} preference (5/5 R)	2^{nd} preference (5/5 R)	1^{st} preference (5/5 R)
Qualification	Feeding on frozen copepods after consuming FM and BSF pellets.	Picking up and consuming FM pellet after consuming BSF pellet.	Attracted and picked up BSF pellet first within least amount of time of feed addition.
60-100 g	3^{rd} preference (5/5 R)	2^{nd} preference (5/5 R)	1^{st} preference (5/5 R)
Qualification	Feeding on frozen copepods after consuming FM and BSF pellets.	Picking up and feeding on FM pellet after consuming BSF pellet.	Attracted and picked up BSF pellet first within least amount of time of feed addition.

Abbreviations: FC- frozen copepods, FM-fishmeal formulated feed pellet, BSF- black soldier fly feed pellet; R- replicates.

3.4. Food consumption in different weight groups of marron

Venn diagram describes the food consumption by different weight groups of marron observed during the one hour of post-feeding observations. Fishmeal formulated feed (FM) pellets and frozen copepod were consumed by all weight groups of marron (Fig. 2). However, BSF pellet was consumed only by >30 g marron weight groups.

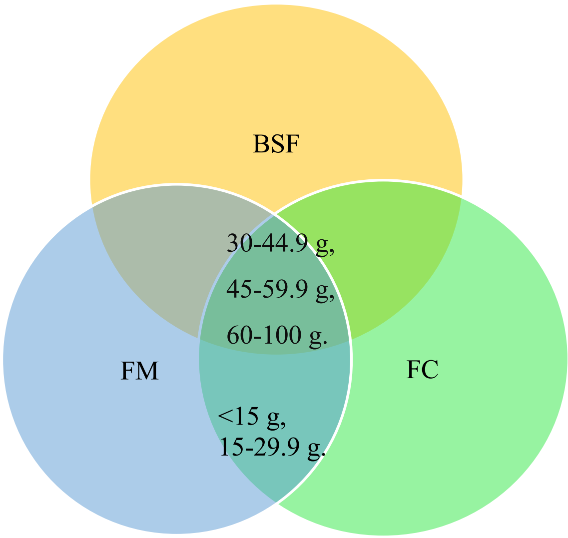

Fig. 2. The FM, BSF and frozen copepods consumption by different weight groups of marron (ranging from <15 g to 100 g) over the one hour of post-feeding observations (n=5). **Abbreviations:** FC- frozen copepods, FM-fishmeal formulated feed pellet, BSF- black soldier fly feed pellet.

3.5. Frozen copepod consumption

The number of frozen copepods eaten by different weight groups of marron during the one hour of post-feeding observations varied between group sizes (Fig. 3). The copepod consumption decreased significantly as the marron size increased between < 15 g and 100 g. The numbers of copepods consumed by <15 g marron were significantly highest than the other weight groups.

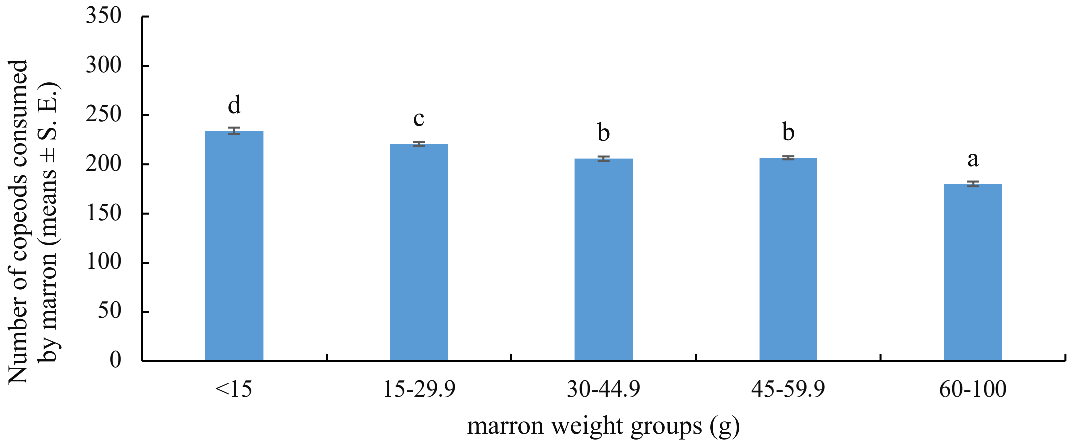

Fig. 3. The number of frozen copepods consumed by different weight groups of marron during the one hour of feeding observation (mean ± S. E.; n=5). The letters a, b, c and d indicate significant difference in the number of frozen copepod consumption between the different weight groups of marron.

4. Discussion

The current study describes the post-feeding activities, feeding behaviour and weight dependent feed preference of marron and allows the comparison of activities displayed by other species. In marron ponds the abundance of copepod adults and copepod nauplii were recorded through-out the year (Tulsankar et al., 2021). Based on those finding, the current experiment was conducted to better understand the feeding preference of different weight groups of marron ranging from <15 g - 100 g, under controlled laboratory conditions when they are provided with frozen copepods and two different formulated feeds.

This is the first attempt to analyse the feed preference and quantifying copepod consumption by the different weight groups of marron in controlled conditions, and it could provide new insights into the role of formulated feed and zooplankton in marron diets. The data showed that the juvenile marron (<15 g) spent more time feeding on copepods, similar to a study by (Alcorlo et al., 2004) where copepods were observed in the stomach contents of red swamp crayfish (*Procambarus clarkii*) with zooplankton consumption being higher in juveniles than in adults.

Marron's ability to consume copepods indicates that copepods may play an important role in the marron diet in pond systems and may provide a source of nutrition especially during the early life stages. Similar observations were made by (Meakin et al., 2009) where juvenile marron were able to capture and consume a large quantity of *Daphnia* in a short period of time i.e. <1 hour. In the case of yabbies, animals up to 45 g were efficient in capturing 400-500 individuals of live *Daphnia* (Meakin et al., 2008). The present study does not suggest any "suppression" of copepod densities, but simply that all weight groups ate frozen copepods. In a study by (Sierp and Qin, 2001) in a field experiment, the adult marron were unable to supress the zooplankton. Another reason for a lower number of copepods eaten by adult marron may indicate the feeding until satiation (Momot, 1995), as marron groups >30 g preferred to feed on formulated feed pellets first, and copepods were their last preference. Feeding until satiation on formulated feed pellets in a short time may have resulted in the significantly longer time on resting by the weight groups >30 g marron, and also caused suspension of feeding in weight group >60 g after 30 minutes of post-feeding as they did not indulge in consuming copepods for a longer time.

As crayfish grow, the precise moment required to capture the zooplankton may decline; they may lose the dexterity (Abrahamsson, 1966), however, we did not find any impaired ability of adult marron movement to feed on frozen copepods. All weight groups were equally capable of collecting and feeding copepods. The observations made on the feeding behaviour indicated that marron feeding on copepod may not be depend on the size, as zooplankton capture and consumption relies on the mouth parts (Meakin et al., 2008). We observed that large marron could effectively use their mouth parts to feed on copepods, despite the overall increase in size, though juvenile marron (<30g) showed greater preference for frozen copepods over formulated feed pellets by spending more time feeding on zooplankton. Our results showed that the marron weight groups ranging from <15 - 100 g can effectively consume frozen copepods at an initial prey density of 170- 243 individuals per aquarium.

Frozen zooplankton improved the growth performance of red swamp crayfish (Sonsupharp and Dahms, 2017) and feeding on fresh or frozen zooplankton resulted in similar growth and survival in juvenile yabbies when compared with the formulated diet (Jones et al., 1995; Verhoef et al., 1998). Marron can consume any food available within a pond ecosystem, including plant and animal material (Alonso, 2009). The direct impact of crayfish on pelagic plankton populations is considered to be relatively weak due to their benthic nature (Sierp and Qin, 2001). In the case of copepods, they migrate vertically to avoid the predation and to find the shade during the sunny hours. This vertical migration towards the pond bottom would allow marron to feed on copepods as marron are benthic animals. During the juvenile stage copepods can be a source of nutrition for marron, even after death as a part of the detritus.

A typical trend of choosing fishmeal formulated feed by all weight groups was observed and BSF formulated feed was not eaten by <30 g marron. Juvenile marron were attracted towards the BSF, but did not consume it, despite spending significant time on exploring the food. BSF has a strong odour which may have caused a negative chemosensory response in <30 g marron (Corotto and O'Brien, 2002). Though BSF was the first preference for >45 g weight groups; the taste of BSF was not attractive to <30 g marron. In aquaculture, the use of formulated feed is also more important to achieve high growth rates in larger sized animals, whereas during their early life stages marron may rely largely on naturally occurring food items. Juvenile marron are generally more active and planktivorous than adults (Sierp and Qin, 2001). Marron in weight group 15-30 g spent more time on walking where the <15 g spent more time on eating copepods and exploring BSF diet. On the other hand, weight groups >30 g consumed the formulated feed within least amount of time and spent more time searching for it.

Our results showed that supplemented formulated feed is utilized by year one (>30 g) marron though their preference was weight dependent. BSF can be an alternative animal protein source to fishmeal based feeds for grow-out marron. In this study we did not investigate the capture efficiency of marron due to the use of frozen copepods. However, using live copepods to examine the capture efficiency of marron can provide more information on marron feeding biology.

3. Conclusion

The post-feeding activities and feed preference are weight dependent in marron. Copepod consumption was highest in juvenile marron and the consumption decreased as weight of marron increased. Larger marron preferred to feed on formulated feed including insect based feed. Further studies investigating feed preference and impact of feed on water quality in outdoor culture systems will provide beneficial information on husbandry management of aquaculture species. Understanding species specific feeding behaviour and nutritional requirements is important for the assessment of animal welfare and ecology. It may also help to solve many feeding related problems and allow animals to maximize the potential of the nutritional value of the feed provided.

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Declaration of interest: none.

CRediT Author Contributions

Smita Sadanand Tulsankar: Conceptualization, designing and set up of experiment, feeding observations, data collection, data analysis and writing of manuscript. Anthony J. Cole: Copepod counting, feeding observation, reviewing and editing manuscript. **Marthe Monique**

Gagnon: Supervision, reviewing and editing manuscript. **Ravi Fotedar** Methodology validation, supervision, review and editing manuscript. The article submission has been approved by co-authors.

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