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Growth Performance of Babylon Snails (*Babylonia areolata* Link, 1807) Fed Formulated Diet in Ponds and Recirculating Aquaculture System

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Abstract: The use of trashfish as food to Babylon snails in grow out farming has recently caused challenges in environment and disease outbreak in Vietnam. This paper is to present the growth, survival, FCR and shell apperance of the stocked snails at size of 0.27 g individual^{-1} fed formulated diet at a daily feeding rate of 0.8 - 1.2% body weight in recirculating aquaculture system (RAS) and ponds. In RAS tanks of 25 m^2, the snails reached to a size of 7.1 ± 0.05 g individual^{-1}, survival of 87.3 ± 1.68% and FCR of 0.77 ± 0.01 after six months. Meanwhile in ponds of 1500 m^2, they reached to a size of 7.21 ± 0.09 g individual^{-1}, survival of 80.53 ± 4.04% and FCR of 0.81 ± 0.01. The significant difference was observed in the survival ($p < 0.05$) and FCR ($p < 0.01$) but not in the growth ($p > 0.05$). The presence of unexpected predatory crabs possibly caused the lower survival and higher FCR in the ponds. For the steamed harvested product, the snail flesh body looked quite similar while their shells showed pale brown in the tanks compared to the common ones in the ponds. This possibly related to the low pH and alkalinity in RAS tanks. The snail growth in this study was roughly similar while their survival was better than those in trashfish based culture previously reported. The growth and survival indicate a high potential to replace trashfish by formulated diet in farming of Babylon snails.

Keywords: *Babylonia areolata*, Formulated Diet, Recirculating System

1. Introduction

Traditional culture of Babylon snails has developed for two decades and become an important industry in Vietnam. This aquaculture has originally started in sea pence and ponds [1] and moved to recirculating system [2, 3] with a production of around 10.000 tonnes of premium grade snails equivalent to USD 90 millions in 2020. However, the snail farming has experienced significant increasing challenges from disease, severe environmental degradation [3]. The use of trashfish as the snail food which has seasonally unstable quality and potentially serious disease agents is a major contributing factor to their poor condition and increased susceptibility to diseases. The preliminary results indicated a possibility to apply formulated diets to Babylon snails. However, these results were just from small scale trials [4-7] and the use of formulated diet in snail farming has not been in practice yet. Furthermore, in severe situation of global climate change, poor environmental condition is another challenge to Babylon snail farming in Viettnam. The suddenly reduced salinity and low temperature in culture ponds due to heavy rain and cold wind in last six months of the year has caused Babylon snail dead in mass. Application of RAS has indicated an interesting result in lobster farming [8, 9] and good performance of Babylon snails in preliminary trials [3, 7, 10-12]. Thus, RAS farming of Babylon snails basing formulated diets is expected to eliminate risk due to sudden environmental condition during windy and heavy rain

season as well as prevent serious disease agent from trashfish.

This paper is to present culture system, husbandry, water quality, and growth and survival of Babylon snails fed formulated diet in RAS tanks and ponds. The results are interesting in development of Babylon snail farming.

2. Materials and Methods

2.1. Culture System

Two separate systems were used including tanks in RAS and ponds. RAS constituted of four culture tanks, a manhole, a settlement tank, a biofilter, two pumps, two blowers and a ultraviolet light (UV) (Figure 1). The square concrete culture tank had a 5 m side and 0.6 m depth (water depth of 0.4 m). The culture tank was installed 5 aerators and filled of a 3 cm sand layer on the bottom. The round concrete settlement tank had a 4 m diameter and 1.4 m depth. The submerged bed biofilter (Figure 2) previously described [3, 9] had three consecutive parts, each had a 2.6 m length, 1.4 m width and 1.4 m depth. The first part connecting to the settlement tank was filled of coarse gravels (15 - 30 mm size). For two remaining parts, each had an empty section (0.5 m length x 1.4 m width x 1.4 m depth) and a biological section. The biological section of the second and third part was filled of medium (10 - 15 mm) and fine (5 - 10 mm) gravels, respectively. The biofilter has been activated and in use for several years. The pumps (12 - 24 m^3 h^{-1}, Taiwan) were used to circulate water from biofilter through a UV (24 m^3 h^{-1}, Camix) to culture tanks. The blowers (3 HP, Taiwan) were used to supply oxygen to culture tanks as well as empty sections of the biofilter.

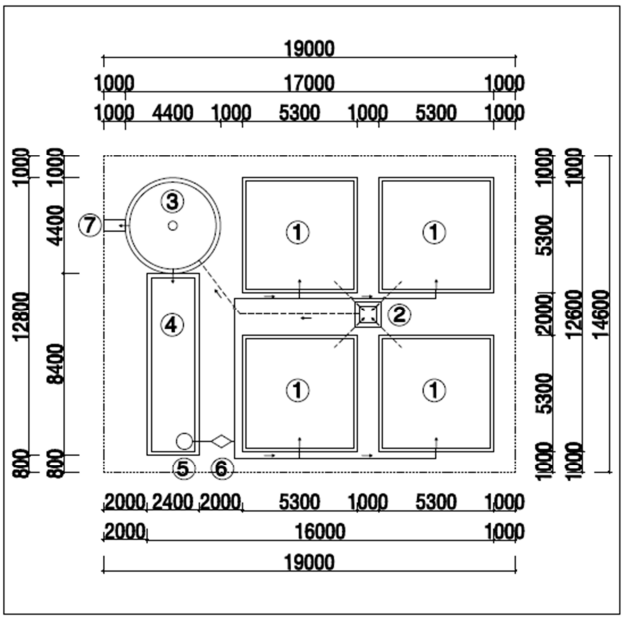

Figure 1. *Horizontal system plan for Babylon snail culture. 1: culture tank; 2: manhole; 3: settlement tank; 4: biofilter; 5: pump; 6: UV.*

The clean seawater with salinity of 30 - 36‰, pH of 7.8 - 8.2 was treated first by chlorine 70% at 10 mg L^{-1} and three days later by thiosulphate to make sure no existence of free chlorine before supplying to the system. In RAS, the water from culture tanks was gathered in the manhole and settlement tank entering the biofilter. Water from the biofilter was pumped through the UV back to the culture tanks.

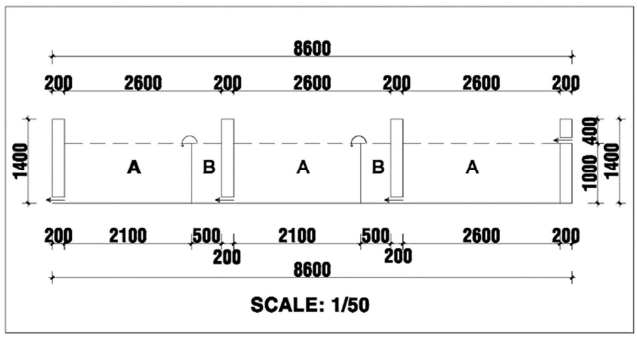

Figure 2. *Design of submerged bed biofilter. A: gravel filled section; B: empty section.*

There were three rectangular culture ponds each had a 30 m width and a 50 m length. A sand layer of 5 cm was initially prepared on the pond bottom. A congruent rectangular area, equivalent to two-third area of the pond was netted for Babylon snail stocking area. The clean seawater with salinity of 30 - 36‰, pH of 7.8 - 8.2 was pumped through a predator preventing netting to a water depth of 1.0 - 1.2 m. Two paddles (3 HP per unit) were installed in each pond to supply dissolved oxygen and circulate the water current in the nighttime.

2.2. Seed Quality and Stocking

The seeds of Babylon snails were selected from a hatchery batch which was acclimatised to formulated diets for two months in a RAS described previously [3]. For seed selection, a random sample of the snails from a batch with clear shell colour was collected and released into a plastic bucket having sand layer on the bottom. The batch was accepted if just after being released into the bucket, most of the observed snails moved quickly and buried themselves in the sand layer. A total of 310 kg seed snails roughly equivalent to 1134600 counted individuals at a size of 3660 individuals kg^{-1} was used for the experiment. Of which 146400 individuals (40 kg) were stocked evenly into four square concrete tanks in RAS. The rest (988200 individuals, 270 kg) was stocked evenly into three rectangular ponds.

2.3. Formulated Diet and Feeding

Formulation and biochemical composition of formulated diet in pellets is given in Table 1. To make the pellets, all flour materials were mixed and ground to 0.8 mm. The flour mixture was added liquids, mixed thoroughly and added clean fresh water to have a dough (approximately 16 - 18% moist). The dough was then pushed through a breaker plate to have noodles of 8 mm in diameters by extruder. The noodles were steamed in 5 - 7 minutes before being pushed

through anothor breaker plate to gain final pellets of 2 - 3 mm in diameter and 1 - 3 cm in length by extruder. The final pellets were dried in 65 - 70°C for 90 minutes before being packed (Figure 3). The proximate compositions and the relative methods carried out by Upscience, Vietnam branch were: highly unsaturated fatty acids (HUFA): ISO 12966-2: 2017 (CH035), ash: GE001 (Ref. AOAC 942.05) (V), carbohydrate: calculation, protein: TCVN 8134: 2009 / ISO 937: 1978 (V), lipid: hydrolysis-extraction.

Table 1. *Formulation and approximate composition of the formulated diet.*

No.	Ingredient	Percentage
1	Super fishmeal (67% of protein), KIHUSEA VN	54.5
2	Wheat flour, Vietnam	18.2
3	Shrimp meal, Vietnam	9.1
4	Soya bean flour	9.1
5	Fish oil, 98%, Vietnam	0.5
6	Lecithin, E322, Argentina	2.2
7	Growmix®shrimp, Bayer	2.3
8	CMC, Yulong, China	1.8
9	Squid liver powder, Vietnam	1.8
	Total	100
1	*HUFA (g 100 g^{-1})*	*0.98*
2	*Carbohydrate (g 100 g^{-1})*	*12.41*
3	*Ash (g 100 g^{-1})*	*19.25*
4	*Protein (g 100 g^{-1})*	*42.12*
5	*Lipid (g 100 g^{-1})*	*10.42*

The formulated pellets were fed to the snails once a day in the morning at a feeding rate of 1.2% of snail body weight in the beginning and gradually reduced to 0.8% in the late period. The pellets were delivered evenly to the bottom of the ponds and the tanks by hand. Each week, all snails were starved one day for cleaning the culture tanks as well as ponds.

Figure 3. *The formulated pellets fed to Babylon snails.*

2.4. Management and Data Recording

For RAS, in the beginning, a pump of 12 m^3 h^{-1} was operated to circulate water current and the daily recycled water was approximately at 200%. In late culture period, the pump power of 24 m^3 h^{-1} was applied. The skimmer of 24 m^3 h^{-1} was daily operated in 6 - 10 hours. Each week, for the culture tanks, cleaned the sand layer by water pump and drained water off. The water percentage removed due to these activities was about 50 - 60% of the total water volume in the whole RAS. The new seawater was added to maintain whole water volume. BZT®, Tetrasul Intlpharmar, a product of *Bacillus* was periodically supplied to maintain the useful bacteria community in the system. The requirement of water quality was T°C: 28 - 30°C; pH: 7.6 - 8.2; S‰: 28 - 38‰; dissolved oxygen (DO) ≥ 4.6 mg L^{-1}; total ammonia nitrogen (TAN) < 0.9 mg L^{-1}; NO_2-N ≤ 1.0 mg L^{-1}; NO_3-N ≤ 50 mg L^{-1}; alkalinity: 90 - 140 mg L^{-1}. The salinity was controlled by exchange of new seawater. The BIO-II (*Bacillus subtilis; Lactobacillus sp; Nitrosomonas sp* and *Nitrobacter sp*) from BIO Nha Trang Vietnam was periodically supplied to the biofilter as monitored TAN or NO_2-N were out of expected range. Bikap C. P. Vietnam was periodically added to maintain the alkalinity. Every day, checked snail activities and immediately removed away all the unhealthy ones laying on sand layer with a swollen mouth trunk.

For pond culture, 30 - 50% seawater was daily drained out during low tide and supplied by pumps at highest tide. Every day, after feeding, checked the feed consumption, snail activities. Every week, cleaned the pond bottom by water pump. BZT® was periodically supplied to maintain the useful bacteria community in the pond. The management of water quality was as described in RAS. Every day, checked and removed the unhealthy snails and trapped crabs in the ponds.

The water quality parameters were measured by Hanna HI83306-02 Environmental Analysis Photometer. The water was periodically sampled at 2 pm from the midle water layer in the settlement tank in RAS and in the bottom water layer in the ponds. At harvest, scored production, body weight of the snails. The picture of steamed snail shells and their soft body were taken by iphone 7.

2.5. Data Analysis

The specific growth rate (SGR) = ln (W_2/W_1)/180*100 (%) where W_1 and W_2 (g) were the average body weight of the snails recorded at the stocking and harvest day after a 180 day culture, respectively.

Survival rate (%) = N_2/N_1*100 where N_1 and N_2 were the number of snails recorded at the stocking and harvest day, respectively.

Feed conversion rate (FCR) = total supplied feed (kg)/ weight gain (kg); where weight gain = final biomas – initial biomas. The average criteria were calculated using the recorded data from four RAS tanks and from three ponds.

3. Results

3.1. Growth Performance of Babylon Snails

The growth and survival of Babylon snails are given in Table 2. In RAS tanks, the stocked snails at a size of 0.27 g reached to 7.14 ± 0.05 g individual^{-1} and got a survival of 87.33 ± 1.68%. These criteria in the ponds were 7.21 ± 0.09 g individual^{-1} and 80.53 ± 4.04%, respectively. The growth of snails in the tanks (final weight and SGR%) was slower than those in the ponds but the difference was not statistically significant ($p > 0.05$). The survival in the tanks was higher than those in the ponds while FCR in the tanks was lower than those in the ponds and these differences are statistically significant ($p < 0.05$ and $p < 0.01$, respectively). A few

unhealthy snails showed a swollen mouth trunk and they were immediately removed away. Other deads left their shells showing no specific disease syndrome in both tanks and ponds. In addition, many snail broken shells were observed in the ponds.

The shells of the fresh snails from the tanks and ponds looked quite similar showing common dark brown spots without any partial removal of outer shell memberance. However, for the steamed snails, while the colour of the soft body looked quite similar, the shells from the tanks were less brown as those from the ponds (Figure 4).

Table 2. *Growth performance of Babylon snails. The different superscripts indicate significant difference at p < 0.05 (*) and p < 0.01 (**).*

Criteria	RAS Tanks (n = 4)	Ponds (n = 3)
Initial weight (g individual^{-1})	0.27	0.27
Final weight (g individual^{-1})	7.10 ± 0.05^{a}	7.21 ± 0.09^{a}
SGR (%)	1.810 ± 0.004^{a}	1.818 ± 0.007^{a}
Survival (%)	$87.33 \pm 1.68^{a*}$	$80.53 \pm 4.04^{b*}$
FCR	$0.77 \pm 0.01^{a**}$	$0.81 \pm 0.01^{b**}$
Productivity (kg m^{-2})	9.08 ± 0.20	1.91 ± 0.11

Figure 4. *Typical appearance of the steamed shells and soft body of harvested snails from RAS tanks (lower) and ponds (upper).*

3.2. Water Quality

The water quality in the RAS tanks and ponds is given in Table 3. The temperature, salinity and DO looked quite similar in two culture systems. Meanwhile pH, alkalinity, TAN and NO_2-N in the tanks were lower than those in the ponds. TAN, NO_2-N and NO_3-N were very low in both systems. For the tanks, in the beginning, when production of snails was still low, pH and alkalinity were higher (7.8 - 7.9 and 118 - 126 mg L^{-1}, respectively) and by the time, reduced gradually and stabilised at low levels (7.5 - 7.6 and 92 - 98 mg L^{-1}, respectively) depending on amount of new seawater supply. In a 0.6 m water depth, it was so clear that the colour shell and movement of the snails and the leftover feed on the tank bottom could be observed clearly by naked eyes.

Table 3. *The seawater quality in the culture of Babylon snails.*

Parameter	RAS tanks	Ponds
T°C	27 - 30	26 - 32
DO (mg L^{-1})	4.6 - 4.8	4.4 - 5.2
Salinity (‰)	32 - 37	30 - 35
pH	7.5 - 7.8	7.8 - 8.0
Alkalinity (mg L^{-1})	92 - 118	114 - 146
TAN (mg L^{-1})	< 0.7	< 0.9
NO_2–N (mg L^{-1})	< 0.08	< 1.2
NO_3– N (mg L^{-1})	< 5.6	< 2.1

4. Discussion

4.1. Growth, Survival and Formulated Diet

In this study, while the growth of the snails was similar, the survival was significantly higher in the tanks than in the ponds ($p < 0.05$). The presence of crabs, an unexpected seasonal predator in the ponds possibly caused this difference. In management, the seawater was pumped directly to the snail ponds through a netting system at high tide randomly taking eggs of crabs into the ponds. These eggs developed well into adults spending most of their life in the mud sand layers of the ponds. They preferred eating flesh of snails rather than the formulated diet. Consequently, a large number of the snails were killed leaving their broken shells in the ponds in turn decreasing survival and increasing FCR. This suggests putting traps to remove immediately all these predatory crabs from the snail culture system.

In our study, the snails at a size of 0.27 g individual^{-1} got a harvested size of 7.1 - 7.21 g, a survival of 80.53 - 87.33% and FCR of 0.77 – 0.81 (Table 2). Their growth in both tanks and ponds was roughly similar whereas the survival was higher than those in trashfish based culture. In a recent study, the snails at size of 0.05 g individual^{-1} fed trashfish reached to a size of 6.0 ± 0.27 g and a survival of 75.7 ± 3% [3]. Moreover, in traditional culture models (fence or pond), Babylon snails fed trashfish approximately reached to a size of 6 - 7 g individual^{-1} and a survival of 70 - 76% after 6 - 7 months and most of the deads showed a swollen mouth trunk. The fresh trashfish used as food to the snails was thought to be the main reason for this specific syndrome due to potential pathogen [1]. In our study, most of the dead snails showed empty shells without any specific syndrome or broken shells and just a few showed syndrome of swollen mouth trunk. This supports that the formulated diet could minimise potential pathogens via food in Babylon snail culture in turn giving higher survival [1, 7].

Similar results in comparison of the formulated diet to trashfish were also reported. The nonsignificant difference in growth and better survivals of Babylon snails were observed for the formulated pellets of 40% protein, 9.18% lipid and 11.17% moist [4] and for 38.2 - 40.12% protein, 8.35 - 9.24% lipid and 9.92 - 10.18% moist [7]. The snails at size of 1.48 g individual^{-1} fed formulated pellets reached to a size of 5.8 g; survival of 95.7% and FCR of 0.98 [4]. The survival and FCR from these studies were higher than those in ours. The lower quality of food might relate to higher FCR. The bigger size of stocked snails and shorter culture time might provide higher survivals. The growth and survival of the snails in our study indicate a potential to apply the formulated diet in culture of Babylon snails.

4.2. Water Quality and Growth Performance

As discussed above, the growth and survival of the snails in our study (tanks and ponds) were similar or in some cases better than reported ones. It was previously obsevered that Babylon snails grew well at temperature: 26 - 34°C, salinity:

30 - 35‰, pH: 7.4 - 8.5, DO: 4 - 7.5 mg L^{-1}, alkalinity: 100 - 160 mg L^{-1}, NH_3-N: 0.19 - 0.28 mg L^{-1}, NO_2-N: 0.11 ± 0.01 mg L^{-1} and NO_3-N: 0.10 - 0.15 mg L^{-1} [1]. The snail growth was better at lower (0.06 - 0.09 mg L^{-1}) than at higher NH_3-N (0.10 - 0.16 mg L^{-1}) [13] and better at higher (75.6, 90.0 mg L^{-1}) than at lower alkalinity (48.4, 62.58 mg L^{-1}) [14, 15]. Thus, it could be said that water quality in our study was suitable to the development of Babylon snails.

The results indicate that the growth of the snails in the ponds was not significantly different from RAS and the steamed soft flesh body looked quite similar. However, the steamed shells were clearly distinguished from each other (Figure 4). The lower pH and alkalinity in RAS tanks versus ponds (Table 3) possibly caused this difference. According to several authors, the reduced pH and carbonate concentrations may result in a loss of fitness [16, 17] as energy is diverted away from growth and reproduction to ensure that basic metabolic processes are maintained [18, 19]. This may in turn affect the organisms ability to cope with other environmental stressors [20, 21] and, ultimately, influence its survival [19, 22]. In our study, the pale brown spots of steamed shells was only recorded in the tanks at lower pH and alkalinity (minimum of 0.76 and 98 mg L^{-1}, respectively) but not in the ponds where these minimums were higher (0.78 and 112 mg L^{-1}, respectively). This suggests that pH of 7.6 did not affect the growth nor the suvival of the snails but possibly caused pale brown spots of their steamed shells from RAS tanks. The severely abnormal shells of the snails fed trashfish were also obsevred in RAS tanks with alkalinity of 72.5 ± 16.4 mg L^{-1}; TAN of 1.36 ± 0.22 mg L^{-1} whereas their normal shells were observed in flow through system with higher alkalinity (112.7 ± 12.9 mg L^{-1}) and lower TAN (0.062 ± 0.03 mg L^{-1}). The rest parameters of water quality observed there were almost similar including T°C (27.3 verus 27.3); salinity (30.9 versus 30.8‰); pH (7.78 versus 7.78); DO (6.1 versus 6.2 mg L^{-1}); NO_2–N (0.062 versus 0.046 mg L^{-1}) and NO_3–N (10.66 versus 12.03 mg L^{-1}). The shell abnormality in the snails characterised by change in shell colour from dark brown to pale brown spots and the partial removal of outer shell membrance [12]. In our study, pH and alkalinity in the tanks were lower compared to those in the ponds (Table 3) but still higher than those at which abnormal shells were previously observed [12]. Thus, lower pH and alkalinity possibly caused more severe abnormality of the shells [12] compared to those at their higher level in our study. This is also supported by previous observation that low alkalinity had negative effect on the growth of Babylon snails [14, 15]. The effect of low pH was also observed in gastropod shell dissolution [23] and abalone shell integrity [24]. The abnormal shells such as pale brown spots or removal of outer memberance will lower the price of the harvested snails. Thus, control of pH and alkalinity should be in high precaution.

In this study, a reduction in pH and alkalinity to low stable levels in the late culture period of RAS tanks could be explained by higher rate of nitrification in biofilters which consumed more alkalinity and in turn released more H+ ion reducing pH [25] as the snail production in the system increased gradually. It is interesting that at a high rate of the recycled water (50 - 60% weekly removal), the levels of TAN, NO_2-N and NO_3-N in RAS were quite low. This exhibits that beside nitrification, a denitrification happened reducing concentration of NO_3-N as reported previously [3, 9, 26].

5. Conclusion

Babylon snails at a size of 0.27 g individual^{-1} fed formulated diet reached to a final size of 7.10 ± 0.05 g and a survival of 87.33 ± 1.68% in RAS tanks, and 7.21 ± 0.09 g individual^{-1} and 80.53 ± 4.04% in the ponds, respectively after six months. The growth and survival indicate a high potential to replace trashfish by formulated diet in farming of Babylon snails. Technical solutions to maintain pH and alkalinity suitable to this species in RAS are of interest.

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