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Effects of feeding treatments on growth and survival of Asian clam (*Corbicula fluminea*) in the hatchery

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ABSTRACT

Asian clam (*Corbicula fluminea*) is potentially reared as aquaculture species but feeding regimes and nutrition for this clam remain questioned. The growth and survival of *C. fluminea* were evaluated with the assessment of feeding regimes raised in captive rearing treatments (with substrates) fed with spirulina (*Arthrospira platensis*). Three feeding methods were applied, Treatment A: deposit-feeding, Treatment B: suspension feeding, and Treatment C: combination deposit and suspension-feeding. Shell length (SL) and weight of the individuals were taken every 7 d which last for 6 wk. Condition index (CI), instantaneous growth rates of weight (K_w), and SL (K_L) were determined after the experiment ends. Water parameters such as temperature (°C), pH, ammonia (NH_4), and dissolved oxygen (DO) also being monitored along the experimental period. Significant weight gained (K_w) and CI was found higher in *C. fluminea* fed in Treatment C, where the increment was recorded at $6.24x10^{-3}\pm2.4x10^{-3}gday^{-1}$ and 4.34 ±0.3 respectively. Whereas, the increment of SL (K_L) was insignificant in all treatments. Survival rates (SR) were greater than 95% in all treatments. The rearing conditions are significant factors that affect the feed utilization for their growth. The growth and survival indicate the specific feeding methods for captive *C. fluminea* and spirulina feasibility as their feed.

Keywords: Asian clam, *Corbicula fluminea*, feeding method, spirulina, growth

INTRODUCTION

Asian clam (*Corbicula* spp.) inhabiting freshwater bodies and significant roles as a food source for human consumption especially in Asia. This species, such as *Corbicula fluminea* has a significant effect on socio-economic growth in Malaysia in particular, and currently, clam stocks obtained from neighboring countries (Zalina, 2011). *Corbicula* was massively harvested from the wild in decades for people's consumption which leads to their depletion in a natural habitat instead of other human activities (Ramli *et al.,* 2020). It was expected that the capture of this clam would be increased to meet market demands in the future. Depletion source in nature may hinder the *C. fluminea* business development. The adoption of *C. fluminea* as aquaculture species that could be hatchery-produced may become an upcoming alternative source. For instance, the restoration program of freshwater mussel plain pocketbook (*Lampsilis cardium*) has successfully been produced in the hatchery in Nebraska, USA. This program produces seeds to compensate for the loss in the nature habitat. Hence, a similar program could be carried out for *C. fluminea,* particularly for food purposes.

Previously, the *Corbicula* was failed to be reared in a field or laboratory for mass production due to feeding regimes (Hwang *et al.,* 2010). The understanding of the *Corbicula* feeding regimes is equivocal since it is known as a suspension and pedal feeder that filters phytoplankton and detritus from the water column (Schmidlin, 2011; Majdi *et al.,* 2014). Besides, the juveniles of *Corbicula* were found accumulating the fine organic materials from sand through the pedal sweep and use cilia to create an anterior suspension-feeding current (Shamsul *et al.,* 2017). The filtration ability and organic matter utilization of *Corbicula* in the water column were highlighted which involved both feeding regimes (Majdi *et al.,* 2014). Filtration in *Corbicula* involves pumping the water across gills layers to capture and confiscate microscopic particles suspended in the water column. Meanwhile, *Corbicula* also uses its pedal to catch food into the mantle. Furthermore, cilia on the pedal surface attached and moving the food particles to be ingested. The food particles are sorted on the gills and labial palps responsible to discard large particles. The material that is not ingested is rejected as pseudofeces, while ingested material is used for growth, respiration, reproduction and is excreted as waste including mucous-bound feces (Cheng, 2015).

Furthermore, bivalves are known as efficient filter feeders that capable of reducing the suspended particles including bacterioplankton, protists, phytoplankton, zooplankton, and other abiotic particles (Hwang *et al.,* 2010; Chang *et al.,* 2017). For instance, spirulina (*Arthrospira plantensis*) is a blue-green cyanobacterium that has complete components such as protein, vitamins, and minerals for ideal food (Falquet, 2017). It is also rich in minerals like iron, magnesium, calcium, and phosphorus (Falquet, 2017). In bivalve hatchery, spirulina was found suitable for feeding the marine bivalves since it is a low cost, easy to find in the market shelve, and has essential nutritional requirements under cultural conditions (Arney *et al.,* 2015). This marine microalga has been conventionally used as live feed for mariculture and certain freshwater mollusk. On the other hand, live marine microalgae such as *Isochrysis galbana* and *Skeletonema costatum* were used to feed *C. fluminea* in the laboratory (King *et al.,* 1986). However, it is involved in high cost, difficult to produce in mass and storage (Catarina & Xavier, 2012). Therefore, a recent experiment attempts to evaluate the growth and survival of captive *C. fluminea,* determine the feeding regime, and observe the spirulina feasibility as feed.

MATERIALS AND METHODS

Sampling Location

Corbicula fluminea was harvested from Pergau River, Jeli, Kelantan (5°41'36.6" N, 101°43'38.1" E) by using a dredger. More than 300 *C. fluminea* with different sizes were collected and brought back to Aquaculture Laboratory, University Malaysia Kelantan Jeli Campus for further analysis. They were placed in a holding tank with circulated water for acclimatization.

Experimental Design

Experimental tanks (122 cm length x 46 cm width x 46 cm height) were filled with dechlorinated water (~168 L) and supplemented with circulated water at a flow rate of 14.79 Lh^{-1}. Furthermore, aeration was accomplished in the rearing tanks. To imitate the natural habitat, 2 cm substrate (99.4% sand, 0.44% clay, and 0.15% silt) was placed in a tray (50cm length x 36cm width x 5 cm height). These experimental tanks were set up in triplicates for each treatment. **Treatment A**: Deposit feeding, **Treatment B**: Suspension feeding, and **Treatment C**: combination deposit and suspension-feeding. After a week of acclimation, 540 individuals with shell length (SL) (<20 mm) (Fig. 1) references were distributed into nine experimental tanks. Each individual was numbered using waterproofed ink. The initial individual weight and shell length (SL) were measured. Sixty individuals were placed in each experimental tank. The earlier report shows filtration rate of *Corbicula* was 2.65 mgL^{-1} (Cheng, 2015). However, in the recent experiment, the amount of the feed given was based on a preliminary study where the filtration rate of *Corbicula* was estimated at 1.02 mgL^{-1} which being a foundation in calculating the amount of feed to give. Commercial dried spirulina powder (100g) was watered down in 1000 mL of water and stored in the chiller. This solution was brought to room temperature then sucked by Pasteur pipette (10mL) and pipetted into the experimental tanks twice per day (0800 h and 2000 h) with a concentration of around 30,000 cells/mL. A similar amount was deposited in the substrate for Treatment B and Treatment C, 5 mL was suspended and deposited, respectively. The experiment was last to 6 wk which represented 20% of their general life span (Sousa *et al.,* 2008). The water parameters were maintained as follows: temperature (°C) at 25-28°C, pH at 6-8, and dissolved oxygen (DO) at 6-7 ppm in the experimental tanks. Temperature, pH, and DO were measured using multiparameter YSI Model 556 (YSI Inc., Yellow Springs, Ohio) while ammonia-nitrogen was determined using Hach DR2400 spectrophotometer (Hach Company, Loveland, Colorado) in every 7 d.

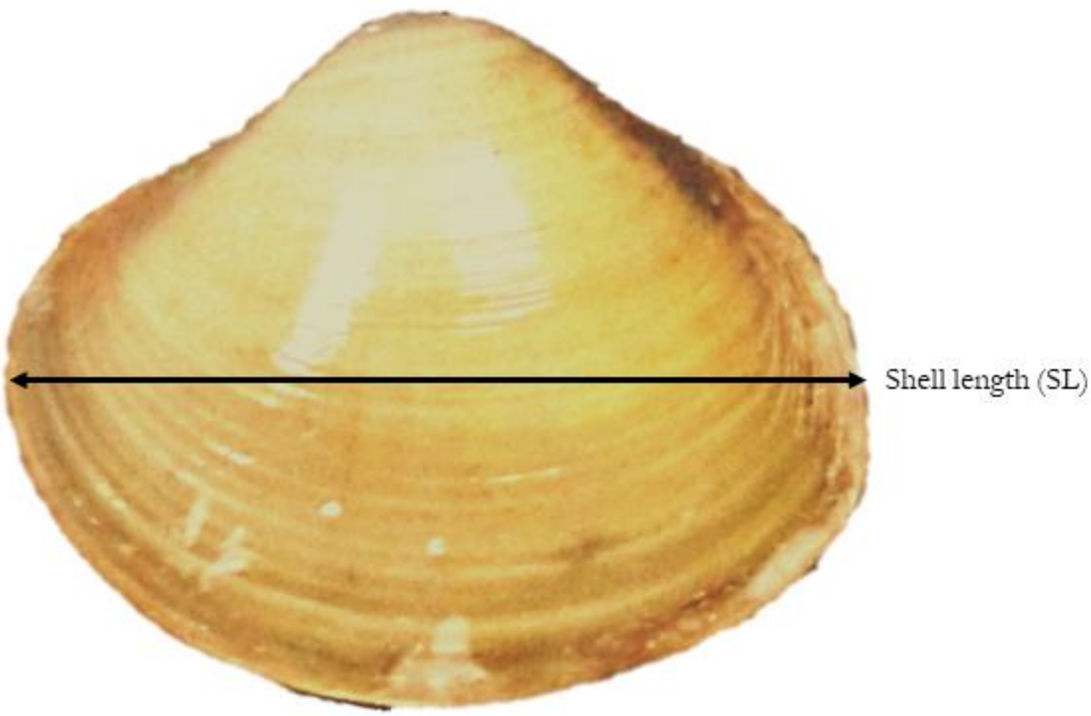

Fig. 1. The shell length (SL) and shell height (SH) measurement of *Corbicula fluminea*

Estimation Growth Indices

Experimental individuals' weight and shell growth were recorded every 7 d. Following indices of growth performance were calculated at the end of the experiment:

(A) Instantaneous growth

I. The instantaneous growth rates (K_L- length) were calculated using the following equations (Serdar *et al.,* 2018):

K_L = ln L_2-ln L_1/ (t_2-t_1)
Where, L_1: initial total length shell length (SL); L_2: final total length shell length (SL)

II. The instantaneous growth rates (K_W- weight)

K_W = ln W_2-ln W_1/ (t_2-t_1)
Where L1: initial wet weight (W_1); L_2: final wet weight (W_2)

(B) Condition Index (CI)

Condition Index (CI) = (W (g) x 100)/S (g)
Where, Weight of dry tissue (g); Dry shell weight (g)

(C) Survival Rate (SR)

Survival Rate (SR) = (I_2/I_1) x 100%
Where I_2=Number of survivors after experimental period; I_1=Initial number of an individual before the experiment

Statistical analysis

The mean values of parameters were analyzed by one-way ANOVA using statistical software (SPSS 22.0) where the significant difference was determined using the Tukey's test at $p<0.05$.

RESULTS

Table 1 shows the mean of instantaneous growth of three treatments in rearing *C.fluminea* after 6 wk experimental periods. The *C.fluminea* has significantly grown in Treatment C where the instantaneous growth of weight (Kw) was recorded at $6.24 \times 10^{-3} \pm 2.4 \times 10^{-3}$ gday^{-1}. Whereas, the insignificant growth of *C. fluminea* in Treatment A and B was found as written in Table 1. Contradict to the instantaneous growth of shell length (K_L) where there was no significant growth.

Table 1. Instantaneous growth of *Corbicula fluminea* reared in two treatments

Treatments	Instantaneous growth (weight) (Kw)	Instantaneous growth (shell length) (K_L)
A	$4.19 \times 10^{-3} \pm 6.7 \times 10^{-4}$ gday^{-1b}	$7.36 \times 10^{-4} \pm 2.2 \times 10^{-4}$ mmday^{-1}
B	$4.13 \times 10^{-3} \pm 3.4 \times 10^{-3}$ gday^{-1b}	$7.30 \times 10^{-4} \pm 3.6 \times 10^{-4}$ mmday-^{1}
C	$6.24 \times 10^{-3} \pm 2.4 \times 10^{-3}$ gday^{-1a}	$7.40 \times 10^{-4} \pm 4.5 \times 10^{-4}$ mmday-^{1}

Table 2 shows the condition index (CI) of *C. fluminea* before and after the experimental period. In this analysis, CI was found insignificant at the beginning of the experiment but significantly differed at the final CI. The CI was increased in all treatments. A significant increment was shown in Treatment C where the final CI was recorded 4.34 ±0.3. Meanwhile, the CI in Treatment A and B was insignificant as in Table 2.

Table 2. Condition index (CI) of *Corbicula fluminea* before and after the experimental period

Treatments	Initial Condition index (CI)	Final Condition index (CI)
A	3.42± 0.4	4.15 ± 0.5^{b}
B	3.34± 0.3	4.08 ± 0.1^{b}
C	3.33±0.3	4.34 ± 0.3^{a}

Table 3 shows no significant difference in the survival rates recorded after the experimental period. The *C.fluminea* highly survived in all treatments which recorded above 95%.

Table 3. Survival rates (%) of *Corbicula fluminea*

Treatments	Survival rates (%)
A	97.22±14.6
B	96.11±16.5
C	98.89±15.5

DISCUSSION

The current experiment was designed by evaluating their feeding methods and the feasibility of nourishing them with spirulina. The feasibility of spirulina as *C. fluminea* feed could be inferred based on the significant increment in instantaneous weight (Kw) and the condition index (CI). As reported earlier, *C. fluminea* is known as a filter feeder where they were efficiently clearing the suspension particles in the water column (Xiao *et al.,* 2014; Falquet, 2017). This understanding agreed with the current finding where the *C. fluminea* filtering the spirulina suspensions (Treatment A and C). The filtration rate is depending on the size of the clam which resulted in different rates of filtration. This observation could be seen in hard clam (*Meretrix lusoria*) where the filtration rate was higher in small clam compared to medium or large clam (Chien and Hsu, 2006). At the beginning of a recent experiment, *Corbicula* with a shell length (SL) less than 20 mm was chosen for evaluating their filtering ability since large clams have a higher mortality rate (McMahon, 2000). Sousa *et al.* (2008) explain the clams with a small SL (<20mm) are considered as juveniles that actively searching food. Juveniles *Corbicula* is a significant parameter since it is highly correlated to metabolism, filtration rate, excretion, and respiration (Xiao *et al.,* 2014). Furthermore, juveniles *Corbicula* gaining tissue biomass whereas adults focused on protein catabolism such as in gametogenesis (Jadhav *et al.,* 2012). Thus, a recent study appropriately used the juvenile's clam for the evaluation.

An insignificant increment of the shell growth (SL) was found in all treatments. Though, the SL increment may result due to high filtration by the *Corbicula* which allows the utilization of nutrients from the spirulina. Previously, *C. fluminea* with small initial SL was significantly grown at a faster rate compared to the larger clams where the juvenile *Corbicula* has to tolerate the fluctuation of water parameter changes such as in salinity (Welch & Joy,1984; Sousa *et al.,* 2008; Xiao *et al.,* 2014). On the other hand, recent findings found that the small clam has a behavior burying within the substrate and substrate-water boundary compared with the large clam. This behavior allows the *Corbicula* to cover up their shells and extended their siphons at the posterior which enabled them to live deeper under the surface. By burying in the substrate, the inhalant siphon allows water flow to the gills where filtering of the suspended particles like microalgae and exit through an exhalant siphon (Gosling, 2003). On the other hand, Majdi *et al.* (2014) elucidated this behavior where juvenile *Corbicula* spends more energy into pedal–feeding to sustain the relatively higher growth rates than larger clams. Therefore, this report explains the importance of substrate and their behavior towards the growth of the clam. In a more recent study, the filtration rate was influenced by several factors such as temperature, salinity, diet size, the concentration of feed despite clam size and behavior (Chien & Hsu, 2006; Xiao *et al.,* 2014). Therefore, an inference could be done as uniform influences experienced the experimental clams.

Other earlier finding has described the weight growth such as in *C. fluminea* is strongly correlated to the metabolism of clam (Xiao *et al.,* 2014). Particularly for *C. fluminea*, the temperature has been the main factor influencing the metabolic activity, growth, filtration rate, and ingestion rate (Rosa *et al.,* 2014; Xiao *et al.,* 2014). In this study, the temperature was found to be a constant since it was reared in captive conditions. Therefore, the influences of temperature maybe not have significantly affected the filtration rates and metabolic activity as above-mentioned. In a recent finding, a significant response in weight gained was found but not in shell increment. It manifested that nutrients in spirulina suffice to nourish the *C. fluminea* even though it is freshwater bivalves. In the meantime, the microalgae nutritional value has been poorly determined for bivalves where the essential macro-and micronutrients had been described for respective bivalves mainly for marine (Catarina & Xavier, 2012). Poorly, there is no nutritional value description or specific requirement for freshwater bivalves' nourishment. Hence, increment in the growth parameters such as in the recent study may suggest the feasibility of spirulina for *C. fluminea* feed.

On the other hand, particle size filtrated by the clam has been one of the factors that affected the growth of *C. fluminea.* According to earlier literature, the large particle size of feed is retained in the gills of clam and release fewer particles in the water which is increasing the feed intake period for the clam (Chien & Hsu, 2006). Meanwhile, the recent finding showed a significant increase in weight which means the size of gills attained a similar response to the retention of the feed for this experiment. The increasing of the suspended particles contributed to lowering the filtration rates, growth, oxygen consumption, and damage the gill which leads to mortality (Tanyaros & Tongnunui, 2011). Suspension of spirulina in the experimental tanks was found not impaired the clam gills but the orientation of the clam on the bed does. The reason for this phenomenon is the proper orientation of the clam in the substrate which favors the clam to orientate vertically for proper feeding (Gosling, 2003). By this orientation, they could siphon the feed from the higher water column and avoid slower water in the boundary layer near the sediment-water interface (O'Beirn *et al.,* 1998). This observation could be seen recently where the clam orients themselves vertically in the substrate while clam without substrate lay on one valve horizontally. In complement to the orientation in the sediment, flowing water in the rearing system may beneficial to the clam survival and feed intake such as in a recent study. For instance, previous research has found clam growth highly correlated to water flow and agitation (Mummert, 2001). Circulated water such as in a recent study is important for the clam to flush wastes and captured the nutrient from the flowing water.

Furthermore, the substrate is essential in rearing freshwater clam where it offers stabilization and protection to the clam from disturbance by the water flow (Gosling, 2003). Without substrates, water current had disturbed the clam for continuously filtering and ingesting the feed where the clam tends to cease feeding and close up (O'Beirn *et al.,* 1998). Hence, feeding cessation and prolong close-up had resulted in slower growth and reduced survival. The substrate also assists the clam to move and feed. Pedal feeding such as in Treatment B reveals the feeding regimes of the clam. The deposited spirulina in the substrate was swept by the clam. Furthermore, the clam moves towards the deposited area to get the feed. In the pedal feeding mechanism, the feed particles are attached to the cilia on the foot, and the particles move into the mantle. In the mantle, the labial palps are responsible to segregate the particle sizes and permitted into the ctenidia. Previous research coined that *C.fluminea* abundantly feeds through pedal feeding instead of filtering the suspension which pedal feeding regimes may assist the clam to get the nutrients deposited in the substrates (Majdi *et al.,* 2014). For Treatment C, the experimental clam benefited through both feeding regimes. As a result, the clam gained weight significantly. The ability to use both feeding regimes increases the utilization of the available feed. This finding explains the mechanism of detritus feeding in the natural habitat. Therefore, the feed particles' existence in the water column or the substrate can be utilized by the clam concurrently.

CONCLUSION

In conclusion, the spirulina was found to be feasible as *C. fluminea* feed as expected. The *C. fluminea* use a filter and pedal to feed. Combination factors described in recent findings may be beneficial in consideration for rearing *C.fluminea* in captive systems for better growth and survival.

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