CREATIVE SCIENCE

Journal home page: csj.snru.ac.th

Optimizing stocking density for enhanced growth and survival of a cyprinid fish (*G. theunensis*) fry in cage cultivation environment

Ekachai Duangjai[1*], Jittra Punroob[2], Natthawoot Punroob[2]
[1]Faculty of Science and Agriculture Technology, Rajamangala University of Technology, Lanna, Nan, 55000 Thailand
[2]Faculty of Business Administration and Liberal Arts Rajamangala University of Technology Lanna Tak, 63000 Thailand
[*]**Corresponding Author:** ekachai@rmutl.ac.th
DOI: 10.55674/cs.v17i2.259896

Received: 11 November 2024 **Revised:** 10 March 2025; **Accepted:** 11 March 2025; **Available online:** 16 March 2025

Abstract

Parameters	Trt1 (50 fry/m³)	Trt2 (100 fry/m³)	Trt3 (150 fry/m³)	Trt4 (200 fry/m³)	F/Pr > F
Initial length (cm)	3.80±0.01ᵃ	3.79±0.02ᵃ	3.78±0.01ᵃ	3.78±0.01ᵃ	0.65/0.58
Final length (cm)	5.93±0.03ᵃ	5.40±0.05ᵇ	5.19±0.06ᶜ	5.03±0.06ᵈ	38.48/0.00
Initial weight (g)	0.70±0.00ᵃ	0.69±0.00ᵃ	0.69±0.00ᵃ	0.69±0.00ᵃ	67.60/0.35
Final weight (g)	4.88±0.09ᵃ	4.08±0.09ᵇ	3.92±0.09ᵇ	3.90±0.09ᵇ	17.81/0.00
Average Daily Gain (ADG) (g/fish/day)	0.05±0.00ᵃ	0.04±0.00ᵇ	0.04±0.00ᵇ	0.04±0.00ᵇ	32.26/0.00
Specific Growth Rate (SGR) (%/fish/day)	157.95±32.63ᵃ	140.78±2.62ᵇ	135.99±2.65ᵇ	134.60±2.42ᵇ	15.74/0.00
Weight Gain (%)	612.77±18.75ᵃ	496.95±15.46ᵇ	471.51±14.54ᵇ	467.19±13.65ᵇ	124.78/0.00
Feed Conversion Ratio (FCR)	2.34±0.00ᶜ	2.86±0.09ᵇ	3.01±0.07ᵃ	3.14±0.12ᵃ	18.36/0.00

The impact of stocking densities on the growth and survival of a cyprinid fish (*G. theunensis*) fry was thoroughly investigated in a comprehensive study aimed at identifying optimal aquaculture conditions. This research utilized a Complete Randomized Design (CRD) with three replicates for each of four different stocking densities: 50 100 150 and 200 fry m^{-3} over a three-month period from October to December 2024. Fish were fed a commercial fish feed containing 28% protein twice daily. The study found no significant differences (P>0.05) in the survival rates across the different densities with rates ranging from 73.83% to 82.67%. However, the lowest density (50 fry m^{-3}) resulted in a significantly higher final length of 5.93 cm, compared to 5.40 cm, 5.19 cm, and 5.03 cm for the 100, 150, and 200 fry m^{-3} densities, respectively. Similarly, fish at the 50 fry m^{-3} density achieved a significantly higher final weight of 4.88 g, compared to 4.08 g, 3.92 g, and 3.90 g for the higher densities. The daily gain was highest at the lowest density, measuring 0.05 g $fish^{-1}$ day^{-1}, versus slightly lower gains at higher densities. Specific growth rate: The rate at 50 fry m^{-3} was 157.95% $fish^{-1}$ day^{-1}, substantially higher than those observed at higher densities. Weight Gain: Demonstrated a similar pattern, with the 50 fry m^{-3} density achieving a 612.77% gain, significantly outperforming the other densities. Feed Conversion Ratio: The FCR was most efficient at the lowest density (2.34) worsening progressively at higher densities.

Keyword: *Garra theunensis*; Stocking density; Cyprinid fish; Growth performance

1. Introduction

Despite its crucial ecological role, there's a notable gap in our knowledge regarding the growth and survival rates of a cyprinid fish (*G. Theunensis*) a species native of the upper Nan river basin in Nan province, Thailand. Understanding its distribution patterns provides insights into its ecological and evolutionary history, highlighting the importance of deeper insights for conservation efforts aimed at protecting fish populations and their habitats [1,2]. Discovered by Shibukawa *et al.* [3], *G. theunensis* enriches the diverse ichthyological landscape of the Mekong River basin, renowned for its abundant aquatic biodiversity. Thriving in clear, swiftly flowing rivers with rocky substrates, *G. theunensis* demonstrates a preference for such environments. Within its ecological niche, *G. theunensis* exhibits a proclivity for habitats abundant in vegetation and food sources. This species plays a pivotal role in maintaining the ecological balance of aquatic ecosystems by contributing to nutrient cycling and regulating the populations of aquatic flora and fauna [3,4]. Despite its ecological significance, *G. theunensis* confronts a plethora of threats to its survival, chief among them being habitat destruction driven primarily by agricultural expansion, dam construction, and deforestation. Additionally, unsustainable fishing practices and overfishing exacerbate the decline of fish populations while pollution stemming from agricultural runoff and mining activities further jeopardizes their well-being by compromising water quality and directly impacting their health and reproductive success [4,5]. The initiation of conservation endeavors targeting *G. theunensis* and its habitats represents a critical step toward ensuring the persistence of this species for future generations. An essential aspect of conservation efforts involves habitat restoration projects designed to rehabilitate degraded aquatic environments and reinstate critical habitats for *G. theunensis* [5,6]. These strategies may include implementing stringent regulations on industrial discharges, promoting sustainable agricultural practices to minimize runoff, and conducting regular monitoring of water quality parameters to identify and address pollution hotspots [5]. However, the successful cultivation of gravid broodfish and fry in ponds hinges on maintaining an optimal stocking density which significantly impacts fish growth and survival of fish in captivity. Each rearing system offers distinct advantages and poses specific challenges. Cage cultivation and earthen ponds capitalize on natural environments but have less control over environmental conditions and potentially higher ecological impacts. In contrast, concrete ponds and Recirculating Aquaculture Systems (RAS) offer greater control over the aquaculture environment, improving predictability in production but at a higher cost and with greater energy requirements [7]. This ideal density varies based on factors like species and fry developmental stage. Higher stocking densities cage cultivation into earthen ponds may boost growth rates in cyprinid fry but can hinder their development. To succeed in fry culture, understanding fish nutritional needs and their ability to adapt to environmental conditions is crucial. While food availability generally correlates with growth rates, the relationship between population density and growth often shows an inverse pattern. Thus, striking a balance between stocking density, nutrition, and environmental factors is essential for maximizing growth and survival rates. Karnatak *et al.* [8] reported that in their study on the impact of stocking density on the growth, feed utilization, and survival of cage-reared minor carp, *Labeo bata*, in Maithon reservoir, India, different stocking densities had significant effects on these parameters. Their research demonstrated that lower stocking densities tended to result in better growth rates, improved feed utilization, and higher survival rates compared to higher densities. The objective of this study is to determine the optimal stocking density for rearing *G. theunensis* fry, The results will offer essential insights for establishing best practices in fish aquaculture, thereby enhancing the sustainability and conservation of fish populations. This initiative aims to support biodiversity preservation and promote ecological balance by refining stocking density conditions and cultivation techniques. Effective management of stocking density is crucial for maintaining fish health, safeguarding environmental sustainability, and ensuring economic viability in aquaculture. Key challenges include optimizing the allocation of resources such as oxygen and feed, controlling the spread of disease and stress due to overcrowding, and managing waste to preserve water quality.

2. Materials and Methods

Place and time of research

The research was conducted at the faculty of science and agriculture technology, Rajamangala University of Technology, Lanna located in Nan province, Thailand. It spanned a period of three months, from October to December 2024. Approval for all experimental procedures, animal care, and biosafety measures was granted by the animal experiment committee and the biosafety committee of Rajamangala University of Technology Lanna, as documented under approval number RMUTL-IACUC 007/2024, dated June 24, 2024 , and valid until June 2 3, 2026. This approval ensured that the research adhered to established ethical standards and regulatory requirements concerning animal welfare and safety protocols.

Animal preparation

Approximately 1500 fry of *G. theunensis* fish, aged around 60 days, were used for the research. Fry were induced through semi-artificial propagation. Female fish were administered LH-RHa at a dosage of 25 µg kg^{-1} BW, along with a dopamine antagonist at a dose of 10 mg kg^{-1} BW. While, male fish underwent induction with LH-RHa at a dosage of 10 µg kg^{-1} BW, combined with a dopamine antagonist at a dose of 10 mg kg^{-1} BW. The process of inducing *G. theunensis* broodfish was modified from the steps outlined by Na-Nakorn [9]. They were then subjected to further study to investigate the effects of stocking density on growth or other relevant parameters (Fig.1).

Fig.1 Morphology of *G. theunensis* brood-fish with distinctions between male (b) and female (a) was illustrated.

Fig.2 Morphology of a cyprinid fish (*G. theunensis*) fry at the initial study.

Experimental Treatment and Design

The fry utilized in the experiment had an average weight of 0.70 ± 0.00, 0.69 ± 0.00, 0.69 ± 0.00, and 0.69 ± 0.00 g for Trt1, Trt2, Trt3, and Trt4, respectively. Meanwhile, the lengths for Trt1, Trt2, Trt3, and Trt4 were 3.80 ± 0.01, 3.79 ± 0.02, 3.78 ± 0.01, and 3.78 ± 0.01 cm, respectively. A completely randomized design (CRD) was conducted to allocate four treatments (Trt) include Trt1 (50 fry m^{-3}), Trt2 (100 fry m^{-3}), Trt3 (150 fry m^{-3}), and Trt4 (200 fry m^{-3}). Each treatment was replicated three times to ensure the reliability and robustness of the experimental data. The fish were stocked in cages, each measuring 1.0 m^3, within an earthen pond measuring 10 × 20 × 2.0 m and lined with chrome pipe. The basic floating part of the cage measured 1 × 1 m, and the bottom of the net with a mesh size of 10 mm was stretched with a chrome pipe frame also measuring 1 × 1 m The top of the cage was covered with a black mesh to protect the fish from intense light. Throughout the study conducted, fish were fed a commercial fish feed containing 28% protein, 5% fat, 12% moisture, and 11% fiber. The feed was provided to the fish at a rate of 3% of their body weight twice a day, specifically at 8:00 am and 5 pm. Leftover feed and waste were removed every day in the morning. Dead fish in each treatment were recorded and removed.

Data collection

Every month, fish were randomly sampled and measured during the experimental period, spanning from about 30 days up to 90 days. They were measured (nearest millimeter of total length) and weighed (nearest 0.01 g) after being gently blotted with a towel. At the end of a experimental period, final weight, final length, feed dispensed, and mortality were recorded. Data on the growth characteristics of the fry were documented at thirty-day intervals, with length being measured using a wooden scale and weight being measured using a digital balance (Sartorius, BSA224S-CW, Max 220 g, d = 0.1 mg). For each treatment, the following growth parameters were determined: average daily gain (ADG: g/fish/day), specific growth rate (SGR: %), feed conversion ratio (FCR), and survival rate (%). These calculations were based on the formulae provided in previous studies [10,11,12].

$$\text{ADG} = \frac{\text{Final weight-Initial weight}}{\text{Number of days}} \quad (1)$$

Where: Final weight is the weight of the fish at the end of the period. Initial weight is the weight of the fish at the beginning of the period. Number of days is the duration of the period over which the weight gain is measured.

$$\text{SGR (\%)} = \left(\frac{\ln W_2 - \ln W_1}{t_2 - t_1}\right) \text{x100} \quad (2)$$

Where: W_1 is the initial weight of the fish. W_2 is the final weight of the fish. t_1 and t_2 are the times at the beginning and end of the period over which growth is measured, respectively.

$$\text{FCR} = \frac{\text{Total feed consumed (g)}}{\text{Total weight gain of fish}} \quad (3)$$

Where: Total feed dispensed is the sum of feed provided during the culture period. Total weight gain is the difference between final and initial weights.

$$\text{Survival rate (\%)} = \left(\frac{\text{Number of fish at the end of experiment}}{\text{Number of fish at the beginning of experiment}}\right) \text{x100} \quad (4)$$

Evaluation of water quality performance

Throughout the three months experimental period, water quality parameters were rigorously monitored at thirty-day intervals to ensure optimal conditions for fish health and growth. The monitoring utilized advanced water quality meters from Horiba, Japan (Horiba U-50 series water quality meter), allowing for comprehensive assessment of parameters including pH, dissolved oxygen, conductivity, water temperature, turbidity, salinity and total dissolved solids. Additionally, the concentration of ammonia nitrite and nitrate in the water was regularly measured using LAQUA twin compact water quality meter (Horiba, Japan).

Statistical analysis

The study employed a statistical analysis to assess differences between treatment groups. Data were presented as mean±SE. One-way analysis of variance (ANOVA) was used to determine overall differences among groups. Post-hoc analysis was conducted using Duncan's multiple range test to identify specific group differences. A significance level of $P < 0.05$ was chosen.

3. Results and Discussion

Results of growth performance of fish

The study focused on evaluating the growth performance of *G. theunensis* fish fry across different stocking densities over a duration of three months. The findings, outlined in Table 1, revealed notable variations in mean weight across the various stocking densities. Particularly, fry stocked at a density of 50 fry/m^3 exhibited the highest mean weight recorded at 4. 88 ± 0. 09 g, showcasing the potential benefits of higher stocking densities. In contrast, fry stocked at a density of 200 fry/m^3 displayed the lowest mean weight, amounting to 3. 90 ± 0. 09 g. The data also illustrated a discernible trend wherein an increase in stocking density corresponded to a rise in lower mean weight. However, it's worth noting that while no significant difference was observed in mean weight gain between fry stocked at 200 fry m^{-3} and those at 150 fry m^{-3} or 100 fry m^{-3}, a notable disparity emerged when compared to fry stocked at 50 fry m^{-3}. In this regard, a significant difference in mean weight gain was noted between fry stocked at 50 fry m^{-3} and those at 100 fry m^{-3}, 150 fry m^{-3}, and 200 fry m^{-3}. These findings underscore the intricate relationship between stocking density and the growth performance of fish fry, highlighting the nuanced interplay between population density and individual weight gain. Moreover, the observed patterns provide valuable insights for optimizing stocking strategies in aquaculture practices, aiming to enhance fry growth and overall productivity while ensuring optimal conditions for fish health and welfare.

The average daily gain (ADG) of fish fry were determined across four treatments, denoted as Trt1 Trt2 Trt3 and Trt4 with corresponding values of 0. 05 ± 0. 00, 0. 04 ± 0. 00, 0. 04 ± 0. 00, and 0. 04 ± 0. 00 g/fish/day, respectively (Table1). In Trt1, where fry was stocked at 50 fry/m^3, the observed ADG was notably higher (0. 04 ± 0. 00 g $fish^{-1}$ day^{-1}) compared to the other treatments. Conversely, in Trt4, where fishes were stocked at 200 fry m^{-3}, ADG was lower (0. 036 ± 0. 00 g $fish^{-1}$ day^{-1}) relative to the other treatments. These findings suggest that stocking density may have a significant impact on ADG of fish fry during the experimental period.

The study examined the specific growth rate (SGR) across various stocking densities, revealing a range from 134.60 ± 2.42 to 157.95 ± 32.63% day^{-1} (Table1). Notably, fry stocked at 200 fry m^{-3} exhibited significantly higher SGR compared to other stocking density treatments (Table1), suggesting a positive correlation between stocking density and growth rate in fish fry. Furthermore, the investigation into survival rates during the initial month of the experiment revealed no significant differences among treatments. However, as the study progressed, a notable disparity emerged, with fry stocked at 200 fry m^{-3} demonstrating significantly higher survival rates compared to those at lower densities of 50 fry m^{-3} and 100 fry m^{-3}. These findings underscore the intricate relationship between stocking density, growth performance,

Table 1 The mean (±SE) growth of *G. theunensis* fry assessed in a cage cultivation environment under different stocking densities over a three-month experimental period

Parameters	Trt1 (50 fry m^{-2})	Trt2 (100 fry m^{-2})	Trt3 (150 fry m^{-2})	Trt4 (200 fry m^{-2})	F/Pr > F
Initial length (cm)	3.80±0.01^a	3.79±0.02^a	3.78±0.01^a	3.78±0.01^a	0.65/0.58
Final length (cm)	5.93±0.03^a	5.40±0.05^b	5.19±0.06^c	5.03±0.06^d	38.48/0.00
Initial weight (g)	0.70±0.00^a	0.69±0.00^a	0.69±0.00^a	0.69±0.00^a	67.60/0.35
Final weight (g)	4.88±0.09^a	4.08±0.09^b	3.92±0.09^b	3.90±0.09^b	17.81/0.00
Average Daily Gain (ADG) (g/fish/day)	0.05±0.00^a	0.04±0.00^b	0.04±0.00^b	0.04±0.00^b	32.26/0.00
Specific Growth Rate (SGR) (%/fish/day)	157.95±32.63^a	140.78±2.62^b	135.99±2.65^b	134.60±2.42^b	15.74/0.00
Weight Gain (%)	612.77±18.75^a	496.95±15.46^b	471.51±14.54^b	467.19±13.65^b	124.78/0.00
Feed Conversion Ratio (FCR)	2.34±0.00^c	2.86±0.09^b	3.01±0.07^a	3.14±0.12^a	18.36/0.00

Note: Data are shown as the mean ± SE, derived from three independent experiments. Means in a row superscripted with different lowercase letters were significantly different.

Fig.3 Morphology of *G. theunensis* fish at the end of a 90-day experiment.

and survival outcomes in fish fry, providing valuable insights for optimizing rearing conditions and informing aquaculture practices aimed at enhancing productivity and sustainability. The feed conversion ratio (FCR) demonstrated an inverse relationship with stocking density, where higher densities led to increased FCR values. Trt1, characterized by the lowest stocking density of 50 fry m^{-3}, exhibited the most favorable FCR. Fish fry stocked at this density displayed a lower FCR of 2.34 ± 0.00, contrasting with those stocked at 200 fry m^{-3}, which showed the highest FCR of 3.14 ± 0.12. Significant differences in FCR were evident between the fry stocked at 50 fry m^{-3}, and the other treatments, highlighting the impact of stocking density on feed efficiency. However, no significant differences were noted in FCR between fry stocked at 100 fish m^{-3} and 150 fry m^{-3}. These findings emphasize the crucial role of stocking density management in optimizing feed utilization and enhancing overall productivity within fish culture systems. A better survival rate was observed in the fry stocked at 50 fry m^{-3} over a three-month experimental period. The survival rate of fry was found to be significantly higher in fry stocked at 50 fry m^{-3} compared to the rest of the treatments. No significant difference was observed in survival rates between the treatments in the first month of the experiment. However, at the end of the experiment, survival rates were found to be not significantly different between fry stocked at 50 fry m^{-3} and fry stocked of 100,150, and 200 fry m^{-3} (Table 2).

Table 2 The mean (±SE) monthly survival rate of *G. theunensis* fry assessed in a cage cultivation environment under different stocking densities over a three-month experimental period

Culture period (month)	Trt1 (50 fry m^{-3})	Trt2 (100 fry m^{-3})	Trt3 (150 fry m^{-3})	Trt4 (200 fry m^{-3})	F/Pr > F
1	95.33±1.33^{a}	93.00±1.53^{ab}	94.44±1.56^{ab}	90.17±1.86^{b}	2.05/0.03
2	88.00±1.15^{a}	81.00±2.65^{bc}	86.22±1.56^{ab}	78.00±0.58^{c}	7.69/0.00
3	82.67±1.76^{a}	76.67±2.33^{a}	79.33±3.06^{a}	73.83±3.42^{a}	1.92/0.20

Note: Data are shown as the mean ± SE, derived from three independent experiments. Means in a row superscripted with different lowercase letters were significantly different.

Water quality parameters

According to Table3, there were no significant differences among treatment groups for certain parameters of water quality performance, including dissolved oxygen, conductivity, total dissolved solids, water temperature, pH, and salinity. Trt1 exhibited the highest pH (5.07 ± 0.01) and dissolved oxygen (5.07 ± 0.01 mg L^{-1}) levels. Conversely, water temperature (25.40 ± 0.03 ^{0}C), conductivity (0.10 ± 0.00 µS cm^{-1}), total dissolved solids (0.07 ± 0.00 mg L^{-1}), turbidity (24.67 ± 0.09 NTU), and salinity (0.01 ± 0.00 ppt) were highest in Trt4. However, these differences were not statistically significant among treatment groups, suggesting that stocking density management had no significant influence on these parameters of water quality performance. On the other hand, significant differences were observed among treatment groups for certain parameters such as un-ionized ammonia (NH_3) nitrite (NO_2^-) and nitrate (NO_3^-). Nitrogenous compounds, originating primarily from feeds, are of concern due to their role in nutrient enrichment in aquaculture systems. In this study, the levels of un-ionized ammonia, nitrite, and nitrate in fish fry cage cultured at different stocking densities (Trt1: 50 fry m^{-3}, Trt2: 100 fry m^{-3}, Trt3: 150 fry m^{-3}, and Trt4: 200 fry m^{-3}) were significantly different within treatment groups (Table3). Higher levels of gaseous nitrogen from fish excretion were observed in Trt4 (200 fry m^{-3}), significantly differing from Trt1, Trt2, and Trt3, respectively.

Table 3 The mean (±SE) water quality parameters assessed in a cage cultivation environment of *G. theunensis* fry under different stocking densities over a three-month experimental period

Parameters	OPW	Trt1 (50 fry m^{-3})	Trt2 (100 fry m^{-3})	Trt3 (150 fry m^{-3})	Trt4 (200 fry m^{-3})	F /Pr > F
Water temperature (^{0}C)	25-29	25.40±0.03^a	25.48±0.02^a	25.47±0.03^a	25.57±0.03^a	5.64/0.56
Dissolved oxygen (mg/L)	3-6	5.07±0.01^a	5.04±0.01^a	5.04±0.01^a	5.04±0.01^a	1.39/0.65
pH	5.5-6.5	5.07±0.01^a	5.07±0.01^a	5.08±0.01^a	5.07±0.01^a	15.38/0.63
Turbidity (NTU)	20-30	24.67±0.09^b	24.70±0.12^b	25.22±0.10^a	25.26±0.07^a	198.90/0.00
Conductivity (µS/cm)	0.05-0.15	0.10±0.00^a	0.10±0.00^a	0.10±0.00^a	0.11±0.00^a	0.55/0.66
Total dissolved solids (mg/L)	0.05-1.00	0.07±0.00^a	0.07±0.00^a	0.07±0.00^a	0.07±0.00^a	9.30/0.58
Salinity (ppt)	0.00-0.05	0.01±0.00^a	0.02±0.00^a	0.02±0.00^a	0.02±0.00^a	0.25/0.85
Nitrates (mg/L)	0.00-0.50	0.24±0.00^d	0.31±0.01^c	0.34±0.01^b	0.37±0.01^a	45.00/0.00
Nitrites (mg/L)	0.00-30.0	20.52±0.53^d	23.71±0.55^c	26.75±0.55^b	29.61±0.68^a	45.47/0.00
Ammonia (mg/L)	0.00-0.40	0.24±0.01^d	0.27±0.01^c	0.30±0.01^b	0.33±0.01^a	45.00/0.00

Note: Data are shown as the mean ± SE, derived from three independent experiments. Means in a row superscripted with different lowercase letters are significantly different. OPW is optimal water quality of fish in the upper river basin in Thailand.

Discussion

In aquaculture, the stocking density of fish plays a crucial role in determining their growth, health, and overall production outcomes. High stocking densities often result in increased competition among fish for food resources, space, and oxygen, leading to stress and compromised growth rates. Additionally, the accumulation of waste products in the water can degrade water quality, further exacerbating stress and potentially leading to mortality among the fry. Conversely, low stocking densities may lead to underutilization of feed resources and lower production output than optimal [13,14]. In the present study, the result revealed that stocking density significantly impacted the growth performance of *G. theunensis* fry in rearing conditions. Furthermore, it demonstrated the effects of stocking density on fish size and how external stress, such as construction noise, affected various welfare indicators in the fish. Maintaining lower stocking densities of 50 fry/m^3 were often yield better growth performance and health outcomes for *G. theunensis* fry. This is because lower stocking densities alleviate competition for food and space, reducing stress levels among the fish population. As a result, fish stocked at lower densities may exhibit higher weight gain, improved feed utilization efficiency, and overall better health status. In this study, maintaining lower stocking densities of 50 fry/m^3 has consistently shown to yield better growth performance and improved health outcomes for *G. theunensis* fry. This positive effect is primarily attributed to the reduction of competition for both food and space among the fish population. With fewer individuals competing for resources, each fish has increased access to essential elements like food and space, consequently leading to lower stress levels within the population. This optimal environment allows the fry to allocate more energy towards growth and development, rather than expending it on coping with high competition levels. As a result, the fish experience enhanced growth rates and overall better health, highlighting the importance of stocking density management in ensuring the welfare and productivity of *G. theunensis* fry in aquaculture settings. The study by Majhi et al. [15] investigated the immune and stress-related gene expression of butter catfish fry in biofloc systems. Their findings revealed that lower stocking densities were associated with higher levels of antioxidant enzymes, indicating better health for the fish. Similarly, Díaz de Otálora *et al.* [14] reported that the importance of stocking density in aquaponic systems and its impact on water quality, crop yield, and fish growth. The findings suggest that while daily water consumption remains consistent regardless of stocking density, dissolved oxygen levels vary significantly, which can affect the overall health of the aquatic organisms. The observed lower specific growth rate (SGR) in this study may have been influenced by various factors, one of which is insufficient space for *G. theunensis* fry. When stocking densities are high, individual fish may have limited access to food and exhibit reduced feeding rates, ultimately leading to lower growth rates. This notion is supported by findings from Magouz *et al.* [16], who highlighted the critical balance required in aquaculture systems between maximizing productivity and maintaining the health and welfare of the fish. Therefore, optimizing stocking densities to minimize overcrowding is essential for promoting healthy growth and maximizing the productivity of fish farms. Despite the challenges posed by stocking density, *G. theunensis* fry showcased a commendable growth rate compared to other species within the same genus. This suggests that *G. theunensis* has inherent qualities that make it a promising candidate for aquaculture endeavors. Its ability to thrive and exhibit satisfactory growth rates even under suboptimal conditions underscores its potential for commercial production. In a similar context, Li *et al.* [17] findings highlight the negative consequences of high stocking densities in aquaculture settings, particularly concerning fish welfare and health. The study suggests that maintaining stocking densities that minimize stress and promote healthy immune responses is crucial for the long-term success and sustainability of aquaculture enterprises. Similarly, Li *et al.* [18] reported that prolonged exposure to high stocking densities can lead to compromised intestinal health, disrupted microbial ecosystems, and altered metabolic and immune functions. This study underscores the need for careful management of stocking densities to maintain the health and welfare of fish in aquaculture systems, suggesting that optimal density settings are crucial for sustainable aquaculture practices. Conversely, Al-Emran *et al.* [19] reported the highest SGR in *Thai Sharpunti* when stocked at 50 fish m^{-3}, indicating species-specific responses to stocking density variations. This suggests that optimal stocking densities may vary depending on the species and environmental conditions. Contrary to the findings of this study, Roy *et al.* [20] observed a lower SGR in *Mystus vittatus* fry stocked at 50 fish m^{-3}. This discrepancy underscores the importance of considering species-specific requirements and environmental factors when determining the ideal stocking densities for aquaculture operations.

The Feed Conversion Ratio (FCR) is a crucial parameter in aquaculture, serving as a key indicator of how efficiently fish utilize feed to gain body mass. Our study delved into the relationship between stocking density and FCR, revealing intriguing insights into feed efficiency dynamics. At its core, our findings unveiled a direct correlation between stocking density and FCR, elucidating that higher stocking densities tend to lead to higher FCR values. This trend implies that as competition for resources increases among densely stocked fish populations, feed conversion becomes less efficient, potentially due to factors such as heightened stress levels and increased energy requirements. This observation resonates with prior research, as studies by Fuentes-Andraca *et al.* [21] and Khanjani *et al.* [22] have previously reported how elevated stocking densities can induce stress in fish, consequently impacting their metabolic processes and feed utilization efficiency. Similarly, Khanjani *et al.* [23] suggested that the optimization of water quality parameters within biofloc technology systems is crucial for maximizing the growth and health of aquaculture species. Their findings emphasize the importance of maintaining specific ranges for dissolved oxygen, pH, and nitrogen compounds to enhance the microbial processes that are central to the efficiency of biofloc systems. Comparing our results to existing literature, it's noteworthy that the FCR values observed in our study were higher than those reported for Nile tilapia juveniles by Fuentes-Andraca *et al.* [24]. This disparity underscores the importance of considering species-specific traits, feeding regimes, and environmental factors when interpreting FCR data across different studies. Ultimately, our findings underscore the intricate interplay between stocking density and feed efficiency in aquaculture systems. By meticulously managing stocking densities, aquaculturists can optimize feed utilization and enhance overall production efficiency, ultimately contributing to the sustainability and profitability of fish farming operations. Throughout the three-month study period, we monitored various water quality parameters to ensure they remained within optimal ranges conducive to the growth and development of *G. theunensis* fry. Notably, parameters such as pH, dissolved oxygen, conductivity, total dissolved solids, water temperature, turbidity, salinity, ammonia, nitrite, and nitrate were regularly assessed. Our analysis revealed significant differences among treatment groups regarding these water quality parameters. For instance, pH levels ranged from 5.07 to 5.08, dissolved oxygen concentrations from 5.04 to 5.07 mg L^{-1}, and conductivity from 0.10 to 0.11 µS cm^{-1}, water temperature from 25.40 to 25.57 °C, turbidity from 24.67 to 25.26 NTU, and salinity from 0.01 to 0.02 ppt. Additionally, ammonia concentrations varied from 0.24 to 0.33 mg L^{-1}, nitrite from 20.52 to 29.61 mg L^{-1}, and nitrate from 0.24 to 0.37 mg L^{-1}.

Our findings align with established literature guidelines, suggesting that the observed water quality parameters fell within acceptable limits for successful fry rearing. Specifically, maintaining dissolved oxygen levels above 3 mg L^{-1}, total ammonia nitrogen between 0.5 to 1.0 mg L^{-1}, and nitrite concentrations below 1.0 mg L^{-1} are crucial for optimal aquaculture conditions. Throughout a three-month experimental period, rigorous monitoring of water quality parameters was carried out to ensure optimal conditions for fry rearing. The observed parameters consistently remained within acceptable ranges, in accordance with the guidelines outlined by Saha *et al.* [6] which emphasize the significant role of water quality in the biology and physiology of fish. It is well-established that water quality can profoundly influence the health and productivity of a culture system.

During the experiment, the water quality was consistently maintained within the optimal range necessary for cyprinid fish, ensuring a controlled environment for accurate assessments. According to the findings by Saha *et al.* [6], the study revealed significant spatial variations in water quality within an urban oxbow lake. Proximity to the parent channel was associated with higher pollution levels, particularly during the pre-monsoon season, adversely impacting the diversity and distribution of fish species. Therefore, any variations in fish growth observed in this study may not solely be attributed to the characteristics of water quality parameters. The study by Is-haak *et al.* [25] investigates the effects of temperature and total ammonia nitrogen (TAN) on the oxygen consumption rate of Asian seabass juveniles. Significant interactions were found, particularly at higher temperatures and TAN levels, impacting OCR adversely. Nonetheless, concentrations of nitrate, nitrites, and phosphates were diligently kept within desirable limits, as recommended by Luo *et al.* [26] for successful culture practices. This steadfast adherence to established standards underscores the paramount importance of maintaining suitable water quality conditions to bolster the health and growth of *G. theunensis* fry throughout their rearing period. Interestingly, none of the cultured environments exceeded acceptable limits, indicating prudent management practices and suggesting that overstocking of fish was effectively mitigated. This underscores the importance of carefully regulating stocking densities to maintain water quality and ensure optimal conditions for fry development. Our study also suggests that Trt1 (50 fry m^{-3}) may have benefited from improved water quality, potentially attributed to enhanced water circulation and the removal of total organic nitrogen accumulation.

4. Conclusion

The study investigated the growth performance, survival rates, feed conversion ratio, and water quality parameters of *G. theunensis* fry across different stocking densities over a three-month period. Results indicated that higher stocking densities led to increased mean weight and specific growth rates, with the highest values observed at 50 fry m^{-3}. Higher survival rates were also observed at this density. However, the feed conversion ratio was found to be inversely related to stocking density, with the best ratio observed at the lowest density. Significant differences in nitrogenous compounds among stocking density groups were shown by water quality parameters. These findings highlight the complex interplay between stocking density, growth, survival, feed efficiency, and water quality in the *G. theunensis* fry in cage cultivation environment.

5. Acknowledgement

Financial support for the year 2024 was provided by the National Research Council of Thailand (NRCT), under the strategic fund N24A670775. The project was categorized within the subject group focusing on reducing risks and impacts of natural disasters and climate change on economic animals. This funding was facilitated through the 2024 Strategic Fund (SF) managed by the Research and Development Institute of Rajamangala University of Technology Lanna.

6. References

[1] A. Lothongkham, S. Arbsuwan, P. Musikasinthorn, *Garra waensis*, a new cyprinid fish (actinopterygii: cypriniformes) from the Nan river basin of the Chao Phraya river system, northern Thailand, Zootaxa. 3790 (2014) 543–554. 10.11646/zootaxa.3790.4.2.

[2] W. Tangjitjaroen, Z.S. Randall, S. Tongnunui, D.A. Boyd, L.M. Page, Species of *Garra* in the Salween river basin with description of an enigmatic new species from the Ataran river drainage of Thailand and Myanmar, Zootaxa. 5311 (2023) 375–392. 10.11646/zootaxa.5311.3.3.

[3] K. Shibukawa, P. Phousavanh, K. Phongsa, A. Iwata, A new species of *Metzia* (cypriniformes: cyprinidae) from Northern Laos, Zootaxa. 3586 (2012) 264–271. 10.11646/zootaxa.3586.1.25.

[4] A. Suvarnaraksha, P. Boonsatien, Habitat preferences and population structure of *Garra theunensis* (Teleostei: cyprinidae) in the Nam Xam and Xe Bangfai Drainages, Laos. Ichthyol. Explor. Freshwaters. 29 (2018) 315–324. 10.23788/IEF-1156.

[5] N.A. Hamidan, Ecology and Conservation of *Garra ghorensis*, an Endangered Freshwater Fish in Jordan; Bournemouth University, 2016.

[6] S. Saha, A.V. Chukwuka, D. Mukherjee, N.C. Saha, A.O. Adeogun, Hydrological connectivity, surface water quality and distribution of fish species within sub-locations of an urban oxbow lake, East India, Watersh. Ecol. Environ. 4(1) (2022) 44 – 58. 10.1016/j.wsee.2022.04.001.

[7] L. Kolek, M. Inglot, Integrated cage-pond carp farming for increased aquaculture production, Fish. Aquat. Life. 31(2) (2023) 87-94. 10.2478/aopf-2023-0010.

[8] G. Karnatak, B.K. Das, P. Mishal, T. Tayung, S. Kumari, U.K. Sarkar, A.K. Das,

Y. Ali, Impact of stocking density on growth, feed utilization and survival of cage reared minor carp, *Labeo bata* (Hamilton, 1822) in Maithon reservoir, India, Aquaculture. 532 (2021) 736078. 10.1016/j.aquaculture.2020.736078.

[9] U. Na-Nakorn, *Clarias* spp.: Breeding and Culture, 2nd ed., The Kasetsart University Press, Bangkok, 2001.

[10] M. Hossain, G. Haylor, M. Beveridge, Effect of feeding time and frequency on the growth and feed utilization of African catfish (*Clarias gariepinus*) fingerlings, Aquac. Res. 32 (2002) 999–1004. 10.1046 /j.1365-2109.2001.00635.x.

[11] S. Sawhney, R. Gandotra, Growth response and feed conversion efficiency of *Tor putitora* (Ham.) fry at varying dietary protein levels, Pak. J. Nutr. 9 (2010) 86–90.10.3923/pjn.2010.86.90.

[12] M.K. Mustapha, T.T. Oladokun, M.M. Salman, I.A. Adeniyi, D. Ojo, Does light duration (photoperiod) have an effect on the mortality and welfare of cultured *Oreochromis niloticus*? Turk, J. Zool. 38 (2014) 1–5. 10.3906/zoo-1309-23.

[13] C. d'Abbadie, , Low-emission beef production in the Southern Rangelands of Western Australia: an analysis of herd structure and stocking rate experiencing droughts, Rangeland J. 46 (3) (2024). RJ24007. doi.org/10.1071/RJ24007.

[14] X. Díaz de Otálora, B. Amon, L. Balaine, F. Dragoni, F. Estellés, G. Ragaglini, M. Kieronczyk, G.H.M. Jørgensen, A. del Prado, Influence of farm diversity on nitrogen and greenhouse gas emission sources from key European dairy cattle systems: a step towards emission mitigation and nutrient circularity, Agric. Syst. 216 (2024) 103902. 10.1016/j.agsy. 2024.103902.

[15] S.S. Majhi, S.K. Singh, P. Biswas, R. Debbarma, J. Parhi, A. Khatei, Y.A. Mangang, G. Waikhom, A.B. Patel, Stocking density affects immune and stress-related gene expression of Butter catfish fry in biofloc landscapes, Fish Shellfish Immunol. Rep. 5 (2023) 100-112. 10.1016 /j.fsirep.2023.100112.

[16] J. Seo, J. Park, Does stocking density affect growth performance and hematological parameters of juvenile Olive flounder in a recirculating aquaculture system?, Animals, 13(1) (2023) 44-55. 10.3390/ ani13010044.

[17] W. Li, D. Li, Q. Yang, L. Liu, J. Liu, J. Lu, Y. Wang, R. Tang, L. Li, X. Zhang, Long-term crowding stress induces chronic inflammatory response and declines the immunity of Grass carp, Aquacult. 577 (2023) 739976. 10.1016/j.aquaculture. 2023.739976.

[18] M. Li, L. Yang, Y. Liu, H. Ma, Long-term crowding stress disrupts intestinal homeostasis in largemouth bass (*Micropterus salmoides*), Aquacult. 599 (2025) 742171. 10.1016/j. aquaculture. 2025.742171.

[19] M. Al-Emran, R. Rahman, M.I. Miah, M. Shahjahan, Effects of stocking density on growth and production in monoculture of Thai sharpunti, Prog. Agric. 28(3) (2017) 249–252. 10.3329/pa. v28i3.34662.

[20] P.S. Roy, B.K. Das, J. Adhikary, S. Chowdhury, R. Samanta, A. Das, B. Chirwatkar, A. Karan, S. Purkait, D. Bhakta, Growth studies of *Systomus sarana*, and *M. rosenbergii* reared under freshwater pond in bamboo-net cage system, J. Exp. Zool. India, 21 (2018) 357–362. 10.20546/ ijcmas.2018.702.339.

[21] V.H. Fuentes-Andraca, S.C. Ángeles Campos, H. Castillo-Juárez, E.J. Marin-Coria, J.C. Quintana-Casares, R. Domínguez-May, G.R. Campos-Montes, Different stocking density effects on growth performance, muscle protein composition, and production cost of Nile tilapia juveniles in on-growing stage in biofloc system, Aquac. Int. 33(2025) 176. 10.1016/j. aquaculture.2023.739713.

[22] M.H. Khanjani, M. Sharifinia, S. Hajirezaee, Biofloc: A sustainable alternative for improving the production of farmed cyprinid species, Aquacult. Rep. 33

(2023) 101748. 10.1016/j.aqrep. 2023.101748.

[23] M.H. Khanjani, A. Mohammadi, M.G.C. Emerenciano, Water quality in biofloc technology (BFT): an applied review for an evolving aquaculture, Aquac. Int. 32(2024) 9321–9374. 10.1016/j.aqrep. 2024.07.019.

[24] V.H. Fuentes-Andraca, S.C. Ángeles Campos, H. Castillo-Juárez, E.J. Marin-Coria, R. Domínguez-May, G.R. Campos-Montes, Different stocking density effects on growth performance, muscle protein composition, and production cost of Nile tilapia (*Oreochromis niloticus*) juveniles in on-growing stage in biofloc system, Aquacult. Int. 33 (2025) 176. 10.1016/j. aquaculture.2025.101748.

[25] J. Is-haak, S. Koydon, Y. Iampichai, C. Ngamphongsai, Effects of ammonia, temperature and their interaction on oxygen consumption rate of Asian seabass (*Lates calcarifer*) juveniles, Agric. Nat. Resour. 56(5) (2022) 917–924. https://li01.tci-thaijo.org/index.php/anres/article/view/256572.

[26] G. Luo, L. Jiayang, X. Jinxiang, W. Liu, H. Tan, Effects of dissolved organic carbon and total ammonia nitrogen concentrations with the same DOC/TAN on biofloc performance, Aquaculture. 574 (2023) 739713. 10.1016/j. aquaculture. 2023.739713