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Effect of stocking density of Snakeskin gourami (*Trichopodus pectoralis*) in polyculture system with Black apple snail (*Pila polita*)

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ABSTRACT

The experiment determined the effect of stocking density of snakeskin gourami in the polyculture model with black apple snails. The experiment included four treatments corresponding to four densities of fish (ind./m^2): (i, Control): 0, (ii) 3, (iii) 5, and (iv) 7; snails were stocked at 150 snails/m^2; each treatment was triplicated. The initial fish's weight was 12 ± 0.3 g, and the snail's weight was 0.15 ± 0.01 g. After 120 days of the experiment, the survival rate of fish was 100% in all treatments, whereas the survival rate of snails ranged from 80.22 to 82.44%, and there were no significant differences ($p>0.05$). Snails in the control treatment obtained the highest specific growth rate (2.68 %/day) and productivity (0.54 kg/m^2). In contrast, the specific growth rate and productivity of fish obtained the highest number in treatment iv, with 0.98 %/day and 0.24 kg/m^2, respectively. The highest profit was recorded in the treatment with 7 fish/m^2, and this was significantly different ($p<0.05$) compared to the control and 3 fish/m^2 treatment. Results from this study showed that the stocking density of 5 fish/m^2 was suitable to enhance productivity of both snail and snakeskin gourami in polyculture system.

1. INTRODUCTION

Black apple snail farming is one of the traditional aquacultures in the Mekong Delta which brings a lot of economic benefits and aids poverty reduction. In nature, snails mainly have been grown in places where there are many aquatic plants, soft soils, and a slight water flow (Pain, 1950, 1960; Andrews, 1965). According to Thao and Binh (2020), the suitable temperature for the growth of the black apple snail is between 22 and 32 °C, when the temperature increased to 37-39 °C, the growth will be decreased and can lead to high mortality. In addition, they also have the ability to live in an environment with a fluctuation of pH from 6 to 10.5 (Thao & Binh, 2018). Previous studies have identified a stocking density of 100 individuals/m² as optimal for commercial black apple snail farming (Dat, 2010; Binh et al., 2012). Binh and Thao (2017a) showed that a stocking density of 150 individuals/m² also ensures growth rates, survival rates, and economic efficiency for black apple snails. Black apple snails do not have a selective diet and eat almost everything available in their surroundings. Their feed includes vegetables, benthos algae, detritus, and organic matter. In recent years, snail farming has been growing strongly, with the model of raising black apple snails in lined tanks bringing high economic efficiency to farmers (TSVN, 2021), because of the growing demand for snails because of their high nutrition and as one of the favorite foods of Vietnamese families. They are focused on by scientists; numerous studies on black apple snails have been conducted to better understand the reproductive characteristics, improve the quality of the seed, and enhance the quality of broodstock conditioning techniques (Roff, 1992; Cowie, 2002; Darby et al., 2008; Binh, 2011; Phuc & Chu, 2014; Thao et al., 2016; Binh & Thao, 2017b; Binh et al., 2022). There is a wide range of research on the combination of feed, optimal density, and suitable substrate for snails to improve the growth rate and enhance productivity (Thao et al., 2013; Swetha & Muthukumar, 2016; Thao et al., 2014; Thao, 2015).

In terms of polyculture, Chinese farmers developed a high-yielding fish culture system based mainly on the mixed stocking of multiple fish species (polyculture) and feeding with manures and organic wastes (Lin, 1954; Tang, 1970; Bardach et al., 1972). In recent years, Vietnamese farmers have become increasingly interested in the idea of polyculture due to their economic benefits. Moreover, polyculture also improves the ecological balance of the pond water, preventing the formation of massive algal blooms (Cohen et al., 1983), saving arable land, increasing income, and allowing the use of manures as a substitute for supplemental feeds (Moav et al., 1977).

Co-culture other species that can thrive under the same conditions as the black apple snail not only diversifies the cultivated species but also has the potential to increase farmers' income. Snakeskin gourami (*Trichopodus pectoralis*) is a prime candidate for co-cultivation due to its economic value (Thanh et al., 2019) and ease of farming in different types of models (Vromant et al., 2001, Vidthayanon, 2002).

In terms of integrated aquaculture systems, it is particularly popular in the Mekong Delta under the type of VAC model, which comprises three components: a garden (V), a pond (A), and a livestock pen (C). Previous research showed that the co-culturing of black apple snails and snakeskin gourami in VAC improves pond water quality by reducing the concentration of organic matter and simultaneously increasing household incomes (Giao et al., 2019). However, existing studies have focused primarily on water quality and profitability, lacking detailed discussions on growth rates. Furthermore, there is a scarcity of research comparing the economic efficiency of fish-snail combination culture models.

This study aimed to determine the appropriate stocking densities of snakeskin gourami fish in the co-culture system with black apple snails. These results could contribute to improving the co-cultivation technique of *Pila polita* and *Trichopodus pectoralis*.

2. MATERIALS AND METHODS

2.1. Experimental design

The experiment was performed with four different density treatments of snakeskin gourami: 0 (control treatment), 3, 5, and 7 fish/m^2. Black apple snails were co-cultured in all treatments at the density of 150 snails/m^2. Each treatment was repeated three times. Snakeskin gourami and black apple snails were co-cultured in a composite tank with a volume of 500L and a water depth of 50 cm, with continuous aeration. Each tank used a combination of the water lettuce and the nylon bundle as the substrate for snails; each component was put in the tank in a 1:1 ratio and covered 0.3 m^2 of the tank surface. The water in the cultured tank was exchanged 30%, and the sediment bottom was siphoned every two days. The experimental period lasted 120 days.

The snail eggs were purchased from a hatchery in Can Tho, transported to the Laboratory of the College of Aquaculture and Fisheries, hatched into seed snails, and nursed for about 35 days for the experimental arrangement to reach the weight of 0.15 ± 0.01 g. The source fish fingerlings were also purchased at the hatchery in Can Tho and nursed to the size needed (12 ± 0.3 g) to contribute to the experimental tank.

The feeds used in the experiment were vegetable and red tilapia commercial floating feed with a protein content of 28% and a particle diameter of 0.1 - 0.2 mm and was fed twice per day (7:00 am and 17:00 pm). The snails were fed by pellet feed and vegetables according to a ratio of 50% for each in the diet. The amount of feed for each tank was provided at 3% of the biomass in each tank and adjusted depending on the needs of the cultured species. Commercial feeds for snails were spread in areas with substrate, followed by feeding vegetables 30 minutes later, while fish pellets were placed in areas without substrate after providing vegetables for snails.

2.2. Sampling and data collection

Water temperature was recorded periodically twice a day by thermometer at 7:00 a.m. and 14:00 p.m. Water environmental parameters, such as concentrations of TAN, NO2, and Alkalinity, were recorded every 15 days by SERA test kit (Germany). Temperature and pH were measured twice a day.

The sampling was conducted every 15 days for snails, while this was applied to fish at 30-day intervals throughout the experiment period. Particularly, the total number of snails was separated into 20 groups of varying sizes, each comprising five individuals of similar size. The average body weight and shell height of each group were represented by measuring three snails in that group. In addition, the shell height was measured by an electronic digital caliper (0.01 mm error), and the snail and fish weights were determined with an electronic balance (0.01 g error).

The data of the growth parameters collected were calculated according to the following formulas:

Daily weight gain, daily shell height gain, (DWG-mg/day; DSG-mm/day;) = (W2, L2 – W1, L1)/t, where: W1, L1: weight and shell height of snails at initial time of experiment; W2, L2: weight and shell height of snails at time collection sample; t: Experiment duration (days).

Specific growth rate in body weight and shell height ($SGR_{W, S}$; %/day) = (ln (W2, L2) – ln (W1, L1))/t × 100.

Survival rate (SR; %) = (Number of alive snails or fish/Initial number of stocked snails or fish) × 100.

Biomass (g/tank) = Body weight × Number of individuals in the tank.

Biomass increase rate (BIR; %) = 100 × (Biomass final – biomass initial increase)/Biomass initial.

Productivity (P; kg/m^3) = Biomass/volume of the tank

Coefficient variance (CV; %) = Standard deviation/The average number of weight and length individuals at the end of the experiment x 100

Feeding ratio (FR) = Total feed intake /Weight gain

Gonadosomatic Index (GSI) = Gonad weight x 100 / fish's body weight

Total cost (VND/m^2) = total cost of feed, breeds, chemicals

Total income (VND/m^2) = Productivity (kg/m^2) × selling price (VND/kg).

Profit (VND/m^2) = Total revenue (VND/m^2) – Total costs (VND/m^2).

Profit Margin (%) = Profit (VND/m^2)/total cost (VND/m^2) × 100.

2.3. Statistical analysis

The data were analysed for mean values, standard deviation by using Excel software, and one-way ANOVA analysis followed by Duncan post hoc test was applied to compare the significant difference of collected parameters among treatments at $p<0.05$ using SPSS program version 20.0.

3. RESULTS AND DISSCUSIONS

3.1. Water quality parameters

The mean of the water parameters was recorded in Table 1. During the experiment, the morning temperature remained between 26 to 27.2°C, and was maintained around 28 to 29°C in the afternoon. There was no significant difference in temperature among treatments ($p>0.05$). TAN and NO_2^- concentrations varied from 0.32 to 0.46 mg/L and 0.67 to 1.07 mg/L, respectively, which raised according to the density of fish because of the increase in waste matters. The highest concentration of total ammonia nitrogen (TAN) was observed in the treatment with a fish density of 7 fish/m^2, and there was no significant difference compared to the treatment with a fish density of 5 fish/m^2 and 3 fish/m^2. The control treatment (without fish stocking) displayed the lowest TAN concentration (0.32 ± 0.02 mg/L) and showed a significant difference from the other treatments ($p<0.05$), except for the treatment with 3 fish/m^2 ($p>0.05$). The concentration of NO_2^- in the treatment with 7 fish/m^2 was the highest (0.67 ± 0.02 mg/L) but did not show a significant difference from the other treatments ($p>0.05$).

The mean values of pH and alkalinity in all treatments had no significant difference ($p>0.05$). The pH levels in the experiments were between 8.17 - 8.19. Alkalinity in the experiment was maintained in the suitable range, which might not affect the growth performance of both snails and fish.

Table 1. Mean values of environmental parameters during culture period

Parameters	Density of fish (fish/m^2)			
	0	3	5	7
Morning temperature (°C)	26.5±0.5^a	26.7±0.5^a	26.7±0.5^a	26.7±0.5^a
Afternoon temperature (°C)	28.1±1.0^a	28.2±0.9^a	28.1±1.0^a	28.1±1.0^a
pH	8.19±0.02^a	8.18±0.05^a	8.17±0.03^a	8.18±0.05^a
TAN (mg/L)	0.32±0.02^b	0.38±0.03^{ab}	0.46±0.06^a	0.46±0.06^a
NO_2^- (mg/L)	0.42±0.03^a	0.51±0.12^a	0.52±0.05^a	0.67±0.02^a
Alkalinity (mgCaCO$_3$/L)	58.18±0.00^a	58.18±0.00^a	58.18±0.00^a	54.60±0.00^a

The values in the same row with different letters indicating the significant difference ($p<0.05$)

Overall, it could be seen that the environmental parameters during the culture period were generally in suitable ranges for the growth of snails and fish. However, the accumulation of uneaten feed and waste matters that grew through the experiment could cause specific adverse effects. It was also clear that with increasing fish stocking density, TAN and NO_2^- concentrations gradually rose, but the differences were insignificant ($p>0.05$) between treatments 5 and 7 fish/m^2.

3.2. Growth performance of Pila polita and Trichopodus pectoralis

3.2.1. Growth of Pila polita

After 120 days of the experiment period, the average body weight of snails in different treatments was increased significantly and reached from 4.74 to 4.97 g/snail. The control treatment (no stocking fish) resulted in the highest average snail body weight (4.97 g), insignificantly differing from treatments of 3 fish/m² and 5 fish/m² ($p>0.05$). Conversely, the treatment with the highest fish stocking density (7 fish/m²) showed the lowest snail body weight (4.74 g/snail), significantly different from the rest ($p<0.05$).

The shell height of snails increased continuous growth throughout the culture period across various treatments. By the end of the experimental period, the control treatment displayed the highest average shell height of snails (25.89 mm), with no significant difference compared to the other treatments ($p>0.05$). In contrast, the treatment with a fish density of 7 fish/m² reported the lowest average shell height (23.44 mm) and showed a significant difference ($p<0.05$) from the other treatments.

According to Table 2, the average daily weight gain of snails increased gradually during the nursing period and slightly decreased from day 76 until the end of the culture period. Notably, an increase in fish density corresponded to a decline in the average daily weight gain of snails. The control treatment exhibited the highest average daily weight gain in snails at 25.48 mg/day, and this was significantly different ($p<0.05$) from the rest. The treatment with the lowest fish density recorded a daily weight gain of 24.98 mg/day, showing no significant difference ($p>0.05$) from the density treatments of 5 and 7 fish/m².

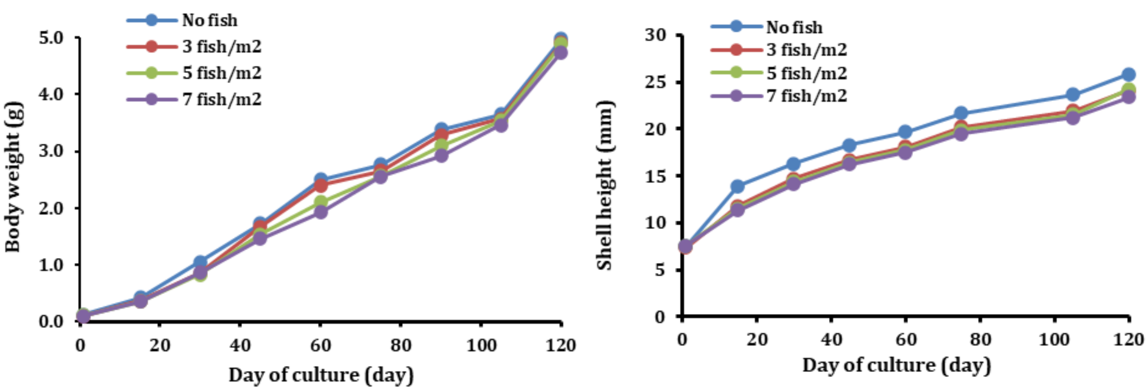

Figure 1. The body weight and shell height of snails in different treatments during the experiment period

Table 2. Daily weight gain of snail (mg/day)

Day of culture	Density of fish (fish/m^2)			
	0	3	5	7
1-15	19.06 ± 0.83^a	19.24 ± 0.97^a	19.58 ± 0.86^a	19.33 ± 0.69^a
16-30	22.23 ± 0.73^a	20.86 ± 0.55^a	19.21 ± 0.53^a	18.96 ± 0.55^a
31-45	26.56 ± 0.35^a	25.34 ± 0.22^a	25.09 ± 0.48^a	24.68 ± 0.42^a
46-60	26.85 ± 0.68^a	25.56 ± 0.51^b	25.53 ± 0.56^b	25.28 ± 0.27^b
61-75	28.60 ± 0.39^a	27.57 ± 0.25^b	27.32 ± 0.17^b	26.93 ± 0.43^b
76-90	27.53 ± 0.29^a	26.31 ± 0.61^b	26.18 ± 0.81^b	25.93 ± 0.45^b
91-105	25.77 ± 0.22^a	24.55 ± 0.32^b	24.42 ± 0.26^b	24.17 ± 0.29^b
106-120	25.07 ± 0.83^a	23.85 ± 0.64^b	23.72 ± 0.52^b	23.47 ± 0.49^b
Mean	26.48 ± 0.36^a	24.98± 0.29^b	24.75 ± 0.37^b	24.65 ± 0.25^b

The values in the same row with different letters indicating the significant difference ($p<0.05$)

Regarding the specific growth rate of snails (Table 3), this was decreased gradually all over the experiment time. The average value of this parameter was recorded as the highest in the control treatment (2.68 g/day), and significantly different from the rest ($p<0.05$). The treatment with a fish density of 3 fish/m² recorded a higher average specific growth rate of 2.58 g/day compared to the treatments with densities of 5 fish/m² (2.54 g/day) and 7 fish/m² (2.51 g/day), but there was no significant difference between these treatments ($p>0.05$).

Table 3. Specific growth rate in snail weight (%/day)

Day of culture	Density of fish (fish/m^2)			
	0	3	5	7
1-15	3.17 ± 0.12^a	3.16± 0.11^b	3.14 ± 0.09^b	3.02 ± 0.07^b
16-30	3.04 ± 0.05^a	2.96 ± 0.04^b	2.88 ± 0.08^b	2.76 ± 0.03^b
31-45	2.97 ± 0.22^a	2.91 ± 0.13^b	2.79 ± 0.11^b	2.67 ± 0.09^b
46-60	2.70 ± 0.17^a	2.65 ± 0.22^b	2.60 ± 0.12^b	2.48 ± 0.14^b
61-75	2.48 ± 0.08^a	2.44 ± 0.05^b	2.38 ± 0.07^b	2.26 ± 0.012^b
76-90	2.36 ± 0.13^a	2.28 ± 0.04^b	2.21 ± 0.09^b	2.09 ± 0.15^b
91-105	2.22 ± 0.01^a	2.14 ± 0.04^b	2.07 ± 0.03^b	1.94 ± 0.05^b
106-120	2.13 ± 0.04^a	2.05 ± 0.06^b	1.98 ± 0.07^b	1.85 ± 0.01^b
Mean	2.68 ± 0.08^a	2.58 ± 0.09^b	2.54 ± 0.07^b	2.51 ± 0.05^b

The values in the same row with different letters indicating the significant difference ($p<0.05$)

In terms of the average daily shell height gains, as presented in Table 4, the mean values gradually decreased over the entire culture period. The highest figure was recorded in the control treatment (0.35 mm/day) and differed significantly from the others ($p<0.05$). However, there was no significant difference among the remaining treatments ($p>0.05$).

Table 4. Daily shell height gain of snails (mm/day)

Day of culture	Density of fish (fish/m^2)			
	0	3	5	7
1-15	0.38 ± 0.01^a	0.37 ± 0.01^b	0.36 ± 0.01^b	0.34 ± 0.01^b
16-30	0.39 ± 0.01^a	0.36 ± 0.01^b	0.36 ± 0.01^b	0.34 ± 0.01^b
31-45	0.34 ± 0.01^a	0.32 ± 0.02^b	0.32 ± 0.02^b	0.30 ± 0.01^b
46-60	0.32 ± 0.01^a	0.31 ± 0.01^b	0.31 ± 0.01^b	0.29 ± 0.01^b
61-75	0.31 ± 0.00^a	0.30 ± 0.00^b	0.29 ± 0.00^b	0.27 ± 0.00^b
76-90	0.29 ± 0.00^a	0.28 ± 0.00^b	0.28 ± 0.00^b	0.26 ± 0.00^b
91-105	0.28 ± 0.01^a	0.27 ± 0.01^b	0.27 ± 0.01^b	0.25 ± 0.00^b
106-120	0.26 ± 0.01^a	0.25 ± 0.01^b	0.24 ± 0.01^b	0.22 ± 0.01^b
Mean	0.35 ± 0.01^a	0.29 ± 0.01^b	0.28 ± 0.00^b	0.27 ± 0.00^b

The values in the same row with different letters indicating the significant difference ($p<0.05$)

The average specific growth rates (SGR) in the shell height of the snail, as illustrated in Table 5, decreased during the experiment period. Especially, the control treatment showed the highest average value of 1.72%/day and significant differences from the remaining treatments ($p<0.05$). However, there was no significant difference ($p>0.05$) between the treatment with a fish density of 3 fish/m^2 and the treatment having fish stocking density of 5 and 7 fish/m^2.

Table 5. Specific growth rate in shell height (%/day)

Day of culture	Density of fish (fish/m^2)			
	0	3	5	7
1-15	2.53 ± 0.03[a]	2.45 ± 0.05[a]	2.40 ± 0.02[a]	2.37 ± 0.03[a]
16-30	2.36 ± 0.03[a]	2.25 ± 0.01[b]	2.22 ± 0.05[b]	2.19 ± 0.03[b]
31-45	2.00 ± 0.00[a]	1.93 ± 0.03[b]	1.90 ± 0.03[b]	1.87 ± 0.01[b]
46-60	1.81 ± 0.05[a]	1.78 ± 0.03[b]	1.76 ± 0.01[b]	1.73 ± 0.03[b]
61-75	1.67 ± 0.01[a]	1.63 ± 0.03[b]	1.62 ± 0.03[b]	1.59 ± 0.02[b]
76-90	1.55 ± 0.01[a]	1.52 ± 0.03[b]	1.51 ± 0.03[b]	1.48 ± 0.03[b]
91-105	1.50 ± 0.00[a]	1.46 ± 0.03[b]	1.45 ± 0.01[b]	1.42 ± 0.03[b]
106-120	1.38 ± 0.01[b]	1.35 ± 0.00[a]	1.34 ± 0.04[a]	1.31 ± 0.05[a]
Mean	1.72 ± 0.01[a]	1.65 ± 0.03[b]	1.63 ± 0.03[b]	1.62 ± 0.01[b]

The values in the same row with different letters indicating the significant difference ($p<0.05$)

After 120 days of the experiment, there was an insignificant difference ($p>0.05$) among treatments, and coefficient variance in weight was around 37.85 to 39.89%. The results showed the snail weight was mostly concentrated in the group of 2 to 3 g, ranging from 16.67% to 40.44%. In which the treatment of 3 fish/m^2 contributed to the lowest number of snails in this group (16.67%), while the treatment of 5 fish/m^2 had the highest number of snails (40.44%), the control treatment and the highest fish stocking density treatment had 27.05% and 30.93%, respectively.

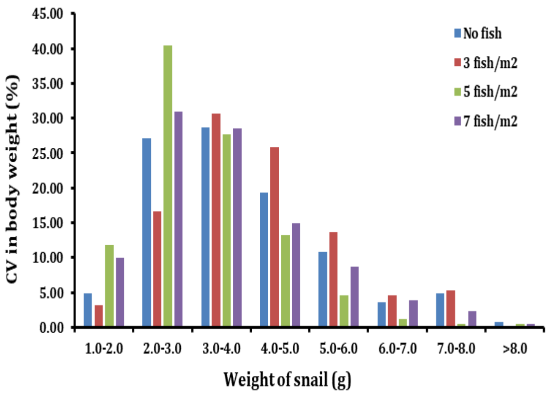

Figure 2. The coefficient variation in body weight of apple snail after 120 days

Followed by that was the group of 3 to 4 g fluctuated from 27.7 to 30.65%, and the treatment density of 3 fish/m^2 contributed the highest number of snails in this group (30.65%). The group of 4 to 5g was the third highest group, which fluctuated from 13.24% to 25.81%, and the lowest stocking density of fish was also recorded in this group, as was the highest number of snails. The rest of the weight group contributed 0.5% to 10.71%. Growth in the snail's weight in all treatments mostly reached the group from 2 to 5g at the end of the experiment.

3.2.2. *Growth of Trichopodus pectoralis*

After 120 days of culture, the weight of fish among treatments varied from 26.47 to 25.41 g/fish; but no significant difference was observed ($p>0.05$). Besides that, the body weight of fish increased continuously during the culture period. In which, the lowest weight of gourami was in the treatment of 3 fish/m^2 (26.44 g/fish) and the highest was in the treatment of 7 fish/m^2 (25.41 g/fish).

Similarly, the growth of fish after 120 days of culture at the stocking density of 3 fish/m^2 was the lowest (11 cm) compared to the other treatments but not significantly different ($p>0,05$), whereas the highest value was in the highest stocking density (11.7 cm), followed by the treatment of 5 fish/m^2 (11.4 cm).

The specific growth rate in different treatments varies from 0.93 to 0.98%/day. In particular, the highest density of fish treatment was recorded the highest specific growth day value of 0.98%/day, followed by that was the density of 5 fish/m^2 treatment with 0. 96%/day.

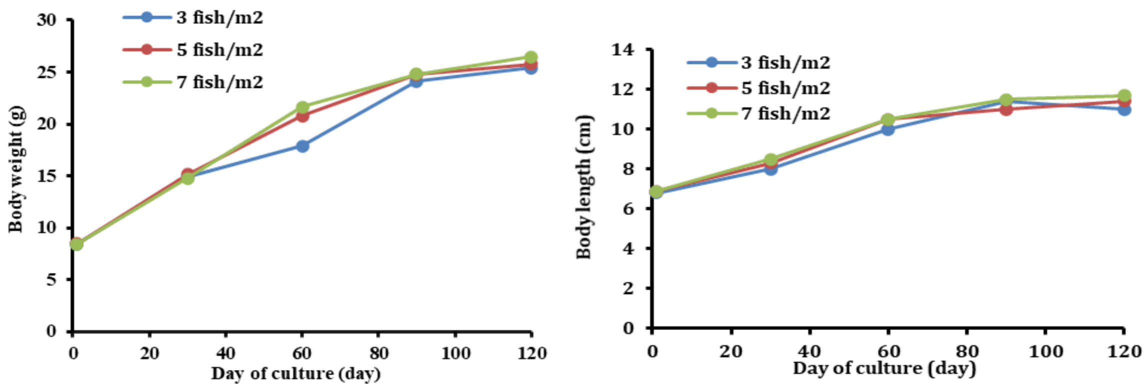

Figure 3. The weight and length of fish in different treatments during culture period

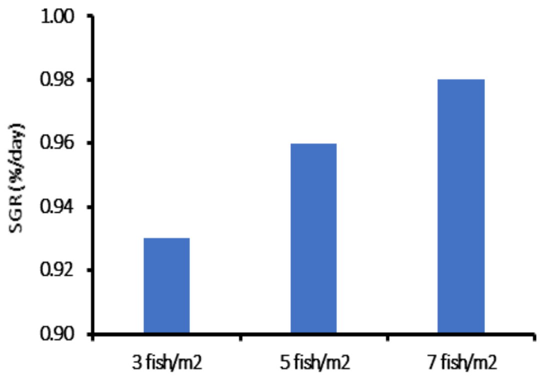

Figure 4. The specific growth rate of fish

The results showed that the gonadosomatic index (GSI) of the fish varied between treatments (5.20% - 5.48%). At the density of 7 fish/m^2, the GSI reached its highest point (5.48%), but there were no noticeable differences among treatments. The observation shows that the most of the fish gonad are developing during the culture period and still do not reach the stage ovulation gonads to ready for spawning.

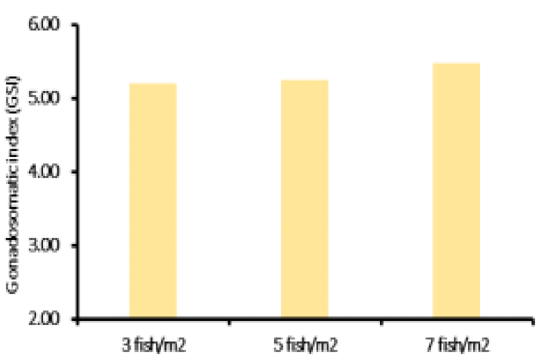

Figure 5. The gonadosomatic index of fish

3.2.3. Survival rate, biomass increasing rate, productivity, and feed ratio of black apple snail and snakeskin gourami

After 120 days of culturing, the average survival rate of black apple snails in different treatments ranged from 80.3% to 82.44%, but there was no significant difference ($p>0.05$). Conversely, increased fish densities also caused a decrease in the survival rate of snails. The average biomass increasing rate of snails reached the highest value in the control treatment (97.32%), with no significant difference compared to the treatments having fish densities of 3, 5, and 7 fish/m^2, and among each of them ($p>0.05$). The highest biomass increasing rate in fish was reported in the treatment with a density of 7 fish/m^2 at 52.98%, showing a significant difference ($p<0.05$) from the treatment with a density of 3 fish/m^2 (48.88%), while there was no significant difference ($p>0.05$) from the treatment with a density of 5 fish/m^2 (50.96%).

The average value of snail productivity was highest in the control treatment with 0.54 kg/m^2 and did not significantly differ from the remaining treatments ($p>0.05$). The highest average fish yield was recorded in the treatment with a density of 7 fish/m^2 at 0.24 kg/m^2, followed by the treatment with a density of 5 fish/m^2 (0.23 kg/m^2), with no significant difference between these two treatments ($p>0.05$), but there was a significant difference compared to the treatment with a fish density of 3 fish/m^2 (0.19 kg/m^2). The average feeding ratio (FR) of snails in all treatments was very low and insignificantly different among treatments ($p>0.05$), ranging from 0.62 to 0.67. The control treatment had the highest average number of FR (0.67), while the treatment with a fish density of 5 fish/m^2 had the lowest average number of FR (0.62). In contrast, the feeding ratio of fish in the treatments was not significantly different (p>0.05), ranging from 3.23 to 3.33. The lowest figure was observed in the treatment with a fish density of 7 fish/m^2 (3.23), but it was not significantly different (p>0.05) from the treatments with fish densities of 5 fish/m^2 (3.27) and 7 fish/m^2 (3.33).

Table 6. The survival rate (SR), Biomass increasing rate (BIR), Productivity (P), and Feeding ratio (FR) of black apple snail and snakeskin gourami

Parameters	Density of fish (fish/m^2)			
	0	3	5	7
Black apple snail				
SR (%)	82.44 ± 1.02^{a}	81.55 ± 1.68^{a}	80.44 ± 0.39^{a}	80.22 ± 1.02^{a}
BIR (%)	97.32 ± 0.99^{a}	97.11 ± 0.24^{a}	96.85 ± 0.06^{a}	96.52 ± 0.24^{a}
P (kg/m^2)	0.54 ± 0.0.1^{a}	0.53 ± 0.05^{a}	0.51 ± 0.01^{a}	0.51 ± 0.06^{a}
FR	0.67 ± 0.03^{a}	0.65 ± 0.04^{a}	0.62 ± 0.05^{a}	0.64 ± 0.08^{a}
Snakeskin gourami				
SR (%)		100	100	100
BIR (%)		48.88 ± 0.40^{b}	50.96 ± 0.56^{a}	52.98 ± 0.51^{a}
P (kg/m^2)		0.19 ± 0.01^{b}	0.23 ± 0.02^{a}	0.24 ± 0.02^{a}
FR		3.33 ± 0.03^{a}	3.27 ± 0.04^{a}	3.23 ± 0.02^{a}

The values in the same row with different letters indicating the significant difference (p<0.05)

3.3. Economic efficiency of co-culture *Pila polita* and *Trichopodus pectoralis*

The economic parameters of this experiment are presented in Table 7. The average value of total cost for the 120-day culture period ranged from 67,575 to 77,471 VND/m^2, showing a significant difference between treatments (p<0.05). Seed expenses made up most of the total cost, with the snail gained from the production facility in Can Tho at a rate of 200 VND/head and the snakeskin gourami at 700 VND/fish. Other expenses included feed, lettuce, chemicals, and supplies. Regarding the total income, this varied across treatments. Particularly, the treatment with a fish density of 7 fish/m² recorded the highest average value at 118,944 VND/m², followed by the treatment with a fish density of 5 fish/m² at 118,128 VND/m². And there was no difference between these two treatments (p>0.05). The treatment with a fish density of 3 fish/m² reported an average total income of 107,580 VND/m², showing a significant difference (p<0.05) from the control treatment, which had an average total income of 98,928 VND/m². After 120 days of culturing, snails were sold at a price of 800 VND/head. The average profits from snails were highest in the control treatment at 31,353 VND/m², followed by the treatment with a fish density of 3 fish/m² at 31,027 VND/m², showing a significant difference (p<0.05) compared to the other treatments. Subsequent treatments with fish densities of 5 fish/m² and 7 fish/m² recorded average profits from snails at 27,984 VND/m² and 27,447 VND/m², respectively, with no significant difference (p>0.05).

Table 7. Economic accounting of co-culture black apple snail and snakeskin gourami at different densities

Parameters	Density of fish (fish/m^2)			
	0	3	0	7
Total cost[1]	67,575 ± 1,026^{d}	71,985 ± 1,127^{c}	74,554 ± 1,023^{b}	77,471 ± 1,107^{a}
Total income[1]	98,928 ± 1,493^{c}	107,580 ± 1,657^{b}	118,128 ± 1,526^{a}	118,944 ± 1,784^{a}
Profits from snails[1]	31,353 ± 1,359^{a}	31,027 ± 1,210^{a}	27,984 ± 1,547^{b}	27,447 ± 1,386^{b}
Profits from fish[1]	0	4,568 ± 789^{b}	14,018 ± 1,439^{a}	15,433 ± 1,205^{a}
Profit margin[2]	45 ± 4.7^{a}	47 ± 3.8^{a}	55 ± 3.1^{b}	57 ± 3.9^{b}

The values in the same row with different letters indicating the significant difference (p<0.05)

Note: [1]unit (VND/m^2); [2]Profit margin (%)

Conversely, the treatment with a density of 7 fish/m² recorded the highest average fish profit at 15,433 VND/m², which was not significantly different (p>0.05) from the treatment with a density of 5 fish/m² (14,018 VND/m²). Meanwhile, the lowest average profit from fish was recorded in the treatment with a density of 3 fish/m² at 4,568 VND/m², showing a significant difference ($p < 0.05$) compared to the other two treatments. Regarding the profit margin, the treatment with a fish density of 7 fish/m² exhibited the highest value at 57%, which was not significantly different ($p>0.05$) from the treatment with a density of 5 fish/m², where the average profit margin was 55%. In contrast, the average profit margin for the control treatment was 45%, and the treatment with a fish density of 3 fish/m² recorded an average profit margin of 47%, with both differing significantly from the other treatments ($p<0.05$).

3.4. Discussion

According to Binh (2011), the snail could grow well in the temperature range from 27 to 30°C during the daytime. However, in other studies, the temperature for snails was 20 - 32°C, when the temperature drops below 15°C or above 40°C, snails switch to hibernation or aestivation (Lum-Kong & Kenny, 1989). The study of Cheng et al. (2023) found that warming lowered the biomass and density of freshwater snail species in the co-habitat fish culture system (*R. swinhoei*) compared to the fish-absent system by 64.4% and 92.5%, respectively. The temperature data in this study was similar to that of the research on black apple snails on varied substrates (Thao, 2012). In our study, the temperature remained in the suitable temperature range (25 - 27°C), therefore it did quietly not affect them. Thus, the experimental temperature was in a sufficient range and could not affect the growth of snail in this experiment.

In other studies, the allowable NO_2^- concentration in aquaculture did not exceed 10 mg/L (preferably less than 2 mg/L) (Boyd, 1998). A study by Dat (2010) on *Pila polita* showed that the NO_2^- can fluctuate between 0.3 - 1.0 mg/L. The result in our study was higher than the experiment of Binh and Thao (2017a), which had an average NO_2^- concentration of 0.45 mg/L, because the feed used in this experiment had a higher protein value (28% versus 18%) and the level of NO_2^- gradually increased as fish density increased. Findings from previous studies showed that surpassing the ideal density of aquatic animals in a specific cultured region results in a substantial elevation of the TAN level, attributed to heightened feed input and waste accumulation in ponds (Al-Harbi & Siddiqui, 2000; Lili et al., 2022). This explains the increase in TAN concentration when increasing the stocking density of snakeskin gourami. In this study, the NO_2^- and TAN level stayed at a suitable level for the growth of fish and snail. According to Linh (2011), in the grow-out culture, the pH level ranged from 7.1 to 8.4, which did not affect to the growth rate of *Pila polita*. Snakeskin gourami also had the peribranchial organ which helped them survive in the eutrophication environment with the pH low around 4.5 to 5 (Biswas, 1993). In addition, the suitable alkalinity level for aquatic species was 50-150 mg/L (Boyd, 1998). The process of increasing carbon dioxide concentration could interfere with gas exchange. According to Nancy and Philip (2009), the calcium concentration had a critical effect on the growth of snails, when the calcium concentration in the environment decreased led to the interruption of shell development, so when the pH of the environment was low, the growth rate in both shell and body weight would decrease.

These growth features were consistent with the body structure of snails because the increase in weight will include both the meat and the shell maintaining normal life functions. Cheng et al. (2023) illustrated that the predation and competition of giant gourami caused reduced biomass and negative effects to the growth rate of two freshwater snail species (*Radix swinhoei* and *Bellamya aeruginosa*). However, snakeskin gourami was less aggressive than the giant gourami, therefore, their impact on the black apple snail was limited in both the competition in food sources and the threat of predation. According to Thao and Chinh (2016), when increased the number of golden apple snails the survival rate of black apple snails decreased (71.1 - 79.4%) after 60 days of culture. Because of the golden apple snail, the competitor of black apple snail, therefore the stronger would be dominant. The results of this experiment show that the survival rate of snails is quite high compared to previous commercial culture studies, in which, the survival rate in the treatment of vegetable and artificial feed combination to cultured black apple snails reached 64.5% - 71.9% (Binh and Thao, 2017b). According to Haque et al. (2014), substrate played an important role in the growth rate and survival rate of freshwater snails in co-habitat with fish species, because of their helpful abilities such as creating a place for snails to cling to hide from enemies or rest, catching the feed supply from outside on the substrate, or enhancing the availability of natural food by improving the water quality and supplying dissolved oxygen.

In addition, the highest growth rate of fish in the treatment with stocking density of 7 fish/m^2 could be explained because of the feeding habit of snakeskin gourami, which can eat both suspended matter and particles (Tacon et al., 2011), the higher the fish density, the more feed sources and organic matter were added to the culture tank. In another case, the study's result of Setijaningsih et al. (2018), showed that the optimal stocking density of snakeskin gourami in commercial pond and in brackish water condition (3 ppt) was extremely important to adapt for the survival rate and the growth rate of the fish. Particularly, the highest survival rate and specific growth rate of fish were observed at a treatment stocking density of 3 fish/m^2, with 93.47% and 1.22 %/day, respectively. In comparison, the survival rate was higher (100% in all treatments) and lower than that of the specific growth rate (0.92 - 0.98%/day), because of the long culture period of 120 days and different cultured environment. The research conducted by Binh and Thao (2014) indicates that as the stocking density of black apple snails in farming increases from 150 snails/m^2 to 200 snails/m^2, the profit margin decreases from 16.4% to 12.2%. This decline is attributed to the fact that if the snail density increases beyond a level suitable for the farming system's area, it adversely affects the growth ability and survival rate, resulting in a reduction of both the profit margin and the overall profit of the farming system. Therefore, the stocking density of snails in this study is appropriate for optimal snail growth. Introducing additional farming species like snakeskin gourami to the model serves to diversify income sources, contributing significantly to the enhancement of profit margins. The FR was not very high from 2.33 to 2.38 in comparison with the recorded in the previous study by Thanh et al. (2019), which had the FR in both selected breeds (2.12) and non-selected breeds (2.29) after 210 days of the experiment. According to Dat (2010), the FR of black apple snails ranged from 1.85-5.59 during 4 - 5 months of culture. Linh (2011) obtained FR results ranging from 2 to 5 when culturing black apple snails for 75 days. The reason for this was that the fish were mature earlier than expected, so they had to stop growing to focus their energy on maturation.

4. CONCLUSION AND RECOMMENDATION

4.1. Conclusions

The average body weight of the snails (4.97 g) and their shell height (24.50 mm) in the control treatment were the highest, although there was no significant difference (p>0.05) compared to the other treatments.

The highest profit margin was recorded in the treatment with a fish density of 7 fish/m^2, and this was significantly different (p<0.05) compared to both the control treatment and the treatment with a fish density of 3 fish/m^2.

Co-culture black apple snails and snakeskin gourami at a density of 5 fish/m^2 ensures better snail growth than a density of 7 fish/m^2 and yields a higher profit margin compared to a density of 3 fish/m^2.

4.2. Recommendations

Polyculture of black apple snails and snakeskin gourami densities of 5 fish/m^2 contributes to improved productivity without affecting the growth rate of snails and fish. Further research is needed under real production conditions and on a larger scale, such as farming in earthen ponds or in hapa nets.

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