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# Feeding juvenile marron (*Cherax cainii* Austin, 2002) exclusively on live mixed plankton improves growth, total haemocyte count and pigmentation

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ABSTRACT

The present study aimed to compare the nutritional effects of a mixture of phytoplankton and zooplankton and a formulated pelleted feed on the growth, survival, moulting, immune and organosomatic indices of juvenile marron (*Cherax cainii* Austin, 2002) cultured for 90 days, under controlled laboratory conditions. The experiment was conducted with two quadruplicated dietary treatments, a pre-selected plankton density and formulated feed pellets (Control). Seventy two juvenile marron of an average weight of 3.73 ± 0.16 g and average total length of 2.57 ± 0.04 cm were collected from a local farm and reared in eight 300 L water capacity tanks. In treatment tanks, the phytoplankton and zooplankton density was maintained at the average of 2.81 ± 0.02 × $10^6$ cells $L^{-1}$ and 228 ± 5.15 individuals $L^{-1}$ respectively. Marron in the control group were fed with formulated feed at 2% of marron's body weight per day during the evening. Marron fed with mixed live plankton achieved a significantly higher growth rate, total haemocyte count and improvement in pigmentation of juvenile marron. Survival rate and organosomatic indices of juvenile marron were similar with the use of mixed plankton, and formulated feed. Therefore, from these results, it can be concluded that the live plankton can be a main source of nutrition for an early stage of juvenile marron, if the plankton density is maintained.

## 1. Introduction

Freshwater crayfish farming is well established worldwide particularly in Europe, and the United States, with the industry still under development in Australia (Hollows, 2016). The global freshwater crayfish production for the year 2019 was 2,162,159 tonnes (FAO, 2021). Australia is home to more than 110 species of freshwater crayfish, of which three *Cherax* species, yabbies (*Cherax destructor*), red claw (*C. quadricarinatus*) and marron (*C. cainii* Austin, 2002) have been identified as ideal candidate species for aquaculture (Lawrence, Jones, 2002; Meakin et al., 2008). The total freshwater crayfish production in Australia for the year 2018 was 166.1 tonnes; marron contributed 65.8 tonnes valued at USD $2.30 million, red claw and yabbies contribution was 48.8 and 51.5 tonnes respectively (FAO, 2018). Marron production showed a marginal increase by ~10 tonnes from 2017−18 (FAO, 2020). Australia is the only significant producer of marron (FAO, 2017; Machin et al., 2008). Native to south west of Western Australia (WA), high market value, and status as gourmet product, disease free and simple life cycle makes marron an ideal species for aquaculture (Machin et al., 2008).

In aquaculture, there is more emphasis on the use of formulated feed than managing the plankton productivity to achieve greater production (Boyd, 2018) despite the drawbacks associated with pelleted feed. The formulated feed may account for the 40–60 % of the total cost of production in semi-intensive aquaculture systems (Correia et al., 2003; Fotedar, 2004). Formulated feed composition is inconsistent as the inclusion of feed ingredients and their percentages depend on various factors such as availability of locally available ingredients, demand and species cultured. The composition, quality and cost of marron feeds also varies greatly, there is no consistent formulated diet that supplies the full nutritional requirements to the marron (Fotedar et al., 2015). Pelleted feeds lose stability after exposure to water, causing loss of nutrition until consumed by marron, and having a negative impact on the water quality (Smith et al., 2002). Moreover, marron ignores the formulated pelleted feed once it has disintegrated into the water (Jussila, Evans, 1998). The main protein source of formulated feeds is fishmeal, and higher demand for fishmeal worldwide has increased the fishmeal cost (Boyd et al., 2007; Olsen, Hasan, 2012).

In the natural environment juvenile narrow clawed crayfish (*Pontastacus leptodactylus* formerly known as *Astacus leptodactylus*) and red

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swamp crayfish (*Procambarus clarkii)* are known to feed mainly on plant, organic detritus and zooplankton (Gutiérrez-Yurrita et al., 1998; Tcherkashina, 1977). Similarly, in a semi-intensive marron pond, a combination of natural productivity and formulated feed provides sufficient nutrition to marron (Fotedar et al., 2015). However availability of natural feed is season dependent (Tulsankar et al., 2020, 2021a). Therefore, analysing the effects of mixed plankton in the absence of formulated feed could be an important research focus not only to minimise the use of fishmeal but also to reduce the formulated feed use by improving the plankton productivity of the ponds, mainly in early juvenile stages as observed in juvenile white leg shrimp (*Litopenaeus vannamei*) (Sánchez et al., 2014); and in juvenile yabbies (Duffy et al., 2011; Jones et al., 1995; Verhoef et al., 1998).

There has been an intensive research conducted on marron nutrition using different protein sources under pond culture and controlled laboratory condition, studying the effect of different diets on marron growth, survival, haemocyte count and organosomatic indices (Fotedar, 1998, 2004; Fotedar et al., 1999; Jussila, Evans, 1996, 1998; Morrissy, 1979, 1976; Morrissy, 1989; O'brien, Davies, 2002; Saputra, Fotedar, 2021; Sommer et al., 1991; Tsvetnenko et al., 1995; Tulsankar et al., 2021; Van den Berg et al., 1990), however there is still a paucity of knowledge on the nutritional requirements of marron. Few studies have been conducted to determine the benefits of dietary zooplankton compared to formulated feed focusing on the growth and survival of Parastacid juvenile crayfish (Austin et al., 1997; Duffy et al., 2011; Morrissy, 1989; Verhoef et al., 1998), however the majority of these studies have been focused on yabbies or red claw crayfish. A study by Morrissy (1989) was conducted using marron, the experimental protocol only used powdered algae extract (*Dunaliella salina*), and the measured parameters were limited to the growth and survival. So far no comparative study has been conducted to analyse the effects of a mixture of both phytoplankton and zooplankton versus formulated diet on the growth, survival, moulting, immune and organosomatic indices of juvenile marron. We hypothesized that the continuous availability of mixed plankton will improve the growth, survival, pigmentation, immune and organosomatic indices of juvenile marron, compared to when the animals are fed formulated feed.

This is the first study investigating the effects of controlled and static density of live plankton mixture on juvenile marron growth, survival, moulting, immune and organosomatic indices, and pigmentation in the absence of formulated feed. The current experiment was a follow-up from the previous indoor trial conducted for 96 days to investigate the impact of supplementing Mn, Si and P in water on plankton density, diversity, and their effect on marron growth, survival, immune and organosomatic indices and gut microbiota (Tulsankar et al., 2021). The experiment presented here was conducted to assess if the juvenile marron could receive the required nutrition from mixed live planktons alone in the absence of formulated feed. As the study focused on the nutritional role played by direct comparison between exclusive dietary planktons and formulated feed, a mixture of these two diets was not considered necessary.

## 2. Materials and methods

### 2.1. Plankton and marron collection

Plankton and experimental animals were collected from a semi-intensive commercial marron farm in Manjimup (34°18′75″ S, 116°06′61″ E), Western Australia. Pond water was filtered separately using plankton net for phytoplankton and zooplankton to obtain the total concentrated quantity of 15 L of sub-sample for phytoplankton and zooplankton each. The plankton samples were brought to the Curtin Aquatic Research Laboratory (CARL) and cultured in outdoor conditions to use in the current experiment. For outdoor plankton culture, planktons were stocked in six 300 L capacity tanks topped up with 200 L of freshwater. The tanks were placed in direct sunlight and aerated continuously using airstones. Aquasol® by Yates Pty Ltd., fertilizer was used to boost and maintain the phytoplankton density at approximately $9.34 \times 10^6$ cells $L^{-1}$. Phytoplankton was used to feed zooplankton at a rate of 10 L of phytoplankton water added every second day to the zooplankton tanks.

Approximately 100 juvenile marron of an average weight of 3.73 ± 0.16 g and average total length of 2.57 ± 0.04 cm were collected and transported to CARL on the same day of collection. On arrival the marron were stocked in 300 L plastic tanks to acclimatise them to laboratory conditions for two weeks.

### 2.2. Formulated feed composition

All feed ingredients were procured from Speciality Feeds Company, Glen Forrest, Western Australia and the feed formulation was conducted in CARL (Table 1).

### 2.3. Experimental set up

A 90 days experiment was conducted under the controlled laboratory conditions in eight 300 L water capacity tanks. Each tank included 9 cages made up of plastic containers with open top for feeding covered by mesh (6 mm size) to stock the marron individually. The cages had a volume of 2 L (170 mm x 115 mm x 135 mm) with two small gaps of 5 mm on sides to allow the plankton exchange directly from the tank water into the cage as described by Tulsankar et al. (2021). The cages were submerged in tank water up to the half of their height by mounting them on PVC pipes. Nine marron were selected randomly and placed individually into the cages. The mixture of live plankton as a feed in treatment tanks was maintained at approximate mean density of $2.73–2.92 \times 10^6$ cells $L^{-1}$ and 213–238 in.ividuals (ind.) $L^{-1}$ of phytoplankton and zooplankton respectively, similar to the highest density found in semi-intensive marron ponds (Tulsankar et al., 2020). Plankton density was maintained by either addition or removal of plankton. Marron in control tanks were fed with formulated pelleted feed at 2% of their body weight once a day in the evening until the satiation. The left over feed was collected and removed the next morning. Both dietary treatments were carried out in four replicates. Continuous aeration was supplied to the tanks.

### 2.4. Water quality analysis

The water quality parameters for plankton growth such as dissolved oxygen (DO), pH and temperature were maintained close to the range observed in marron ponds during summer, the season with highest phytoplankton abundance (Tulsankar et al., 2021a). Water parameters

**Table 1**
Ingredients and proximate composition (in percent) of formulated feed used in this study.

| Ingredients | Basal diet |
|---|---|
| Fishmeal | 40.00 |
| Wheat (10 CP) | 22.14 |
| Corn/wheat starch | 10.00 |
| Lecithin- Soy (70 %) | 1.00 |
| Vitamin C | 0.05 |
| Dicalcium Phosphate | 0.02 |
| Vitamin Premix | 0.29 |
| Canola oil | 8 |
| Cholesterol | 0.5 |
| Fish oil | 10 |
| Barley | 8 |
| Total | 100 |
| Crude Protein (%)* | 28.5 |
| Crude Lipid %* | 10 |

* The proximate composition (Crude protein and crude lipid) of final diet (% dry matter).

such as DO, pH and temperature were monitored daily. An Oxyguard® digital DO meter (Handy Polaris 2, Norway) was used to record DO and temperature, and an Ecoscan pH 5 m (Eutech instruments, Singapore) was used to record pH. A DR/890 portable colorimeter with Permachem reagents (Hach, USA) was used to analyse total ammonia nitrogen (TAN), nitrite ($NO_2$-N), nitrate ($NO_3$-N), and reactive phosphate ($PO_4$) once a week. The experiment was conducted with no water exchange. Tank water level was maintained at 150 L throughout the experiment by adding water to compensate for losses due to the evaporation.

### 2.5. Plankton analysis

During the experiment the plankton density was measured every two days and maintained at the constant preselected density. Plastic containers (100 mL volume) were used to collect and store the samples. For phytoplankton samples, 1 L of tank water was filtered through the plankton net to obtain 100 mL of sample. The zooplankton samples were collected by filtering 5 L of tank water through a 60 μm net to obtain 100 mL of sample. The plankton species were identified to the lowest possible taxonomic level using keys from a book by Canter-Lund, Lund (1995) and a manual by Ingram et al. (1997). The phytoplankton density (cells $L^{-1}$) and zooplankton density (individuals (ind.) $L^{-1}$) was calculated by using the equations from Tulsankar et al. (2021a) and Ingram et al. (1997) respectively.

### 2.6. Marron growth, survival and moulting analyses

The marron growth data were recorded fortnightly, by weighing and measuring individual marron. Marron mortality was recorded on a daily basis. Marron specific growth rate (SGR), weight gain percentage (WG) and survival rate (SR) was calculated by using the following formulae as described by Evans, Jussila (1997):

$$SGR = 100 \text{ X } (\text{Ln } (W_t) - \text{Ln } (W_i))/T,$$

$$WG = (W_t - W_i)/W_i \text{ x } 100$$

$$SR = 100 \text{ x } (n_t / n_0)$$

Where, $W_t$ is final weight (kg), $W_i$ is initial weight, $n_t$ is the number of marron alive at (T) days and $n_0$ is the number of marron stocked initially.

Weight, length and moulting date of marron was recorded to calculate the weight increment (Wm, %), length increment (Lm, %) and moult interval (Tm, days) by using the following equations;

Moult interval (Tm, days):

$$T_m = T_{n+1} - T_n$$

Weight increment at a moult (Wm):

$$Wm = (W_a - W_b) \text{ x } 100/W_b$$

Length increment at moult (Lm):

$$Lm = (TL_a - TL_b) \text{ x } 100/TL_b$$

Where, $T_n$= date of n moult, $T_{n+1}$= date of n+1 moult, $W_a$ = total weight after second moult (g), and $W_b$= total weight after first moult (g), TLa - total length after second moult; $TL_b$ - total length after first moult.

### 2.7. Marron immune and organosomatic indices analyses

The immune indices such as, total haemocyte count (THC) and differential haemocyte count (DHC) were analysed at the end of the experiment. Three marron per replicate were randomly selected to collect the haemolymph samples. The haemolymph was drawn by using a 1 mL syringe inserted ventrally in between the third and fourth pair of pereopods. A method described by Nugroho, Fotedar (2013) was used to analyse THC and DHC.

At the end of the experiment a total of 12 marron per treatment (three per replicate) were randomly selected to analyse the hepatopancreas and tail muscle moisture content, wet weight and dry weight indices. The hepatopancreas and tail muscle samples from every individual marron were weighed; to obtain the dry weight the samples were dried at 105 °C in an oven until the constant weight was achieved. The moisture content and organosomatic indices were calculated as described by Lindqvist, Louekari (1975); Jussila, Mannonen (1997) and Fotedar (2004) using the following equations:

Tail muscle moisture (TM %) = (Tw-Td) X 100/Tw

Hepatopancreas moisture (HM %) = (Hw- Hd) X 100/Hw

Wet tail muscle indices (TMiw) = Tw X 100/BW

Wet hepatopancreas indices (Hiw) = Hw X 100/BW

Dry tail muscle indices (TMid) = Td X 100/BW

Dry hepatopancreas indices (Hid) = Hd X 100/BW.

Where, Tw: Tail muscle wet weight; Hw: Hepatopancreas wet weight; Td: Tail muscle dry weight; Hd: Hepatopancreas dry weight; BW: Body Weight.

### 2.8. Pigmentation observations

At the end of the experiment, the pigmentation of the individual marron was visually assessed as previously reported by, Jussila, Evans (1998). Colour for individual marron was recorded and the most common colour of marron per tank was considered as the colour representation for each tank.

### 2.9. Statistical analyses

All the data were analysed using statistical IBM® SPSS version 26 and are presented as mean ± standard error (S. E.). Independent *t*-test was used to determine significant differences between treatments. Mann-Whitney *U* test was used when the data did lack normality. All tests were considered statistically significant at $p < 0.05$.

## 3. Results

### 3.1. Water quality parameters of juvenile culture tanks

All the water quality parameters were maintained in an optimum range for marron growth and survival (Fotedar et al., 1999; Morrissy et al., 1984, 1990; Policy, 2003; Villarreal, Peláez, 1999) (Table 2).

### 3.2. Plankton density and community

The plankton density was maintained at an average of 2.73–2.92 ×

**Table 2**
The water quality parameters dissolved oxygen (mg $L^{-1}$), temperature (°C), pH, total ammonia nitrogen (TAN; mg $L^{-1}$), nitrite ($NO_2$-N; mg $L^{-1}$), nitrate ($NO_3$-N; mg $L^{-1}$), and reactive phosphate ($PO_4$; mg $L^{-1}$) of the tank water holding juvenile marron throughout the culture under controlled laboratory conditions for 90 days (mean ± S. E.; n = 4).

| Treatments | Mixed plankton | Control |
|---|---|---|
| DO | 9.00 ± 0.01 | 9.01 ± 0.01 |
| Temperature | 21.5 ± 0.01 | 21.4 ± 0.01 |
| pH | 7.67 ± 0.00 | 7.69 ± 0.00 |
| TAN | 0.02 ± 0.00 | 0.02 ± 0.00 |
| $NO_2$-N | 0.03 ± 0.00 | 0.03 ± 0.00 |
| $NO_3$-N | 1.29 ± 0.02 | 1.27 ± 0.02 |
| $PO_4$ | 0.23 ± 0.00 | 0.22 ± 0.00 |

$10^6$ cells $L^{-1}$ and 213–238 ind. $L^{-1}$ for phytoplankton and zooplankton respectively (Table 3). There was no significant difference in plankton density over time, and to avoid a plankton crash a constant density was maintained by either addition or removal of planktons. The culture of phytoplankton in the outdoor tanks consisted of *Scenedesmus* spp.; *Chlorella* spp.; *Closterium* spp.; *Volvox* spp.; *Navicula* spp.; *Nitzschia* spp. while zooplankton tanks had *Calanoida* spp.; *Cyclopoida* spp.; *Keratella quadrata; Keratella cochlearis* and *Daphnia* spp. And these plankton species were observed in marron tanks during the experiment.

### 3.3. Marron growth, survival and moulting

SGR and WG (%) of juvenile marron were significantly higher for marron cultured in tanks fed with mixed plankton than marron fed with formulated pelleted feed (Fig. 1). The SR was similar for both treatment tanks ($p > 0.05$) (mean ± S. E; n = 4). The increment in weight and length at moult was significantly higher in juvenile marron fed with the mixed live plankton compared to those fed with the formulated feed (Fig. 2). Live plankton mixture or formulated feed provided to the juvenile marron did not affect the moult interval. No specific pattern of moulting time was observed, marron moulted during morning, afternoon sometimes during the night.

### 3.4. Marron immune and organosomatic indices

THC was higher in juvenile marron fed with live plankton mixture however the organosomatic indices did not show any significant differences between the two dietary treatments (Table 4) (mean ± S. E.; n = 4).

### 3.5. Pigmentation

At the end of the experiment marron pigmentation was affected by the dietary treatment (Table 5) where plankton fed marron had a dark brown colour and marron fed formulated feed were bluish (Fig. 3).

## 4. Discussion

At the end of this study, live plankton mixture significantly improved the SGR and WG % of marron, similar result of improved SGR were observed in yabbies fed with zooplankton (Austin et al., 1997; Jones et al., 1995; Verhoef et al., 1998). Improved growth rate of marron feed on plankton mixture are the reflection of the constant supply of nutrients, whereas the formulated feed has a tendency to leach out most of the water-soluble nutrients once immersed in pond water (Smith et al., 2002). Plankton are rich source of protein, amino acids, lipids, fatty acids, minerals, chlorophyll, carotenoids, trace elements, enzymes and vitamins (Kibria et al., 1997; Napiórkowska-Krzebietke, 2017) which are essential for marron growth (Fotedar et al., 2015). A constant supply of plankton would have allowed for continuous feeding through filter feeding and scavenging of detritus (Van den Berg et al., 1990). SGR > 0.6 is widely accepted for a commercial marron farm (Evans, Jussila, 1997), however the current work was conducted under controlled laboratory conditions, limiting marron to feed on only plankton entering the cage which may have restricted the growth. And use of cage may have restricted the marron from feeding on detritus formed on tank bottom. Use of containers and artificial diet under controlled laboratory conditions can supress the growth (Geddes et al., 1988).

Although the static plankton density provided to marron was enough to improve growth and health under controlled laboratory conditions, it should be noted that it would be difficult to maintain a continuous plankton productivity in semi-intensive aquaculture facilities. In both dietary treatments the SR was high (>70 %) compared to the SR observed by Celada et al. (1989), where 53.33 % SR was observed for juvenile crayfish (*Pacifastacus leniusculus*) fed phytoplankton and zooplankton. However, similar SR as our study (~70 %) was observed by Carreño-León et al. (2014) for juvenile red claw crayfish fed with microalgae.

The immune and organosomatic parameters have been used as an indicator of immune health in crustaceans (Fotedar et al., 2001; Haefner, Spaargaren, 1993; McClain, 1995a; b). Higher THC represents the higher immune status (Sharma et al., 2009), with higher THC being observed in juvenile marron fed with live plankton mixture, relative to those fed with formulated diet. In decapod crustaceans the food intake and nutritional status affects the haemocyte count in terms of quantity and quality (Le Moullac, Haffner, 2000; Persson et al., 1987). Studies analysing the plankton effect on the immune and organosomatic indices of crayfish have not been reported yet except in a study by Tulsankar et al. (2021a), where plankton supplementation improved hepatopancreas condition. Marron fed fishmeal formulated feed showed similar immune and organosomatic indices as observed by Sang, Fotedar (2010).

In red swamp crayfish (*Procambarus clarkii*) and noble crayfish (*Astacus astacus*) moulting was observed predominantly during the daytime (Culley, Duobinis-Gray, 1987; Franke et al., 2013), and in yabbies mostly at night (Sokal, 1988). In juvenile marron no specific pattern of moulting time was detected during the moulting process. The mean moulting cycle was 14.6 ± 0.43 days for those fed with live plankton and 15.0 ± 0.33 days for the marron fed with formulated feed. While the moulting cycle was reported at 35.4 ± 2.2 days in a study conducted by Mai, Fotedar (2017). The moulting days in our study ranged from 14 to 16 days, similar to the findings of Ackefors et al. (1995) for noble crayfish. The moult interval was decreased significantly in plankton fed marron, indicating that they had better nutrition (Lemos, Weissman, 2021).

The ideal crayfish diet should improve growth, survival and pigmentation (Verhoef et al., 1998), however various studies have found that marron formulated diets were often lacking in carotenoids, resulting in pigmentation loss (Jussila, Mannonen, 1997). Carotenoids are common in algae and are necessary for the good pigmentation in crustaceans, suggesting that farmed marron obtain at least some of their nutrition from natural sources (Goddard, 1988). The pigmentation of juveniles fed with live plankton mixture was dark brown but the pigmentation of those fed with the formulated pelleted feed were light blue, indicating pigmentation loss. This reflects the presence of carotenoids and astaxanthin in the plankton mixture which improved the pigmentation. Increased dietary asthaxanthin through feed for red king crab (*Paralithodes camtschaticus*) achieved darker pigmentation (Daly et al., 2013).

The micronutrional requirements of marron under semi-intensive or extensive farming can be partly fulfilled from the natural productivity with the presence of healthy phytoplankton and zooplankton communities, and detritus, but their macronutritional requirements should be fulfilled by the supplementation of formulated feed (Fotedar et al.,

**Table 3**
Plankton densities controlled in tanks to culture juvenile marron for 90 days under controlled laboratory conditions (mean ± S. E.; n = 4 per treatment).

| Week | Phytoplankton abundance (x$10^6$ cells $L^{-1}$) | Zooplankton abundance (Ind. $L^{-1}$) |
|---|---|---|
| 1 | 2.73 ± 0.03 | 213 ± 12.5 |
| 2 | 2.92 ± 0.10 | 238 ± 23.9 |
| 3 | 2.78 ± 0.06 | 225 ± 10.2 |
| 4 | 2.73 ± 0.03 | 238 ± 12.5 |
| 5 | 2.92 ± 0.09 | 225 ± 20.4 |
| 6 | 2.78 ± 0.06 | 219 ± 6.3 |
| 7 | 2.86 ± 0.06 | 231 ± 21.3 |
| 8 | 2.75 ± 0.06 | 225 ± 10.2 |
| 9 | 2.81 ± 0.05 | 238 ± 23.9 |
| 10 | 2.93 ± 0.05 | 239 ± 6.70 |
| 11 | 2.85 ± 0.07 | 235 ± 5.70 |
| 12 | 2.89 ± 0.06 | 215 ± 10.2 |
| 13 | 2.90 ± 0.07 | 231 ± 0.98 |

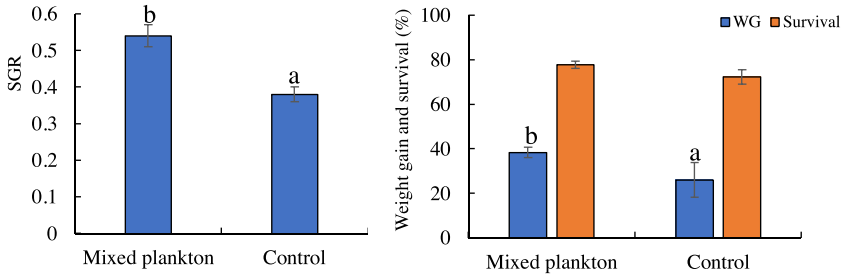



**Fig. 1.** SGR, WG and SR (%) of the juvenile marron cultured under controlled laboratory conditions feeding mixed plankton or formulated feed (control) for 90 days (mean ± S. E.). Letters a and b shows the statistically significant difference between the treatments ($p < 0.05$).

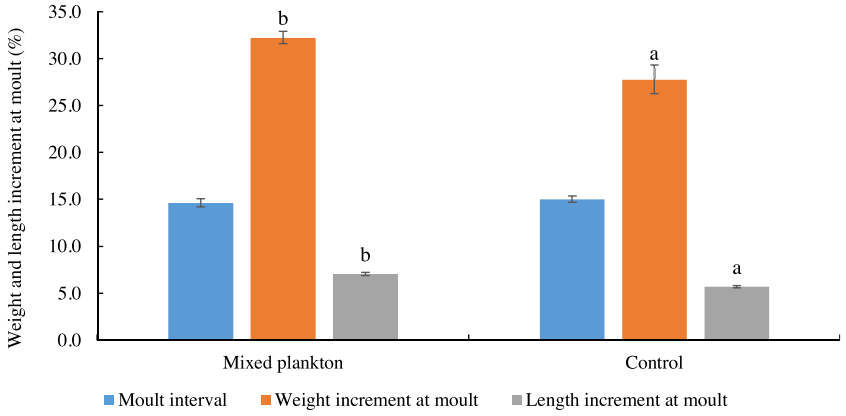



**Fig. 2.** Weight and length increment of juvenile marron on moulting cultured for 90 days under controlled laboratory conditions. Letters a and b show significant differences in weight and length increments of juvenile marron when fed with mixed live plankton or formulated feed (control) (mean ± S. E.).

**Table 4**
Immune and organosomatic indices of juvenile marron fed with mixed live plankton or formulated pelleted feed for 90 days under controlled laboratory conditions (mean ± S. E.; n = 12 per treatment).

| Treatments | Mixed plankton | Control |
|---|---|---|
| THC (million cells $mL^{-1}$) | $2.70 \pm 0.01^{b}$ | $1.81 \pm 0.01^{a}$ |
| Hyaline (%) | 63.3 ± 0.76 | 62.2 ± 1.65 |
| Granular cells (%) | 31.0 ± 1.05 | 31.7 ± 1.16 |
| Semi-granular cells (%) | 5.65 ± 0.72 | 6.23 ± 0.66 |
| TM (%) | 79.25 ± 1.18 | 78.75 ± 1.11 |
| TMiw | 20.7 ± 0.90 | 19.9 ± 0.87 |
| TMid | 4.21 ± 0.11 | 4.26 ± 0.37 |
| HM (%) | 80.0 ± 1.94 | 79.1 ± 1.53 |
| Hiw | 7.90 ± 0.24 | 7.81 ± 0.55 |
| Hid | 1.59 ± 0.17 | 1.63 ± 0.13 |

Superscript [a] and [b] show the significant difference between the treatments within a row ($p < 0.05$).

**Table 5**
Colour representation of marron of each replicate tank fed with mixed plankton and formulated pelleted feed for 90 days under controlled laboratory conditions. (n = 16 per treatment).

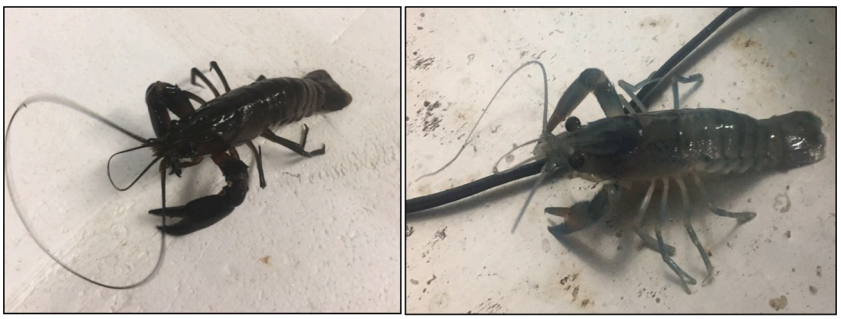
| Treatments | Mixed plankton | Control |
|---|---|---|
| Tank 1 | Reddish Brown | Blue |
| Tank 2 | Dark brown | Light Purple |
| Tank 3 | Dark brown | Light Blue |
| Tank 4 | Dark brown | Light Blue |
| Tank 5 | Reddish Brown | Blue |
| Tank 6 | Dark brown | Blue |
| Tank 7 | Dark brown | Light Blue |
| Tank 8 | Dark brown | Light Blue |

2015). While plankton are rich in vitamins and minerals and can sustain juvenile marron, they may not provide the bulk of nutrients needed for the adult marron to grow, for example marron cannot synthesise cholesterol and it should be included in formulated feed (Fotedar et al., 2015). Juveniles are less benthic and more motile than adults, therefore they have greater ability to capture planktonic prey (Goddard, 1988), whereas adults are less active and can capture larger zooplankton and pelleted feed with pereopods and can achieve their nutritional requirements from formulated feed. Juvenile marron spent longer time in feeding on frozen copepods (Tulsankar et al., 2021b), yabbies <15- 45 g fed zooplankton and formulated feed spent significantly longer time feeding on zooplankton (Meakin et al., 2008); which suggests that they find live-feed more attractive than formulated feed.

The inclusion of live plankton in juvenile marron diet may provide essential nutrition improving the growth rate and survival in aquaculture systems. Better management of the plankton density and diversity will help to lower the use of formulated feed reducing the operational cost. Evaluating the impact of each individual contribution of phytoplankton and zooplankton to the marron growth will put more light on marron nutritional gain from each source.

## 5. Conclusion

In this study, it was possible to maintain a constant plankton density, as the experiment was conducted under controlled laboratory conditions. While recreating the experiment under outdoor conditions the environmental parameters should be considered. Continuous availability of plankton in treatment tanks may have allowed marron to consume the plankton anytime resulting in improved growth rate, total haemocyte count and pigmentation of juvenile marron when compared with the marron fed with formulated feed only. In conclusion, juvenile marron growth rate, total haemocyte count and pigmentation of juvenile individuals was dependant on the supplied feed type.



Mixed Plankton Formulated feed

**Fig. 3.** Difference in pigmentation of juvenile marron fed with formulated diet and live mixed plankton separately under controlled laboratory conditions for 90 days.

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## Animal ethics statement

Animal ethics approval was not required to conduct the experiment as marron are invertebrates.

## CRediT authorship contribution statement

**Smita Sadanand Tulsankar:** Conceptualization, designing and set up of the experiment, day to day feeding, data collection, data analysis and writing of the manuscript. **Anthony J. Cole:** Plankton data collection, reviewing and editing manuscript. **Marthe Monique Gagnon:** Supervision, editing and reviewing the manuscript. **Ravi Fotedar:** Conceptualization, supervision, editing and reviewing the manuscript.

## Declaration of Competing Interest

This work was conducted as a part of PhD course of Mrs. Smita Sadanand Tulsankar, and the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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