Effects of different stocking densities on growth and survival rate of Dau Nhim snakehead (*Channa* sp.) fingerlings

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Abstract. Dau Nhim snakehead (*Channa* sp.), a variant strain of the black snakehead (*Channa striata* Bloch, 1793), has been contributing significantly to freshwater farming in Vietnam. This study was conducted to determine the suitable stocking density of the fingerling stage of the Dau Nhim snakehead in order to supply breeding sources for farming. The fish (mean weight of 4.62 g) were randomly stocked at four densities of 50, 100, 150 and 200 ind m^{-3} with three replicates in 12 net hapas in an outdoor concrete tank and satisfactorily fed twice a day with pellet feed (35% crude protein) for 56 days. The fish growth performance was insignificantly different among the stocking densities, during the first 28 culturing days ($p>0.05$). Data at the experiment end showed a reduction in culturing efficiency with an increasing stocking density. In particular, the growth performance at 100 ind m^{-3} was significantly higher than that at 150 and 200 ind m^{-3} ($p<0.05$), while the feed conversion ratio (FCR) and the feed cost per production unit at the density of 100 ind m^{-3} were significantly lower ($p<0.05$). The survival rate ranged from 89.86 to 94.20% and no significant difference was found between stocking densities of 100 and 200 ind m^{-3} ($p>0.05$). All of the observed parameters had insignificant differences between stocking densities of 50 and 100 ind m^{-3} ($p>0.05$) during the experimental period. The present study indicated that 100 ind m^{-3} was the most suitable density for growing Dau Nhim snakehead fingerlings. It is recommended to culture Dau Nhim snakehead fingerlings through two stages, the first four weeks with a stocking density of 150–200 ind m^{-3}, followed by 100 ind m^{-3} in the coming weeks.

Key Words: *Channa* genus, variant strain, hapa, feed conversion ratio, Vietnam.

Introduction. Vietnam, as a country, possesses a great potential for aquaculture, with a total water surface area of more than 1.7 million ha, and is currently one of the largest producers of aquaculture products worldwide (VASEP 2019). In 2019, aquaculture products reached over 54.31% of the total nation's fishing sector output of 8.27 million tons, of which cultured fish accounted for 69.86%. Due to its advantageous conditions in climate and water surface, aquaculture in the Mekong delta region of Vietnam has been strongly developed for a long time. In 2019, this region accounted for 72% of the nation's aquaculture area with 70% of production, of which 71% of fish (Tri et al 2021). Black snakehead (*Channa striata*, Bloch 1793) is a freshwater species that is naturally encountered throughout Asia and is commercially raised in many countries in Asia, such as Thailand, Cambodia, China, Hong Kong and Taiwan (Pillay 1990). In Vietnam, black snakeheads have been traditionally cultured in freshwater regions and have crucially contributed to national aquaculture (Hien et al 2015; Nguyen & Duong 2016). Recently, two new phenotypes of the *Channa* genus have been discovered in the Mekong Delta, Vietnam (the local names are Dau Nhim snakehead/triangle-head snakehead and square-head snakehead), which possess a similar body shape and appearance to wild black snakeheads. They have been popularly farmed for commercial purposes in this area. A taxonomic study based on morphological characteristics and Cytochrome C oxidase subunit I (COI) for these two new phenotypes of the *Channa* genus was conducted by Nguyen & Duong (2016), who revealed that they were morphological variants of wild black snakehead. Although black snakeheads, Dau Nhim snakeheads, and square-head snakeheads are difficult to differentiate by eye in terms of body shape and appearance, they are different in morphometric ratios, the shape of their heads and the length of their guts. It is believed that wild black snakehead species has been a target of aquaculture in Vietnam for about 20 years; perhaps differences in living environments and feeding types between cultured and wild conditions are the major factors that result in morphological variation among their populations.

Dau Nhim snakehead is known to have numerous superior characteristics of the *Channa* genus, such as a high quality of meat, a large size, a fast growth rate, a wide food spectrum and a good environmental adaptability to adverse conditions, especially an excellent endurance in low oxygen conditions because of possessing gills as an accessory respiratory organ (Ngo & Thai 2003), and consuming pellet feed (Huynh 2004). As a result, farmers in the Mekong Delta commercially culture at high density in many intensive farming systems, including earth ponds, nylon tanks, cement tanks, composite tanks and cages for high productivity (Mai et al 2019).

In culturing aquatic species, besides feeding sufficient quantity and quality feed, optimal stocking density at different growth stages of the animals for each farming model is really important to increase production (Jerez-Cepa & Ruiz-Jarabo 2021; Álvarez et al 2020; Jerez-Cepa et al 2020). Stress deriving from stocking density in aquaculture may result in spent energy for homeostasis and restoration of tissue and immune systems, which affect the culturing productivity. In addition, cannibalistic behavior in predatory fish such as snakehead may decrease the growth and survival of farmed fish (Garr et al 2011; Mazlum 2007; Zhu et al 2011). Previous research on technical aspects of the Dau Nhim snakehead culture has been conducted (Lam et al 2009; Le et al 2017; Mai et al 2019), however, studies on fingerlings are currently scarce. The present study aimed to find a suitable stocking density for the culture of the Dau Nhim snakehead at the fingerling stage, with the culturing model of the hapa placed in the cement tank. As a result, there will be recommendation for optimal technical aspects for the farming of this fish strain, which is expanding in Vietnam.

Material and Method

Source and acclimatization of experimental fish. Dau Nhim snakehead (*Channa* sp.) fingerlings were purchased from a local private shrimp hatchery and held in aerated composite tanks (1 m^3) upon arrival at the Wet Laboratory of Tien Giang University, Vietnam. The fish were acclimatized to the experimental conditions for one week, during which the fish were fed with pellets.

Experimental setup and management. The study was conducted at Tien Giang University, Vietnam from March to May 2019. The experiment was randomly designed with three replicates in 12 net hapas (0.8×0.8×0.9 m), installed in an outdoor cement tank (3.0×2.8×1.0 m). The acclimatized snakehead fingerlings (mean weight of 4.62 g, mean total length of 7.83 cm) were stocked in net hapas at four densities of 50, 100, 150 and 200 ind m^{-3}, and provided with continuous aeration. The fish were satisfactorily fed with floating artificial pellets (35% crude protein; energy 2.8 Kcal g^{-1}) twice a day (at 7:00 AM and 2:00 PM). Water exchange was carried out every three days with 20–30% of the tank volume. The experiment lasted for 56 days.

Data collection*.* Growth parameters such as mean weight, mean length, weight gain, length gain, as well as daily gain of weight and length were collected every fortnight. At that time, ten fingerlings from each hapa (30 of each stocking density) were weighed using an electrical analytical balance calibrated to 0.01 g and measured on a divided ruler of a millimeter unit.

The data for the calculations on survival rate, feed conversion ratio, coefficient of variation and feed cost per unit production were collected at the end of the experiment.

Water quality was checked prior to fish stock and during the culture period. Parameters of pH, temperature and dissolved oxygen were measured twice a day (at 7:00 AM and 2:00 PM) by a sensor meter. Water samples were collected once a week to determine nitrite and ammonium concentrations using standard water sampling and analyzing protocols as outlined by APHA (1995).

Data calculation. The monitored data of experimental fish consist of weight gain, length gain, daily weight gain, daily length gain, survival rate, feed conversion ratio, coefficient of variation, and feed cost per production unit, calculated using the following equations:

- Weight gain = final weight – initial weight;
- Length gain = final length – initial length;
- Daily weight gain (g day^{-1}) = (final weight – initial weight)/experiment days;
- Daily length gain (cm day^{-1}) = (final length – initial length)/experiment days;
- Survival rate (%) = (Final fish number/Initial fish number) × 100;
- Feed conversion ratio - FCR = total dry feed fed (g)/total wet weight gain (g);
- Coefficient of variation - CV (%) = the standard deviation/the mean weight or length × 100;
- Feed cost per production unit (1,000 USD kg^{-1}) = (amount of feed used by fish × unit price)/(final mean weight – initial mean weight).

Statistical analysis. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software for Windows version 20.0. All data were analyzed by one-way analysis of variance (ANOVA), followed by the Duncan test, to identify significant differences between mean values, at a significance level of $p<0.05$.

Results. The fortnightly recorded data on the growth performance of Dau Nhim snakehead at four stocking densities are presented in Figure 1 and Figure 2, and Table 1 and Table 2, which revealed that stocking density had no considerable effect on the fish's growth performance during the first 28 culturing days. After 14 days of culture, the fish reached a mean weight of 7.96–8.09 g ind^{-1} with a weight gain ranging from 3.34 to 3.43 g ind^{-1} and a daily weight gain of 0.24 g day^{-1}. The mean length of fish was 9.37–9.49 cm ind^{-1} with a length gain of 1.62–1.73 cm ind^{-1} and a daily length gain of 0.11–0.12 cm day^{-1}, all of which were insignificantly different among the stocking densities ($p>0.05$). The same growth pattern was observed in the following two culturing weeks. After 28 day culture, the fish achieved a mean weight of 16.22–16.39 g ind^{-1} with a weight gain of 8.16–8.34 g ind^{-1} and a daily weight gain of 0.59 g day^{-1}. The mean length was 11.52–11.60 cm ind^{-1} with a length gain of 2.03–2.22 cm ind^{-1} and a daily length gain of 0.15–0.16 cm day^{-1}. There were insignificant differences in the monitored parameters between the culturing densities. From day 42 onwards, the growth performance was closely related to the stocking densities. On day 42 of the trial, the mean values of weight (31.56–34.44 g ind^{-1}), length (13.52–14.20 cm ind^{-1}), weight gain (15.30–18.19 g ind^{-1}), length gain (2.01–2.63 cm ind^{-1}), daily weight gain (1.09–1.30 g day^{-1}) and daily length gain (0.14–0.20 cm day^{-1}) were measured. Statistical analysis indicated significantly higher growth performances of fish stocking at 100 ind m^{-3} compared to those at 150 and 200 ind m^{-3} ($p<0.05$), but no significant differences were found between the stocking densities of 50 and 100 ind m^{-3} or 150 and 200 ind m^{-3}. The same growth pattern lasted to the end of the culture period, with the values of mean weight of 48.55–53.54 g ind^{-1}, mean length of 15.63–17.02 cm ind^{-1}, weight gain of 17.12–19.09 g ind^{-1}, length gain of 2.08–2.82 cm ind^{-1}, daily weight gain of 1.21–1.37 g day^{-1} and daily length gain of 0.14–0.20 cm day^{-1}.

At the termination of the experiment, the fish survival rates were determined, demonstrating a higher survival rate in lower stocking densities. Fish survival was the highest at 50 ind m^{-3} (94.20%) and significantly higher than those stocking at 150 and 200 ind m^{-3} (90.82% and 89.86%, respectively), but there was no significant difference compared to the density of 100 ind m^{-3} (92.03%) (Table 3).

The feed conversion ratio (FCR) ranged from 1.26 to 1.42, which proportionally increased with the stocking densities. The FCRs at 50, 100, 150 and 200 ind m^{-3}

were 1.26, 1.27, 1.34 and 1.42, respectively, with a significant difference detected among the three highest densities ($p<0.05$). No significant difference was found between the stocking densities of 50 and 100 ind m^{-3} ($p>0.05$) (Table 3).

The coefficient of variation on the final weight and final length deviated from the rules and insignificantly differed among the four stocking densities ($p>0.05$), ranging from 19.93±0.05 to 20.54±1.48 and 5.61±0.51 to 6.99±0.48%, respectively (Table 3).

Following the same pattern with FCR, the feed cost per unit of production in four stocking densities (50, 100, 150 and 200 ind m^{-3}) rose with an increasing stocking density ($p<0.05$), its respective values reaching 0.83, 0.83, 0.88 and 0.94 USD kg^{-1}. Statistical analysis revealed a significant difference among three stocking levels: 100, 150 and 200 ind m^{-3} (Table 3).

The water quality parameters in the cement tank varied within a suitable range for the survival and growth of the snakehead fingerlings and they were insignificantly different among the treatments, during the experimentation period: a temperature range of 29.20–30.03ºC, a pH range of 8.00–8.21, a dissolved oxygen range of 3.74–4.15 mg L^{-1}, a nitrite range of 0.03–1.55 mg L^{-1} and an ammonium range of 0.02–1.00 mg L^{-1}.

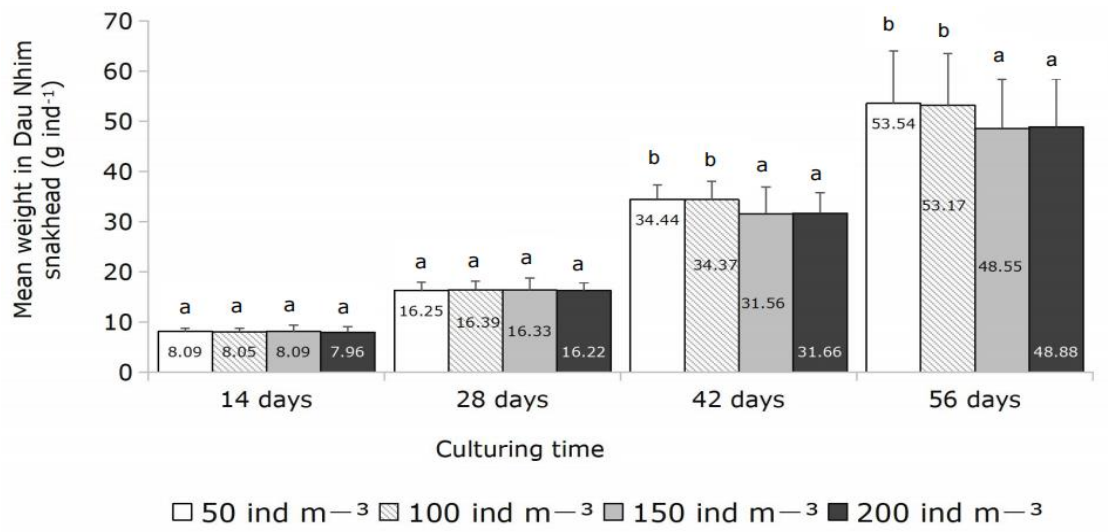

Figure 1. Mean weight of the fish for the four stocking densities during the experimental period.

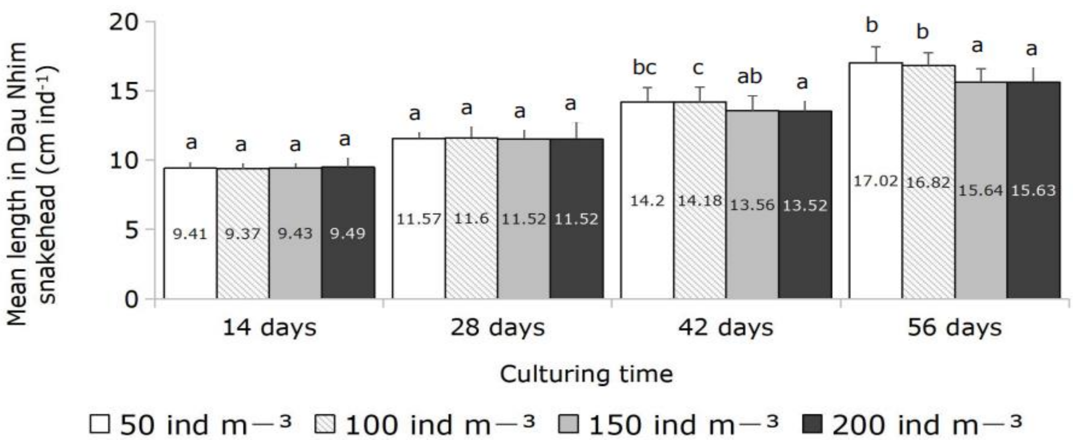

Figure 2. Mean length of fish for the four stocking densities during the experimental period.

Rows in the same sampling time with different superscript letters (a, b, c) in Figures 1 and 2 show a significant difference ($p<0.05$).

Table 1

Weight gain and daily weight gain of the fish

Experimental time	*Stocking densities (ind m^{-3})*			
	50	*100*	*150*	*200*
	Weight gain (g ind^{-1})			
14 days	3.43±0.04^{a}	3.42±0.06^{a}	3.40±0.09^{a}	3.34±0.09^{a}
28 days	8.16±0.09^{a}	8.34±0.04^{a}	8.24±0.07^{a}	8.26±0.08^{a}
42 days	18.19±0.13^{b}	17.98±0.39^{b}	15.30±0.06^{a}	15.44±0.12^{a}
56 days	19.09±0.60^{b}	18.80±0.48^{b}	17.12±0.20^{a}	17.22±0.10^{a}
	Daily weight gain (g day^{-1})			
14 days	0.24±0.01^{a}	0.24±0.01^{a}	0.24±0.01^{a}	0.24±0.01^{a}
28 days	0.59±0.01^{a}	0.59±0.01^{a}	0.59±0.01^{a}	0.59±0.01^{a}
42 days	1.30±0.01^{b}	1.29±0.03^{b}	1.09±0.01^{a}	1.10±0.01^{a}
56 days	1.37±0.04^{b}	1.34±0.04^{b}	1.21±0.01^{a}	1.22±0.01^{a}

Values are presented as mean±SE. Values with different superscript letters (a. b. c) in the same rows show a significant difference ($p<0.05$).

Table 2

Length gain and daily length gain of the fish

Experimental time	*Stocking densities (ind m^{-3})*			
	50	*100*	*150*	*200*
	Length gain (cm ind^{-1})			
14 days	1.62±0.15^{a}	1.62±0.11^{a}	1.62±0.03^{a}	1.67±0.03^{a}
28 days	2.16±0.10^{a}	2.22±0.05^{a}	2.09±0.10^{a}	2.03±0.15^{a}
42 days	2.63±0.13^{b}	2.58±0.03^{b}	2.04±0.18^{a}	2.01±0.21^{a}
56 days	2.82±0.17^{b}	2.64±0.05^{b}	2.08±0.10^{a}	2.11±0.32^{a}
	Daily length gain (cm day^{-1})			
14 days	0.12±0.01^{a}	0.12±0.01^{a}	0.11±0.01^{a}	0.12±0.01^{a}
28 days	0.15±0.01^{a}	0.16±0.01^{a}	0.15±0.01^{a}	0.15±0.01^{a}
42 days	0.20±0.02^{b}	0.19±0.01^{b}	0.14±0.02^{a}	0.14±0.01^{a}
56 days	0.20±0.02^{b}	0.19±0.01^{b}	0.14±0.01^{a}	0.15±0.02^{a}

Values are presented as mean±SE. Values with different superscript letters (a, b, c) in the same rows show a significant difference ($p<0.05$).

Table 3

Survival rate, feed conversion ratio, coefficient of variation and feed cost per unit production (Mean±SE)

Stocking densities (ind m^{-3})	*50*	*100*	*150*	*200*
Survival rate (%)	94.20±1.45^{b}	92.03± 0.73^{ab}	90.82±0.96^{a}	89.86±0.36^{a}
Feed conversion ratio	1.26±0.01^{a}	1.27±0.01^{a}	1.34±0.01^{b}	1.42±0.01^{c}
Final weight CV (%)	20.54±1.48^{a}	19.93±0.55^{a}	20.53±0.53^{a}	19.84±0.52^{a}
Final length CV (%)	6.77±1.29^{a}	5.61±0.51^{a}	5.94±0.76^{a}	6.99±0.48^{a}
Feed cost per unit production (USD kg^{-1})	0.83±0.01^{a}	0.83±0.00^{a}	0.88±0.01^{b}	0.94±0.01^{c}

Values are presented as mean±SE. Values with different superscript letters (a, b, c) in the same rows show a significant difference ($p<0.05$).

Discussion. Inadequate stocking densities are most likely the source of ongoing stress for farmed teleost species (Jerez-Cepa & Ruiz-Jarabo 2021). Stress responses can be induced by both low (Álvarez et al 2020) or high (Jerez-Cepa et al 2020) stocking densities. This effect directly impacts the consumption of energy and stored metabolites in muscle and can also modulate immune responses (Yang et al 2020; Jia et al 2016), which has long-term negative influences on fish health and growth (Jia et al 2016). Besides, the territoriality of fish tends to increase in high density and may result in cannibalism (Hecht & Pienaar 1993). The snakehead fish is a cannibalistic species that consume parts or the entire body. It potentially affects aquaculture production (Folkvord 1997). A snakehead juvenile can catch specimens of its own species when the size variance is 60–80% (Srivastava et al 2012). Furthermore, crowding may cause the fish to move for catching the feed and to compete for it (Stickney 1994). All of the above influence the growth performance and survival of the snakehead reared at high density (Rahman et al 2011; Qin & Fast 1998). The present study shows that the growth performance, feed conversion ratio and feed cost per product unit were related to the stocking densities.

During the first 28 rearing days, when fish were small, the crowding stress, competition for feed and living space were not yet significantly different. Therefore, there were no significant differences in the growth characteristics of the fish (mean weight, mean length, weight gain, length gain, daily weight gain and daily length gain) among the stocking densities ($p>0.05$). From day 42 to the end of the experiment, as the size of the fish (weight and length) increased at each hapa, their living space was reduced, leading to an increase in feeding competition, physiological stress and cannibalizing habits. The feed conversion ratio indicates the rate of feed amount converted into a production unit (1 kg weight gain in farmed fish). The lower the feed conversion ratio, the fewer the feed required to achieve 1 kg gain in fish biomass. It was also noticed that stress conditions caused by crowding induced a supplementary energy expenditure for body balance (Yeh et al 2010; Leland et al 2013; Pederzoli & Mola 2016). In a stressful condition, a higher metabolic energy consumption occured in respiration, movement, tissue recovery and hydromineral regulation processes (Arifin et al 2014), resulting in a poor feed energy conversion efficiency for fish development. In the present study, the FCR values in two lower stocking density treatments (50 and 100 ind m^{-3}) were rather similar ($p>0.05$), but both recorded significantly higher values than those in the higher densities of 150 and 200 ind m^{-3}. Significant differences in FCR values were also found between densities of 150 and 200 ind m^{-3} ($p<0.05$) (Table 3). The FCR variation in this study was in agreement with Amin et al (2015), Saputra et al (2018) and Mai et al (2019), who all recorded higher FCR in higher stocking densities. In contrast to FCR, the growth parameters of fish (weight and length) decreased when stocking densities increased. The growth values (mean weight, mean length, weight gain, length gain, daily gain of weight and length) at 50 and 100 ind m^{-3} were significantly higher from those of 150 and 200 ind m^{-3} ($p<0.05$), but no significant differences were found between the densities of 50 and 100 ind m^{-3} ($p>0.05$) (Figures 1 and 2, Tables 1 and 2).

The FCR was proportionally related to the feed cost per unit of production. The high FCR may result in an increase in the feed cost per unit production. This study on Dau Nhim snakehead also discovered that the feed cost per unit of production tended to increase with density. There was a significantly lower feed cost per unit production in the densities of 50 and 100 ind m^{-3} compared to 150 and 200 ind m^{-3}, while its value was significantly higher at 200 ind m^{-3} ($p<0.05$). However, there was no significant difference between the densities of 50 and 100 ind m^{-3} (Table 3) ($p>0.05$).

The survival rate of Dau Nhim snakehead in this study was rather high (89.86–94.20%) and tended to slightly decrease with increased density. The highest survival rate was observed in the lowest density treatment (50 ind m^{-3}), which was significantly different from other groups ($p<0.05$), though no significant difference was found among the three higher densities ($p>0.05$) (Table 3). This could be explained by the capability of snakeheads to thrive in crowding conditions and low dissolved oxygen concentration, owing to their accessory respiratory organ. Our results conformed to the study data of Lam et al (2009), for nylon tanks, of Mai et al (2019), for composite tanks, and of Saputra et al (2018), for recirculation systems, who found that the survival rate of the snakehead appears to be unrelated to the stocking density. Qin & Fast (1998) indicated that the feeding regime was one of the main causes of mortality, due to insufficient feed, in snakehead farmed at high stocking densities. In the study of Amin et al (2015), poor water quality is also a factor that increases the mortality rate in snakeheads. However, in the present study, experimental specimens were adequately fed, based on their demand, and water quality parameters were kept within a suitable range for a normal growth of snakehead. This was supported by the high survival rate and an insignificant difference in the coefficient of variation of the final weight and length among the four stocking densities (Table 3). The data of water quality parameters showed that the values of nitrite and ammonium increased gradually as the experiment progressed. However, important water factors such as pH, temperature, dissolved oxygen concentration, nitrite and ammonium varied within a suitable range for the survival and growth of the snakehead (Mat Jais et al 1997; Lam et al 2009; Rahman et al 2011). Therefore, the recorded data of the experimental fish is almost unaffected by the environmental factors.

Conclusions. The high stocking densities adversely affected the growth performance, survival rate, feed conversion ratio and feed cost per unit production of Dau Nhim snakehead fingerlings. The stocking density of 100 ind m^{-3} was recommended as suitable for culturing *Channa* sp. (Dau Nhim snakehead) fingerlings in a two-stage system, the first period with stocking densities of 150–200 ind m^{-3}, and the next period with a stocking density of 100 ind m^{-3}.

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Conflict of interest. The authors declare no conflict of interest.

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