

Effect of Salinities and Dietary Patterns toward Fullness of Gut and Gut Evacuation Time of the Newly Introduced *Penaeus merguensis* Larvae

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Abstract

The gut capacity of shrimp larvae is minimal, and their digestion is often challenged by the inevitable fact that they tend to develop slowly during the zoea stage. Many studies approved that the digestive capacity of shrimp larvae could be improved by increasing the retention time of food in the intestine. Gut evacuation time and fullness of the gut are crucial parameters in assessing the growth of shrimp larvae, and the diet as well as environmental conditions indeed influence the activity of these parameters. Although many species of shrimps have a wide salinity tolerance, more specific research on salinity and its relation to the type of diet is necessary to find the optimum condition supporting the growth of shrimp larvae. By employing *Penaeus merguensis* larvae, this study evaluates the effect of three nominal salinities (28, 32, and 36 ppt) and types of diets (Diet A: 100% live feed; Diet B: 100% FRIPPAK; Diet C: a combination of Diet A and Diet C, 50 % each) toward the fullness of gut and gut evacuation time of the newly introduced *Penaeus merguensis* larvae culture. The result showed that the longest gut evacuation time and the highest percentage of gut's fullness were found in all Zoea reared with Diet A at salinity 28 and 32 ppt; Zoea-1 at 28 ppt with Diet A; Zoea-2 at 32 ppt with Diet A; Zoea-3 at 32 ppt with Diet A. Longer gut evacuation time would have an impact on the higher percentage of the fullness of gut. The higher fullness of the gut also indicates that the larvae have the best capacity to produce energy and achieve optimum growth.

Keywords: Optimum development, gut, growth, diet

Introduction

Several diseases that recently attacked shrimp farming have caused considerable losses in the shrimp industry (Zhang *et al.*, 2014). Thus, searching for a more potent alternative that can stand against those diseases is needed. A more powerful species of shrimps for better commercial activities should be empowered with a proper environment for more business coverage.

Two vital organs that primarily influence food ingestion are the stomach and gut. The movement rate of food through the intestines and bowels is used interchangeably to show the pace at which food is processed and moved when food is being digested. Several researchers have examined the relationship between gut evacuation, gastric emptying, and food

retention (Abubakr, 2001; Rocha *et al.*, 2018). The evacuation time of the intestines allows observers to assess the rate of food consumption, estimate feeding rates, and define food conversion (Abrunhosa *et al.*, 2003; Abrunhosa *et al.*, 2006; Lima *et al.*, 2016). Gut evacuation time in shrimp is closely related to the overall shrimp growth process. Slower gut evacuation time will provide an opportunity for the intestinal lining to absorb nutrients from food. However, faster gut evacuation indicates that the process of nutrient absorption by shrimp may not happen completely (Relyea and Auld, 2004).

The ability of an organism to successfully assimilate food depends on two factors: (1) the gut evacuation time between the first intake and the first consumption by assessing the object before the food is digested (the appearance of the stool); (2) digestive

enzyme activity (Carvalho *et al.*, 2011). In the Zoea stage, the larvae consume phytoplankton, accompanied by the occurrence of protease activity. Protease activity appears in the zoea phase because the larvae in that phase have started consuming phytoplankton. Proteolytic activity is a condition in which trypsin and chymotrypsin contribute to the diet and anatomical development of larvae (Hernandez-Cortes *et al.*, 2017).

Similar to other organisms, shrimp as invertebrates experience development in anatomy, physiology, and behavior. Feeding behavior in shrimp is influenced by habitat, and the Penaeidae, such as *Penaeus merguensis*, is a family with a complex development (Poulin *et al.*, 2001). Intestinal behavior can be affected by low salinity, and its effect has a particular impact on the osmoregulation process, yet in many cases, young shrimp could tolerate a wider variety of salinity. The lower the osmoregulatory capacity, the greater the effect on pylorus contraction and food transit rate. It can be clarified that low salinity is interesting to be studied; not only does it affect the rate of food transit, but also the osmoregulatory ability of the concerned organism (Relyea and Auld, 2004). Thus, it can be inferred that salinity would affect the fullness of gut and gut evacuation time.

A study by Piyakarnchana (1987) found that *P. merguensis* can grow ideally in salinity ranging from 20-30 ppt and optimally at 27 ppt. They also found that *P. merguensis* and *P. indicus* required saltwater beyond 10 ppt despite a fairly broad resistance of *P. monodon* across a wide range of salinity. The life cycle of shrimp larvae starts with the nauplius stage. In this phase, nauplius does not need proteolytic activity since they consume yolk to survive. When entering the Zoea stage, larvae begin to consume planktonic algae even though their digestive system is not yet functioning properly, which causes the presence of enzymes needed to completely break down food particles (Lovett and Felder, 1989). Besides, the digestive capacity of larvae is minimal, and indigestion is often exacerbated by the fact that the larvae tend to develop slowly during the zoea stage. One way to improve the digestive capacity of shrimp larvae is by increasing food retention time in the intestine in favor of increasing the absorption of food nutrients. Measurement of shrimp gut evacuation time is highly relevant to water quality maintenance; slower gut evacuation time gives more opportunity for the gut to process food before defecation, while faster gut evacuation time leads to defecation of poorly processed food in the intestines (Relyea and Auld, 2004).

In terms of the overall environment, feces secreted by shrimp larvae due to rapid gut evacuation time can be deposited quickly and can cause various types of diseases and decrease water quality. This study aims to assess the fullness of gut and gut evacuation time of the newly introduced *P. merguensis* larvae, a better configuration for larva farming, specifically for *P. merguensis*, could be hopefully achieved.

Materials and Methods

The materials used in this study were shrimp larvae (*P. merguensis*) produced by Jepara's Center for Brackishwater-Aquaculture Development. The larvae tested were only in the Zoea stage considering that Zoea is the youngest shrimp phase and its transparency makes it easier for researchers to see the food movement being studied. The larvae were stocked at 250 individuals in every 2.5 L beaker glass. Each treatment consisted of 750 individuals that were distributed in three beaker glasses (250 individuals each). With a total of nine beaker glasses, the experiment was carried out in three different salinity nominals (28, 32, and 36 ppt) and three different feeding types (Diet A containing 2,000 cells mL⁻¹ of *Skeletonema costatum*; Diet B containing 100% FRIPPAK Microencapsulated of 4 mg L⁻¹; Diet C is the combination of Diet A and Diet B with 50% each). Those dietary patterns were administered once a day at 8 a.m. to avoid overeating which leads to death.

As many as 250 individuals of zoea larvae were first fed with live microalgae (*Chaetoceros gracilis* and *Tetraselmis chuii*), and artificial microencapsulated was later introduced containing a carbon charcoal solution. After applying the carbon charcoal for 1 min as a marker, the anterior midgut became full of carbon charcoal. All larvae were then treated with regular feeding terms, and the process of food ingestion in the gut was observed using a high-power microscope with high-magnification objectives (100 times). The fullness of the gut was observed within 5 h, and the figures observed were later processed by ImageJ and presented in percentages. ImageJ works by getting information from an image by making use of the intensity of the pixels. Time measurements (mins) for finding gut evacuation time were counted using a manual stopwatch.

Results and Discussions

Several previous studies (Brestoff and Artis, 2013; Cornejo-Granados *et al.*, 2018) proved that gut development was influenced by biological and environmental factors, such as development stage, lifestyle, immune response, metabolism, and general

environment. Observations using zoea in this study indicate that zoea seems to prefer live microalgae (Diet A): Zoea-1 grew optimally at a salinity of 28 ppt with Diet A; Zoea-2 grew optimally at a salinity of 32 ppt with Diet A; Zoea-3 grows optimally at a salinity of 32 ppt with Diet A. Therefore, all zoea grows well with Diet A, especially at the salinity of 28 and 32 ppt. The digestive capabilities of the larvae are very limited and aggravated by the fact that their digestive systems are underdeveloped. Therefore, the only way to develop their digestive capacity is by increasing food retention so that larvae can digest and assimilate food and nutrients to their fullest; reaching a better percentage of the gut's fullness by maximizing their consumption toward prey resources.

The type of feed generally influences the gut evacuation time. Since live microalgae in this study were identified as Diet A, it can be concluded that Diet A with gut evacuation time of 8.98 to 9.45 minutes worked well for zoea. The gut evacuation time for Diets B and C at various salinities occurred relatively short, ranging from 6.7 to 7.1 min. However, at a salinity of 36 ppt, Diet C (8.7 min) seemed to get as good as Diet A at 28 and 32 ppt. According to Beseres *et al.* (2005), feed took between 48.3 to 90.5 minutes to pass through the intestines, regardless of the type of food content (fiber, protein, or fat). However, some types of food that have stiff compositions, such as the sclerotized capsule of mosquito larvae and the type of cuticle and chaeta of oligochaetes, resisted digestion and may prolong the time of gut clearance; might take 4-6 h to pass through the intestines (Carvalho *et al.*, 2011). The structure of the food can affect the length of the gut

clearance time. The gut evacuation time in this study tended to be faster than the research results by Carvalho *et al.* (2011) since this study used live microalgae (*C. gracilis* and *T. chuii*), which is easier for the shrimp larvae to digest (Lovett and Felder, 1989).

In light of the above understanding, zoea's gut evacuation time with Diet A at salinity 28 and 32 ppt were slower than those in other salinities and diets. A slower gut evacuation time indicates that the food was adequately digested by the shrimp larvae and converted into energy before proceeding to disposing of food scraps (Relyea and Auld, 2004). As shown in Figure 4, the longer gut evacuation time is directly proportional to the length of the larvae (Figure 5).

From Figure 5, salinity 28 ppt happens to have the best average fullness of gut with Diet A (100%). Meanwhile, the average fullness of gut in salinity 32 ppt also performs best with Diet A (92%). Those high values of guts are in line with more prolonged gut evacuation. Growth is the product of the digestion process, which becomes an essential product in addition to the energy needed to survive (Silva *et al.*, 2016). Several researchers (Zacharia and Kakati, 2002; Murtaugh, 2004; Beseres *et al.*, 2005) have investigated the survival and optimal growth of shrimp larvae at different salinity, but the fact that shrimp can survive in a wide salinity range is inevitable. Seawater shrimp, such as *P. merguensis*, is believed to have a high tolerance in a wide salinity range. Besides, shrimp growth in this research is not too striking, especially with the salinities ranging from 28 to 36 ppt.

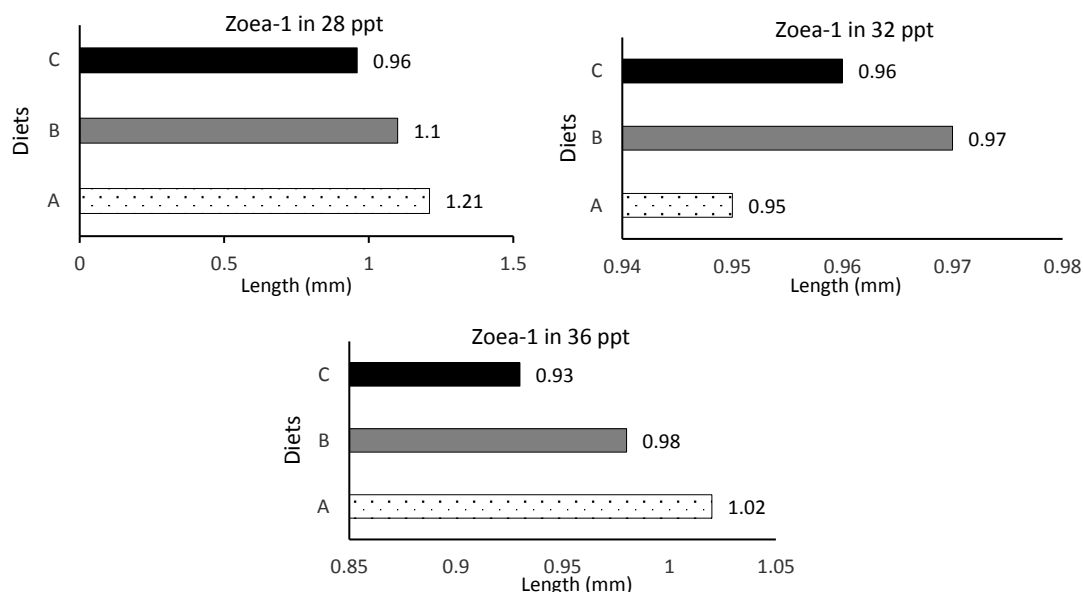


Figure 1. The length of Zoea-1 in different salinities was found to be optimum in 28 ppt with Diet A.

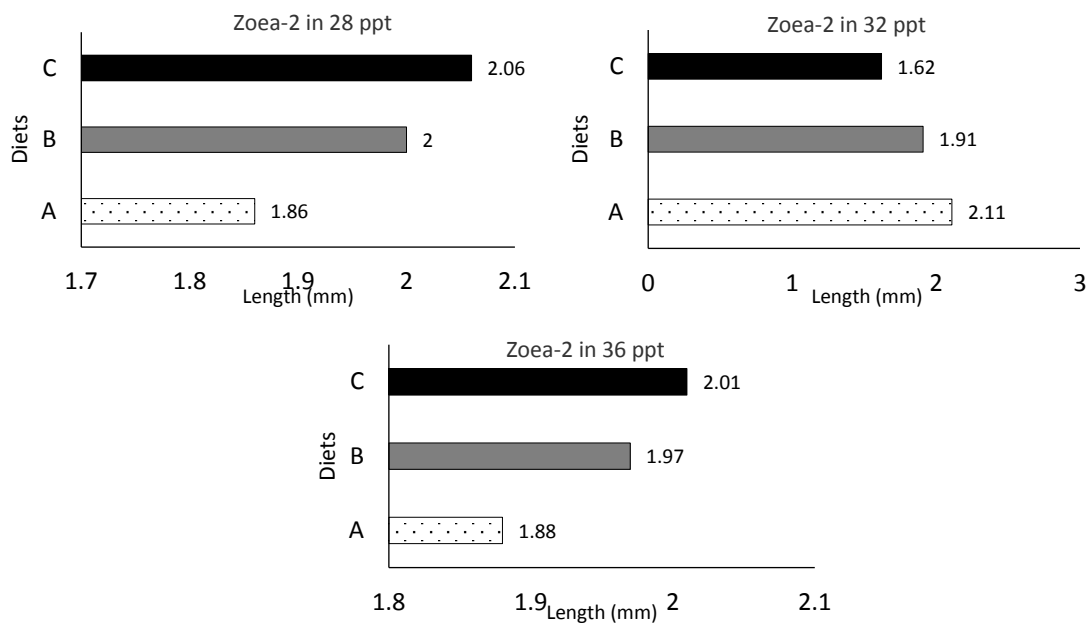


Figure 2. The length of Zoea-2 in different salinities was found to be optimum in 32 ppt with Diet A.

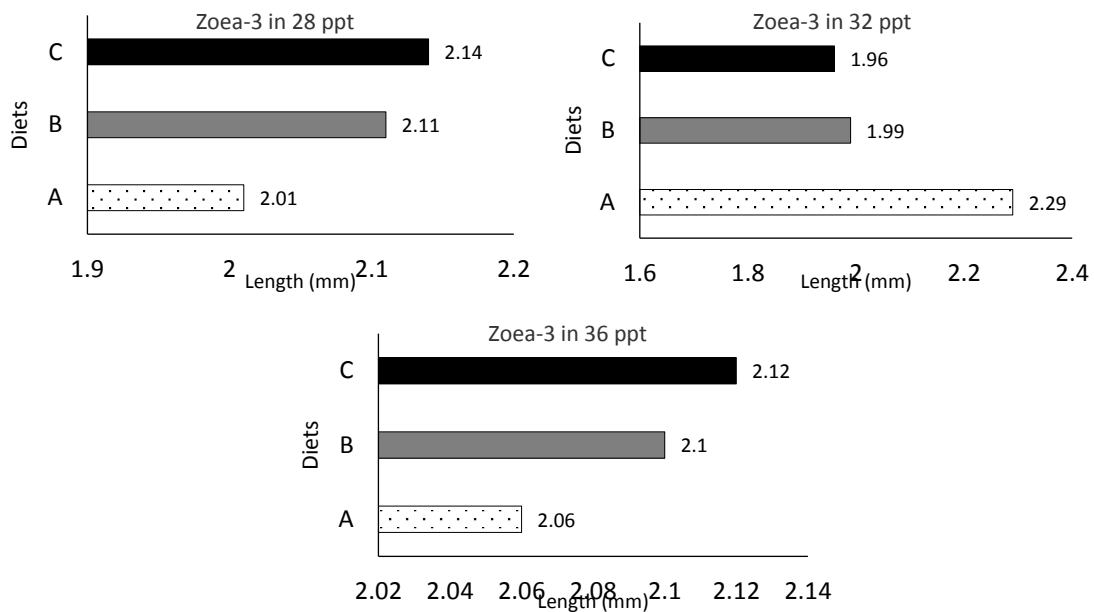


Figure 3. The length of Zoea-3 in different salinities was found to be optimum in 32 ppt with Diet A.

In Figure 6, observations using ImageJ show that 100% fullness of gut was found at a salinity of 28 ppt with Diet A; this fullness of gut was the highest over other observations across various salinities and diets. A high fullness of the gut indicates that the larvae have been eating optimally, and this, together with the gut evacuation time, affects larval growth. The second highest gut fullness was found with Diet A at a salinity of 32 ppt and followed by Diet C at a

salinity of 36 ppt (Figure 7). Although Diet C at 36 ppt appears to have a relatively good fullness of gut, it can be concluded that optimum fullness of gut was still found at salinity 28-32 ppt with Diet A; the overall growth of zoea confirms this judgment. According to Murtaugh (2004), food evacuation occurs faster during continuous feeding as the feed was given intermittently. The movement of food in this study can be observed because the treatment of the charcoal

solution was done only once. Also, this study employed shrimp larvae (zoea) which have a transparent body structure making it easier to observe, especially on the gut.

The fullness of gut observation helps to understand whether the gut is empty or full and reveals whether or not the food have eaten. An empty middle gut indicates that the shrimp is not eating; meanwhile, middle-full means that the shrimp are eating well. From the present study, the obtained fullness of the gut is in line with the findings of gut

evacuation time and optimum length of Zoea-1, Zoea-2, and Zoea-3 (Figure 1-3); meaning that the consumption rate suited with the expansion of the larva's body. The gut is an essential part of the body of living entities; it is a place where various activities that play an important role in general development occur until an organism dies (Sekirov et al., 2010). The present study is concomitant with the study by Relyea and Auld (2004), saying that longer gut evacuation times will result in longer larval growth as they have more chance of absorbing nutrients passing the gut lining.

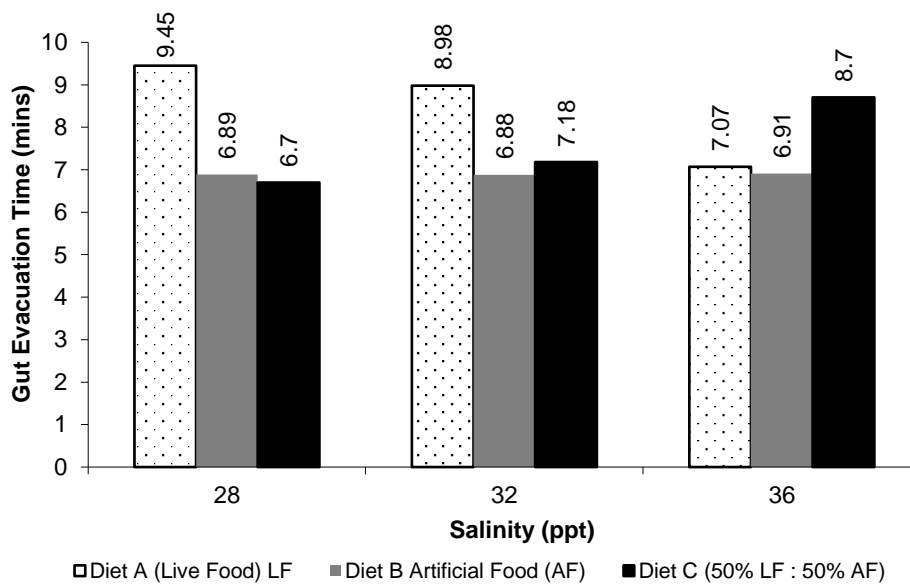


Figure 4. The longest gut evacuation time of *Penaeus merguensis* (9.45 minutes) was found when Diet A was administered at a salinity of 28 ppt

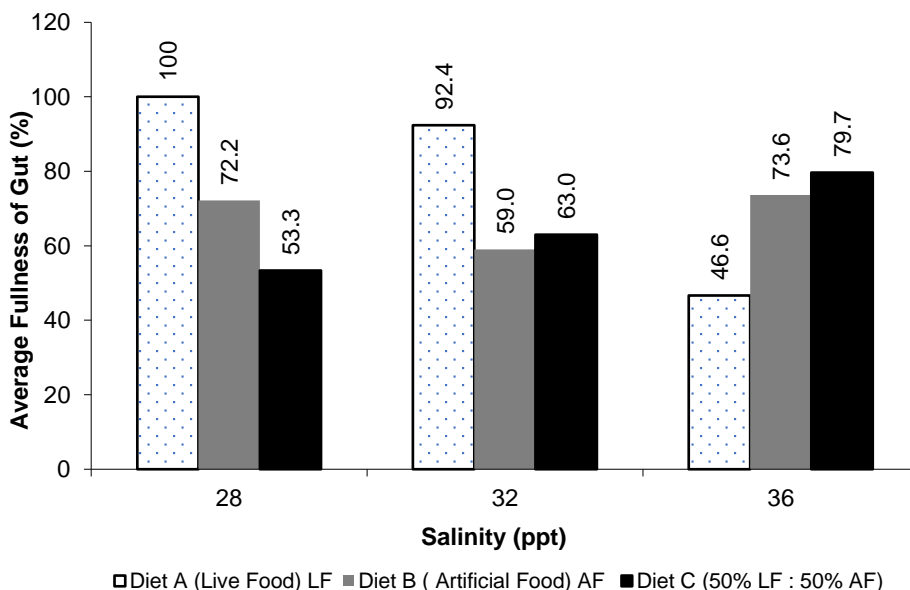


Figure 5. The highest percentage of gut evacuation time of *Penaeus merguensis* (%) was found when Diet A was administered at a salinity of 28 ppt



before ImageJ processing



after ImageJ processing

Figure 6. A 100% fullness of gut of *Penaeus merguensis* larvae in 28 ppt with Diet A.



Before ImageJ processing



after ImageJ processing

Figure 7. A 92% fullness of gut of *Penaeus merguensis* larvae in 32 ppt with Diet A.



before ImageJ processing



after ImageJ processing

Figure 8. A 79.7% fullness of gut of *Penaeus merguensis* larvae in 36 ppt with Diet C.

Studying gut activity is vital to know the gut's response to the food intake given to organisms. Also, daily consumption of a protein-rich diet will provide more net energy, which in turn will contribute to better

performance to benefits. Gallardo *et al.* (2013) stated that the transition at each phase forces shrimp larvae to start acting as predators, usually found between mysis to postlarvae as they will begin to eat

zooplankton more than when the larvae are in the zoea and mysis phases (Cornejo-Granados *et al.*, 2018). The present study showed that shrimp larvae mostly accepted Diet A in 28 and 32 ppt. This result is confirmed by the study by Zacharia and Kakati (2002), saying that *P. merguensis* larvae, like many other penaeid shrimps, performed excellently in seawater with natural food in lower salinity; the salinity of more than 15 ppt was found to be optimum for producing *P. merguensis* postlarvae.

Conclusion

The present study concluded that gut evacuation time has a positive correlation with the fullness of the gut. The longest gut evacuation time and highest fullness of gut were found in Zoea administered with Diet A at salinity 28 and 32 ppt; Zoea-1 at 28 ppt with Diet A; Zoea-2 at 32 ppt with Diet A; Zoea-3 at 32 ppt with Diet A. The higher fullness of the gut indicates that the larvae have the best capacity to generate energy and achieve optimum growth. Gut evacuation time is an essential parameter in studying the level of consumption because the gut is a vital organ for food digestion, nutrient absorption, and immune resistance. Further research examining the gut evacuation time and fullness of gut larvae of *P. merguensis* in other phases with more diverse salinity and diets is a highly recommended study in the future.

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