J.Natn.Sci.Foundation Sri Lanka 2025 53 (2): 151 - 164
DOI: http://dx.doi.org/10.4038/jnsfsr.v53i2.12442

RESEARCH ARTICLE

Environmental Science

Impact of probiotics mixture as a water additive on water quality, growth performance and survival of *Catla catla* fry

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Submitted: 16 November 2024; Revised: 19 March 2025; Accepted: 16 May 2025

Abstract: The main objective of this study was to assess the impact of a commercial probiotic as a water additive on the survival, growth performance, and water quality of *C. catla.* A field trial was conducted for 66 days. About 360 *C. catla* fry (0.25 ± 0.01 g) were distributed randomly among 12 quadruplicate-designed cemented tanks, each containing 30 healthy fish in 0.15 m^3 of water. Control tanks were not treated with probiotics while the treatment tanks received 0.012 g m^{-2} $week^{-1}$ and 0.03 g m^{-2} $week^{-1}$ of the probiotic. Principal Component Analysis was carried out to identify key water quality factors. One-way ANOVA followed by Tukey's test was used to compare statistical differences. The results showed that adding probiotics significantly decreased ammonia concentration ($p < 0.05$). No significant differences were found in water temperature, total dissolved solids, pH, and electrical conductivity between treatments and control. The fish in tanks treated with probiotics showed a substantial increase in survival rate ($p < 0.05$), with the highest value of $92 \pm 1.5\%$. The survival of *C. catla* fry was enhanced when a high dosage of 0.03 g m^{-2} $week^{-1}$ was administered. Despite the positive impact on survival, probiotic treatment did not influence the overall growth performance.

Keywords: Aquaculture, survival, *Catla catla*, probiotics as a water additive, water quality improvement

INTRODUCTION

Aquaculture has emerged as one of the fastest-growing industries, accounting for around 50 % of the food fish production (Rathnachandra *et al.*, 2024; Samarawardane *et al.*, 2021). However, the intensification of aquaculture practices has led to significant challenges, including the decline of the survival of the host species, disease outbreaks, poor water quality, and the excessive use of antibiotics, which contribute to the development of antibiotic-resistant bacteria (Manage, 2018). The aquaculture systems generate wastewater containing significant components such as ammonia, phosphate, and suspended particles. Phosphorus and nitrogen are two excretory products of farmed fish, which greatly affect the quality of water and harm fish health (Yang *et al.*, 2021; Zabidi *et al.*, 2021; Ali *et al.*, 2021). These compounds affect the survival of the culture organism, ultimately leading to significant decline of production as well (Ali *et al.*, 2021). Various biological, physical, and chemical approaches have been developed and used subsequently to manage water pollution in aquaculture (Dawood & Koshio, 2016; El-Saadony *et al.,* 2021; Hai, 2015). Nevertheless, the accumulation of these harmful chemicals in living organisms over time and their potential risks when consumed by humans are noteworthy issues (Jahangiri, 2018). As a result of the detrimental impact of chemicals and antibiotics on the environment, which leads to the prevalence of mutagenic microbial strains and negatively affects fish health, it is no longer advisable to use them for disease control (Jahangiri, 2018; Manage, 2018). Hence, using environmentally

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friendly feed additives, such as microbial supplements, to enhance aquaculture-related species' physiology, growth performance, and immune responses has garnered significant interest recently (Jahangiri, 2018; Mishra & Sharma, 2021). The role of probiotics in fish reproduction is a new area of research (Jahangiri, 2018; Nayak, 2020; Olmos-soto, 2015). Probiotic supplementation refers to the use of microorganisms in the aquaculture industry and has become a practical and extensively used method in aquaculture (El-Saadony *et al.*, 2021; Pandiyan *et al.*, 2013).

Sri Lanka has limited but stable fish production with an expanding aquaculture sector for finfish (Athukorala, 2008). The focus has been on initiating small-scale fish farming to assist local communities with income and food supply (Hansika *et al.*, 2024). In Sri Lanka, the inland fisheries sector contributes around 0.2% to the Gross Domestic Production (GDP), showing a 70% growth in inland fishing contribution (Hansika *et al.*, 2024; Rathnachandra *et al.*, 2024). Further, it is responsible for about 20% of the total fish production in the country (Adikari *et al.*, 2017). The sector provides direct and indirect employment for a considerable number of people. During the last few years, the demand for cultured Carp species has significantly increased owing to their substantial market value (Adikari *et al.*, 2017). Besides, the cultivation of *C. catla* and its demand in Sri Lanka has significantly increased due to it being a delicious and cost-effective protein source, and its appearance (Athukorala, 2008). Nevertheless, the Carp Breeding Centre of the National Aquaculture Development Authority (NAQDA) in Udawalawe (Figure 2) has detected an issue related to their *C. catla* farming. This problem may involved in the production decline and the spread of diseases.

The Udawalawe Reservoir serves as the primary water source for this centre (Figure 1), playing a crucial role in meeting the region's water demands (Athukorala, 2008). However, the reservoir is subjected to various forms of pollution, primarily driven by rapid urbanization. These factors may contribute to increased mortality rates among *C. catla* fry. Inadequate water quality management could negatively impact the survival rate of *C. catla* during the transition stage from fry to fingerling. Therefore, the present experiment was undertaken to find an efficient, environmentally friendly method to increase the survival rate of *C. catla* and improve the water quality parameters in the rearing water.

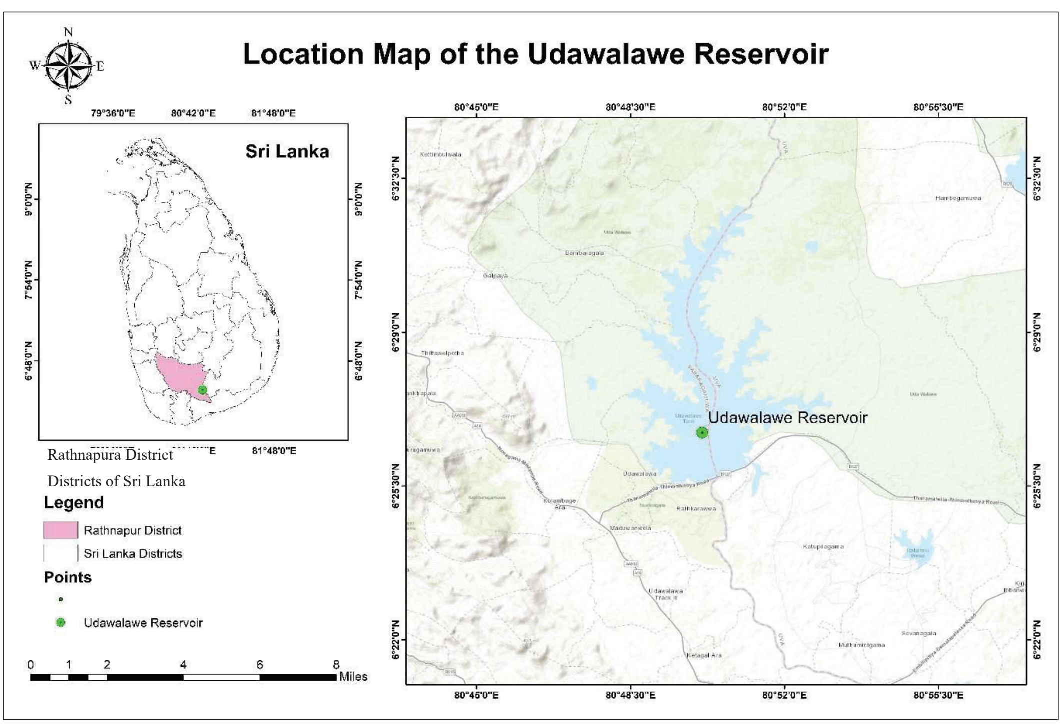

Figure 1: The location map of the Reservoir, Udawalawe Sri Lanka, was generated using ArcMap 10.8. The map highlights the reservoir's role in providing water supply to nearby regions and shows the area's distribution of water bodies.

This study selected a commercially available probiotic supplement, and it was employed to ascertain the effect of probiotics administered to the closed system. Besides, there have been limited investigations on the direct application of multi-strain probiotics in fish farming, as commercial probiotic products are predominantly employed as a supplement in fish feed to enhance fish growth (Ali *et al.*, 2021; El-kady *et al.*, 2022; Hassan *et al.*, 2022; Jahangiri, 2018). Therefore, the administration method of probiotics in aquaculture plays a crucial role in determining their favourable performance (Jahangiri, 2018). Hence, the objective of this study was to evaluate the current methods of probiotic delivery through water in finfish aquaculture. The primary goal of this study was to assess the impact of directly introducing a commercially available probiotic mixture on *C. catla* fish water quality, growth performance, and survival. To the best of the author's knowledge, this is the first study done to assess how multi-strain probiotics enhance *C. catla* survival in Sri Lanka.

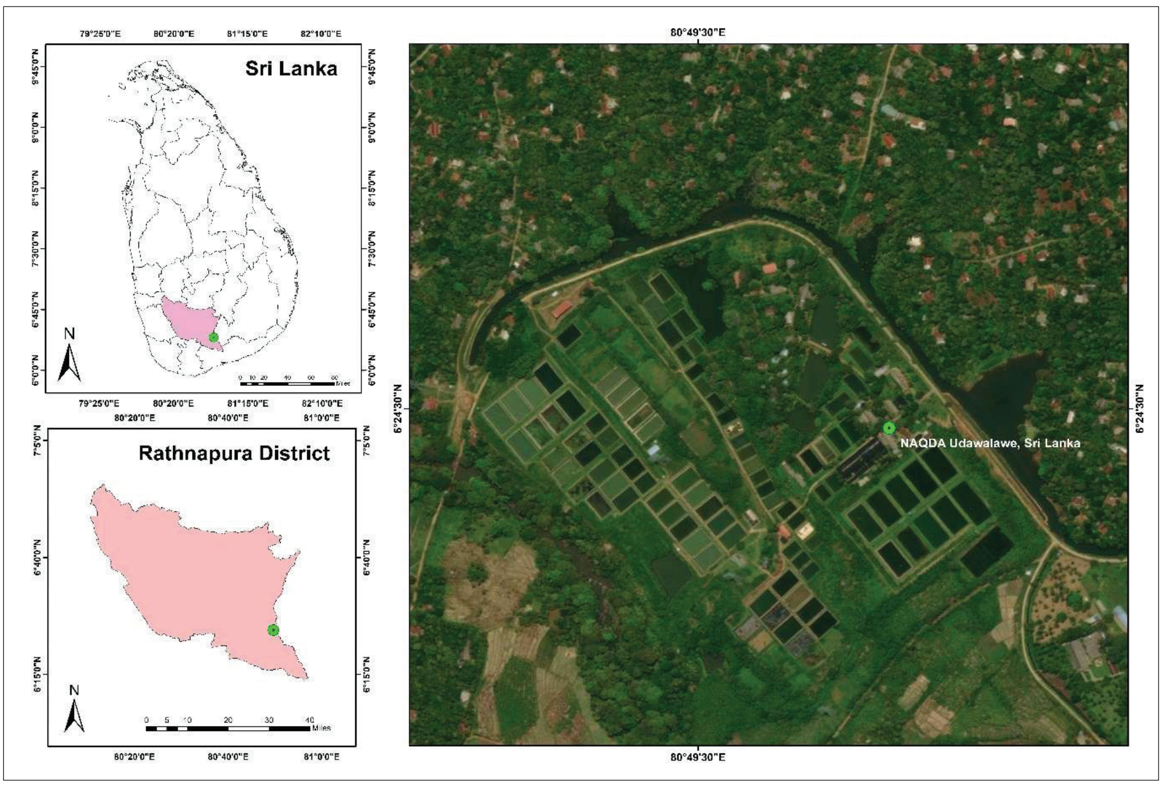

Figure 2: The location map of the Carp Breeding Centre of NAQDA in Udawalawe, Sri Lanka generated using ArcMap 10.8. The map illustrates the location of the Centre and surrounding water bodies. It also highlights the water supply, which is sourced from the Udawalawe Reservoir

MATERIALS AND METHODS

Study site and experimental fish

C. catla fry were obtained from the NAQDA Carp Breeding Centre, Udawalawe, Sri Lanka (6°24'29.1 "N, 80°49'39.8"E), as illustrated in Figure 2. The experiments were also carried out at this Centre and Centre for Water Quality and Algae Research of the University of Sri Jayewardenepura, Sri Lanka.

Experimental set-up

The experiment employed 12 rectangular cement tanks (0.58 × 0.69 × 0.26 m) each with 0.15 m^3 capacity. The tanks were continuously aerated for 66 ds. Each tank was stocked with 30 fry with an average body weight (BW) of 0.25 ± 0.01 g, standard length of 3.15 ± 0.04 cm and total length of 3.97 ± 0.05 cm.

Each treatment was done in quadruplicate. A commercially available probiotic was purchased at the local market. Fry were acclimated to experimental conditions for 14 ds following the procedure described by Das *et al*. (2021).

Experimental design

Cemented tanks were used for the experiment. The control tanks (TC - TC_{-1}, TC_{-2}, TC_{-3}, and TC_{-4}) received only commercial feed. C. *calta* fried in treatment tanks (T_1 - T_{1-1}, T_{1-2}, T_{1-3}, and T_{1-4}) were fed commercial feed supplemented with probiotics at the manufacturer-recommended dose (0.012 g m^{-2} $week^{-1}$). Another set of treatment tanks (T_2 - T_{2-1}, T_{2-2}, T_{2-3} and T_{2-4}) received commercial feed with a higher probiotic dose (0.030 g m^{-2} $week^{-1}$).

The commercial probiotic and the use of it as a water additive

According to the manufacturer, the powdered probiotic used for the present study contains spore-forming *Bacillus* spp. (1.0×10^9 CFU g^{-1}), *B. subtilis* (1.0×10^9 CFU g^{-1}), and *B. licheniformis* (1.0×10^9 CFU g^{-1}), and non-spore-forming *Nitrosomonas* sp. (0.25×10^9 CFU g^{-1}) and *Pediococcus acidilactici* (1.0×10^9 CFU g^{-1}) bacteria. The probiotic was directly added to the rearing water following the manufacturer's instructions. The water in the experimental tanks (T_1 & T_2) were treated with the probiotic at a rate of 0.012 g m^{-2} (lower dose for T_1) and 0.03 g m^{-2} (higher dose for T_2) weekly, while the water in the control tanks (Tc) received no probiotic.

Feeding protocol

Following Withanage (2022), *C. catla* fries in each tank (experimental and control) were fed a recommended commercial feed 3 times a day at 9.00 am, 12.00 noon, and 4.00 pm. According to Withanage (2022), the daily feed ration was adjusted biweekly based on the live body weight of *C. catla* fry, starting from 12.0% of body weight initially; this was then adjusted to 8% and 6% (Mohanty, 2003; Biswas *et al*., 2006). The water volumes (water levels) in the control and treatment tanks were maintained constant for the experimental period; the water lost through evaporation was refilled every 2 ds to maintain a constant water level in each tank (Yang *et al*., 2021).

Fish performance and feed utilizations

The growth of *C. catla* fry was assessed biweekly for 66 ds. Random fish samples were collected from each tank; excess water was removed using clean tissue paper, and the weight of each fish was recorded using an electronic balance (OHASU Adventure AR 2130, USA). After recording each fish's standard length (SL) and total length (TL) separately, each was released back to the relevant tank very carefully. Following Banu *et al*. (2020), conventional methods were employed to compare growth performance under different treatments. (Hossain *et al*., 2017; Banu *et al*., 2020).

To determine the growth performance of *C. catla*, the following parameters, *viz*., weight gain rate (WGR%), final body weight (FBW), condition factor (k), average daily gain (ADG), length gain rate (LGR), specific growth rate (SGR), survival rate (SR%), length gain (LG), feed conservation ratio (FCR), and feed conservation efficiency (FCE) were measured based on the following equations (Froese *et al*., 2013; M. Y. Hossain *et al*., 2015; Shah *et al*., 2021; Tabassum *et al*., 2021):

$$\text{Condition factor (k)} = \frac{\text{Body weight in gm}}{(\text{Body length in cm})^3} \times 100$$

$$\text{ADG} = \frac{(\text{Mean final fish weight} - \text{Mean initial fish weight})}{\text{Time (final time} - \text{initial time)}}$$

$$\text{SGR}(\%) = \frac{(\ln W_t - \ln W_1)}{(\text{Final Time} - \text{Initial Time})} \times 100$$

$$\text{FCR} = \frac{\text{Feed (g) consumed by the fish}}{\text{Weight (g) grain of the fish (Final weight} - \text{Initial weight)}}$$

$$\text{Survival rate (\%)} = \frac{\text{No of actual fish survived}}{\text{No actual fish stocked}} \times 100$$

$$\text{Weight gain (WG, g/fish)} = W_t - W_0$$

Where W_0 = initial mean weight of fish in grams.
W_t = the final mean weight of fish in grams.

$$\text{Length gain Rate} = \frac{L_t - L_0}{L_0} \times 100$$

Where L_0 = initial mean length of fish in cm.
L_t = Final length of fish in cm.

$$\text{Weight gain (\%)} = \frac{\text{mean final weight} - \text{mean initial weight}}{\text{mean initial weight}} \times 100$$

Total feed intake (g) = Total food consumed (g) in each group.

Length-Weight Relationship (LWR) and condition factors of *C. catla* were calculated using the following formula.

The relationship between weight (W) and length (L) in fish has the form:

$W = aL^b$

Where a = scaling coefficient for the weight at the length of the fish species.
b = shape parameter for the body form of the fish species.

When the above model is logarithmically transformed

In (W) = In (a) + b In (L) or Y = A + b X

Where ln(a) is the intercept and (b) is the slope or regression coefficient of the graph of Ln W against Ln L

The above relationship is linear and was used to calculate ordinary linear regression using Microsoft Excel (Kadhar *et al.*, 2014).

Monitoring water quality

The physicochemical parameters of each tank's culture water (pH, DO, TDS, and EC) were recorded daily at 10:00 hrs. using a multiparameter (Thermo Scientific –Star A221). Nitrate and ammonia were measured using APHA Standard Methods (APHA, 2017).

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the significant variation between the different tested groups, followed by the Tukey HSD test. Statistical significance was settled at a probability value of $p < 0.05$. Principal Component Analysis (PCA) was applied to reduce the dimensionality of the data set and to analyze the water quality indicators, including pH, TDS, Temperature, EC, DO, NO_3^-, and NH_4^+, of all treatment samples. The first four principal components (PCs) were selected based on their eigenvalues above the threshold of unity, which is a widely used standard. Four PCs were obtained by extracting the correlation matrix. Statistical analysis was performed using Minitab version 21 for Windows.

RESULTS AND DISCUSSION

Growth performance and survival

At the end of the study, the survival of *C. catla* fry showed a significant difference between the control and treatment groups *($p < 0.05$)*. The results indicated that treatments T_2 and T_1 exhibited the highest survival rates (Figure 3) compared to all other treatments, while the control group showed a slightly lower survival rate ($p < 0.05$). However, during the initial trial stage, statistical analysis indicated no significant differences in fish weight between the treatment and control tanks ($p > 0.05$, Table 1). The growth performance parameters FCR, SGR, FBW, ADG, k, FL, BWG, and TL did not show significant differences ($p >0.05$).

The T_2 group exhibited the highest survival rate (Figure 3) of 92 ± 1.5%, followed by the T_1 group, with a rate of 87 ± 0.4%, and the T_C group, with a rate of 80 ± 0.3%. Furthermore, the (mean) survivability rate of *C. catla* was significantly increased in the T_2 (95.5 ± 0.2%) treatment as compared to the T_1 (93.02 ± 0.3%) and T_C (90.00 ± 0.1%) treatments.

The logarithmic analysis of the length-weight relationship (LWR) and determination coefficient values (R2) are shown in Figures 4, 5, and 6. The experiment revealed a significant positive correlation between length and weight with r (102) = 0.71, $p < 0.001$. Additionally, Figures 4, 5 and 6 demonstrate substantial and strong positive correlation ($p < 0.05$) between length and weight across all experimental groups, with correlation coefficients of 0.712, 0.862, and 0.855 for TC, T1, and T2 treatments, respectively (Table 1). Moreover, no significant changes were recorded in the average growth quality parameters ($p > 0.05$).

Table 1: Growth performance and biometric indices of *C. catla* fry Mean ± SE).

Parameters	T_C Control	T_1 Treatment	T_2 Treatment	P value
Initial body weight (g)	0.25 ± 0.00^a	0.25 ± 0.00^a	0.26 ± 0.00^a	0.109
Final body weight (g)	0.84 ± 0.04^a	0.87 ± 0.03^a	0.92 ± 0.03^a	0.193
Average daily weight gain (g)	0.009 ± 0.00^a	0.009 ± 0.00^a	0.009 ± 0.00^a	0.957
Body weight gain (g)	0.38 ± 0.10^a	0.39 ± 0.13^a	0.40 ± 0.14^a	0.996
SGR (%)	2.29 ± 0.15^a	2.27 ± 0.23^a	2.21 ± 0.26^a	0.969
Weight gain rate (%)	149.9 ± 40.2^a	153.3 ± 51.8^a	151.9 ± 54.0^a	0.997
FCR	1.49 ± 0.33^a	1.47 ± 0.41^a	1.57 ± 0.50^a	0.985
Final Total length (cm)	3.97 ± 0.05^a	4.03 ± 0.05^a	4.03 ± 0.05^a	0.700
Final Standard length (cm)	3.15 ± 0.04^a	3.24 ± 0.04^a	3.24 ± 0.04^a	0.307
Condition factor (k)	1.06 ± 0.06^a	1.02 ± 0.02^a	1.02 ± 0.02^a	0.731
Total feed intake (g)	62.95 ± 4.09^a	60.57 ± 5.33^a	65.57 ± 7.15^a	0.841
Weekly survival rate (%)	90.00 ± 1.66^b	93.02 ± 1.18^{ab}	95.50 ± 5.52^a	0.010

The different superscript letters in the same row indicate significant differences ($p < 0.05$).

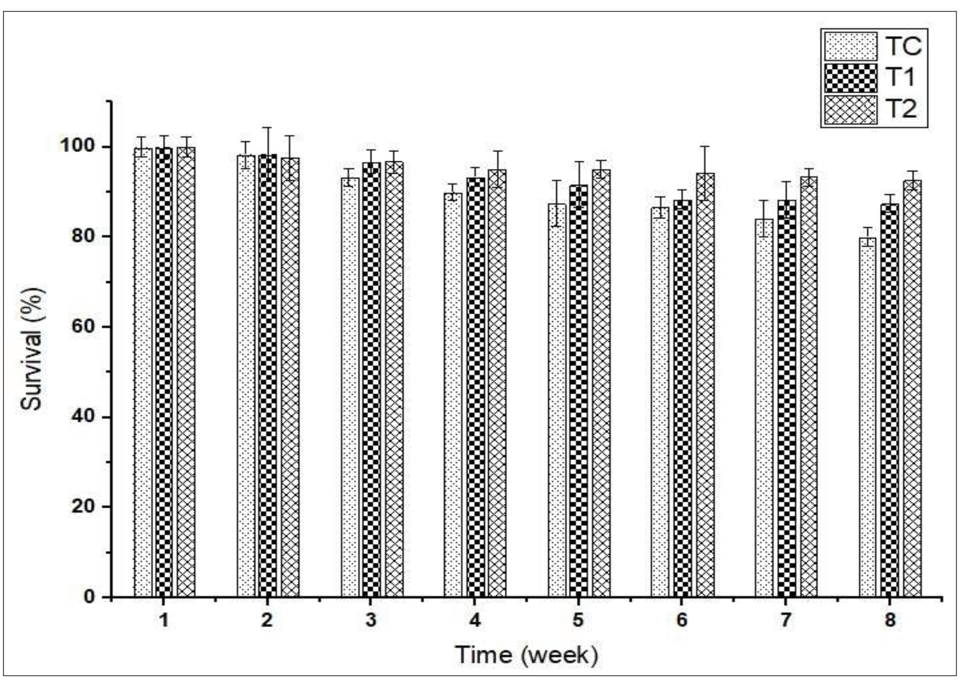

Figure 3: The survival rate among treatments (Initially, treatment T_1, T_2, and control tanks showed similar survival percentages. At the end of the study, control tanks showed the lowest survival and probiotic-treated tanks showed the highest survival compared to control tanks).

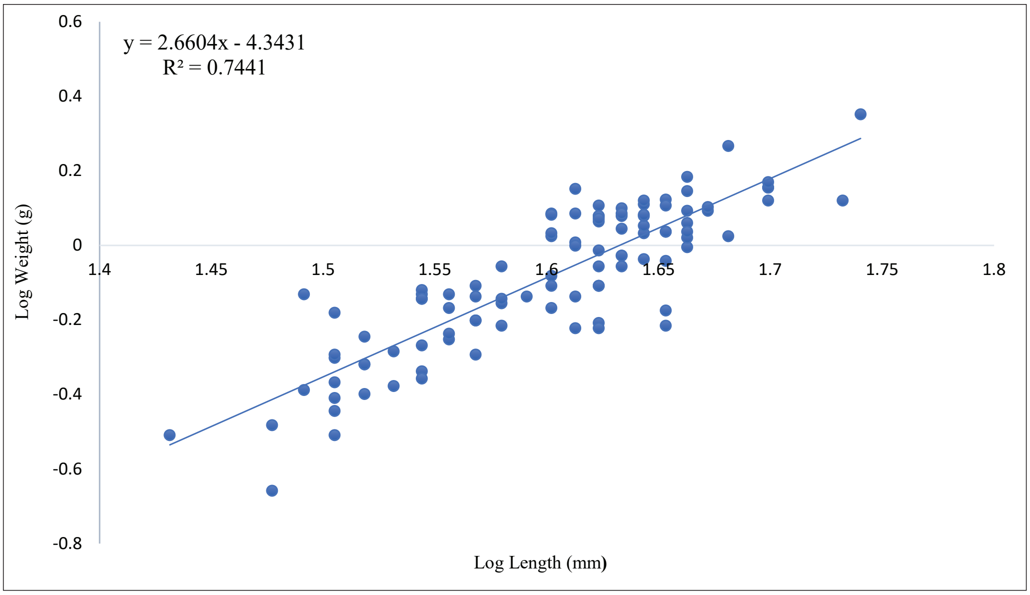

Figure 4: Length-weight relationship of *C. catla* supplement probiotic in T_1 tanks.

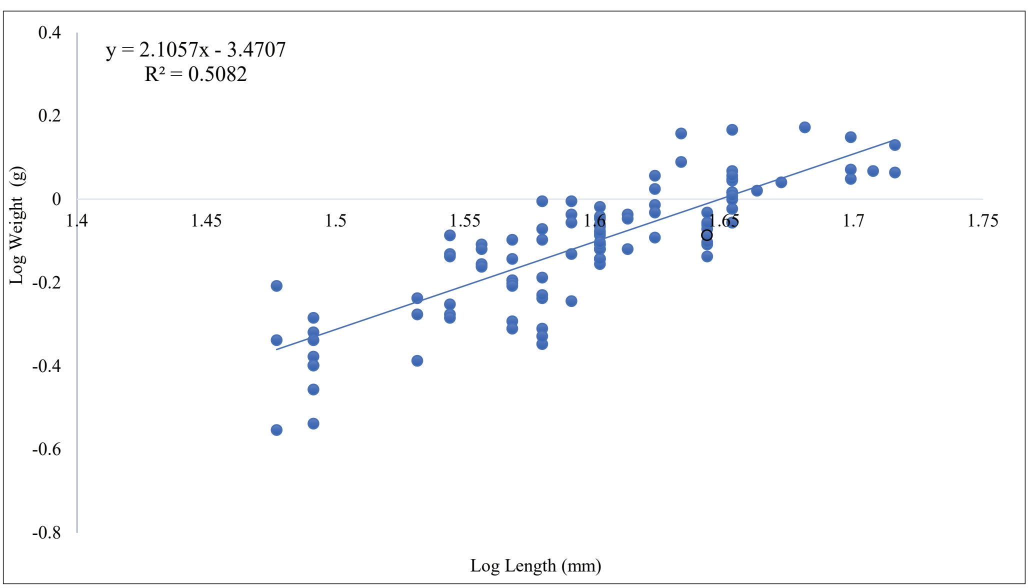

Figure 5: Length-weight relationship of *C. catla* supplement probiotic in T_C tanks.

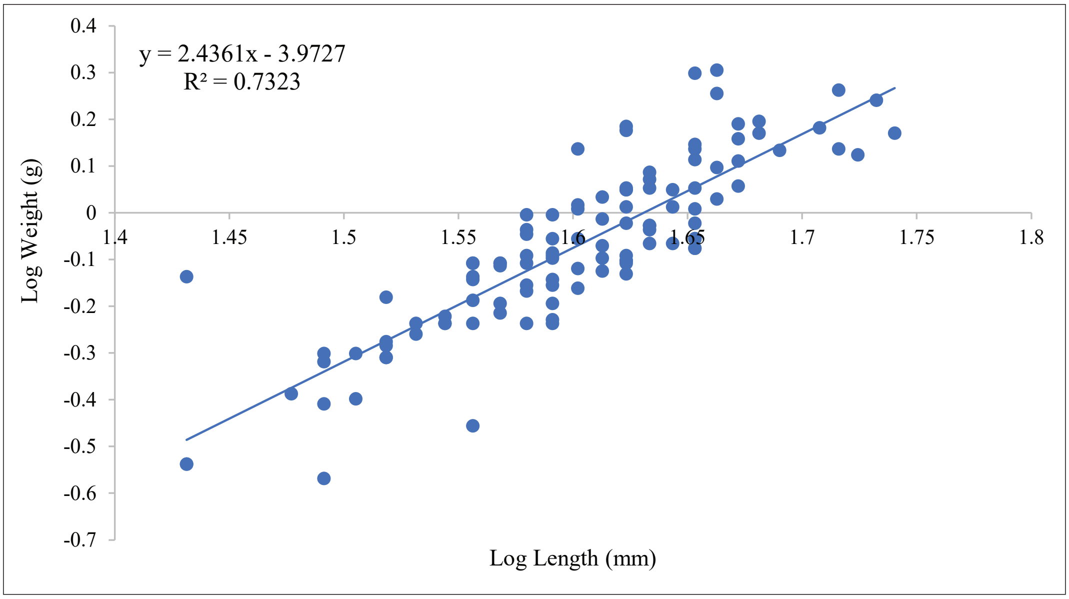

Figure 6: Length-weight relationship of *C. catla* supplement probiotic in T_2 tanks.

Water quality

PCA was employed to analyze the water quality indicators, including pH, TDS, Temperature, EC, DO, NO_3^-, and NH_4^+, of all treatment samples. The first four components were selected based on their eigenvalues above the threshold of unity, a widely used standard. Four PCs were obtained by extracting the correlation matrix. These PCs represent the processes that influence the chemical composition of water and account for approximately 84.0% of the total variance in the sample. The details can be found in Table 2. The PC variance is as follows: 34.9% for PC1, 21.4% for PC2, 14.6% for PC3, and 13.1% for PC4. All PCs that had eigenvalues larger than 0.9 were kept in this investigation. The first principal component strongly relates to two of the original variables. The physicochemical attributes exhibited minimal positive loadings to the factor (PC1) in the data sets. PCA assesses the availability of either a positive or negative correlation. A value close to 1 suggests a strong correlation between the parameter and the PC. The number exceeding 0.75 signifies a strong association. The study revealed a strong correlation among values ranging from 0.5 to 0.74. The first principal component positively correlates with rising TDS and EC scores. This indicates that these two criteria exhibit a correlation. The variables exhibit a positively correlated pattern, whereby the increase of one corresponds to the increase of others. Table 2 demonstrates that TDS and EC have strong positive loadings on PC 1, while DO and pH significantly contribute to PC2. In addition, NH_4^+ exhibits the most significant favourable loading on PC3, while temperature and NO_3^- display the most excellent favourable loading on PC4.

Additionally, it has been shown that the first principal component exhibits the highest correlation with the TDS. With a correlation coefficient 0.553, this primary component predominantly indicates the TDS. Parameters with high values typically indicate a significant amount of TDS available for treatment. The second principal component is positively correlated with none of the variables but negatively correlated with pH and DO. The data set showed a strong positive correlation (0.898) between the factor PC3 and the physicochemical feature of NH_4^+.

Additionally, the biplot derived from the PCA analysis illustrates the connections between different treatment groups in the two-dimensional space defined by the first two components. Principal component 1 has similar heavy loadings for NO_3^-, NH_4^+, DO, temperature, TDS, EC, and pH. The biplot reveals that the ammonia, TDS, EC, and nitrate water quality parameters indicate higher levels of differences from the origin than the pH, temperature, and DO parameters.

Table 2: PCA loadings and percentage of variance were explained for water quality parameters in the study on the impact of a probiotic mixture on *C. catla*.

Parameter	PC1	PC2	PC3	PC4
PH	0.126	-0.588	0.252	0.449
TDS	0.553	0.287	0.040	-0.085
Temperature	0.337	-0.380	-0.028	-0.568
DO	0.398	-0.518	-0.101	0.059
EC	0.545	0.320	-0.016	-0.109
NO_3^-	0.307	0.181	-0.342	0.663
NH^+_4	0.121	0.156	0.898	0.117
Eigenvalue	2.4449	1.4997	1.0225	0.9197
% Cumulative variance	34.9	56.4	71.0	84.1
% variance explained	34.9	21.4	14.6	13.1

Table 3: Effect of the probiotic supplement on water quality in *C. catla* tanks (Mean±SE).

Parameters	T_C Control	T_1 Treatment	T_2 Treatment	p value
pH	6.81 ± 0.07^a	6.74 ± 0.07^a	6.63 ± 0.07^a	0.261
Temperature (ºC)	24.99 ± 0.16^a	25.16 ± 0.15^a	25.09 ± 0.15^a	0.761
TDS (mg L^{-1})	71.77 ± 4.27^a	74.43 ± 2.90^a	74.65 ± 3.50^a	0.821
DO (mg L^{-1})	6.19 ± 0.14^b	6.69 ± 0.09^b	6.85 ± 0.12^a	0.010
EC (µS cm^{-1})	146.34 ± 7.43^a	159.97 ± 7.24^a	148.03 ± 7.81^a	0.194
TAN (mg L^{-1})	0.083 ± 0.07^a	0.052 ± 0.04^b	0.064 ± 0.05^b	0.006
NO_3^--N (mg L^{-1})	1.76 ± 0.18^b	2.56 ± 0.34^a	2.54 ± 0.32^a	0.006

The different superscript letters in the same row indicate different subsets identified by analysis of variance (ANOVA) followed by Tukey post-hoc multiple comparisons of significant differences among groups. Means ± SE in the same row superscripted with varying lowercase letters are significantly ($p < 0.05$) different.

The water quality parameters of the control and treatment tanks are given in Table 3. The control tanks had the lowest average DO levels (6.19 ± 0.14 mg L-1), while the T2 had the highest average DO levels (6.85 ± 0.12 mg L-1). The DO level at the treatment tanks was significantly higher than that of the control ($p < 0.05$). After two weeks, the tanks treated with probiotics exhibited notably elevated concentrations of DO, and this increase persisted over time (Figure 08). No statistically significant difference ($p > 0.05$) in the mean values for pH, temperature, TDS, and EC was observed between the treatment and control groups.Nevertheless, the transparency levels of the groups treated with probiotics (T_1 and T_2) remained consistently low. The concentrations of ammonia differed significantly between the two treatment groups that received probiotics and the control group ($p < 0.05$). The control groups (Tc) had the highest average TAN concentration of 0.083 ± 0.01 mg L^{-1}, while the treatments with probiotics had the lowest average TAN concentrations (T_1 = 0.052 ± 0.04 mg L^{-1} and T_2 = 0.064 ± 0.05 mg L^{-1}, $p < 0.05$). In addition, NO_3^- concentrations in the T_1 and T_2 treatments were significantly higher compared to the control Tc groups ($p < 0.05$). The concentration of NO_3^- increased as the culture age progressed in all treatments (Figure 09), ranging from 1.76 to 2.54 mg L^{-1}. In addition, probiotic supplement-treated T_2 groups had the highest mean NO_3^- (2.56 ± 0.34 mg L^{-1}), whereas control treatments had the lowest mean NO_3^- (1.76 ± 0.18 mg L^{-1}). The water temperature in the treatment and control groups varied between 24.99 ºC and

25.16 ºC. The temperature exhibited uniform fluctuations throughout the entire duration of the experiment. The pH remained consistent and within acceptable limits throughout the study. Nevertheless, the result indicated a diminished concentration of water colour in the groups that received probiotic treatment (T_1 and T_2). A statistically significant difference ($p < 0.05$) was found in the concentration of TAN between the two groups treated with probiotics and the control group (Figure 07). In addition, the control group Tc had the highest average TAN concentration of 0.083 ± 0.007 mg L^{-1}. In contrast, the probiotic treatments ($p < 0.05$, T_2 = 0.064 ± 0.005 mg L^{-1} and T_1 = 0.052 ± 0.004 mg L^{-1}) had the lowest average TAN concentration. The nitrate concentration in the T_1 (2.56 ± 0.34 mg L^{-1}) and T_2 (2.54 ± 0.32 mg L^{-1}) groups were significantly higher (p = 0.006, $p < 0.05$) compared to the control Tc groups.

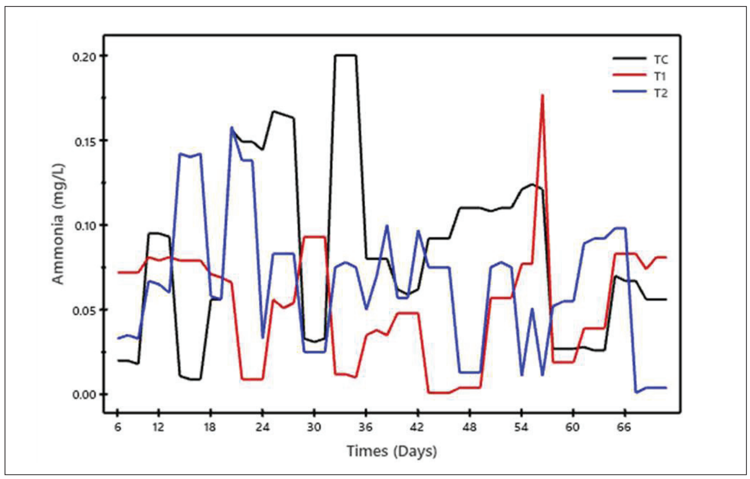

Figure 7: Variation of Ammonia (NH_4^+) concentration during the experimental period.

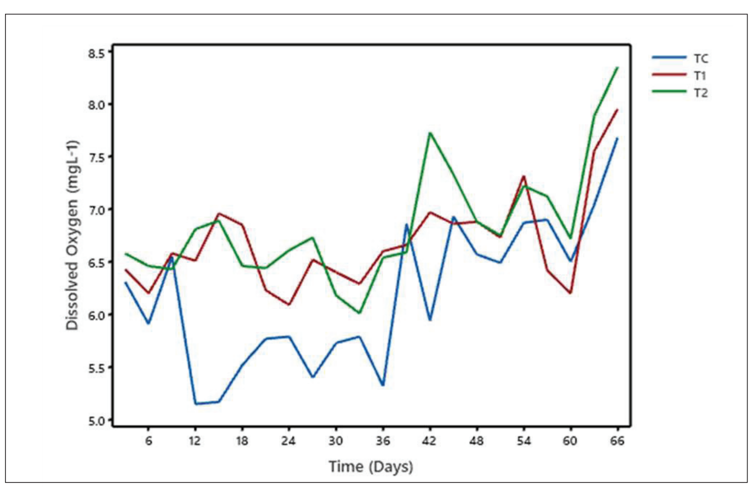

Figure 8: Variation of dissolved oxygen (DO) concentration during the experimental period.

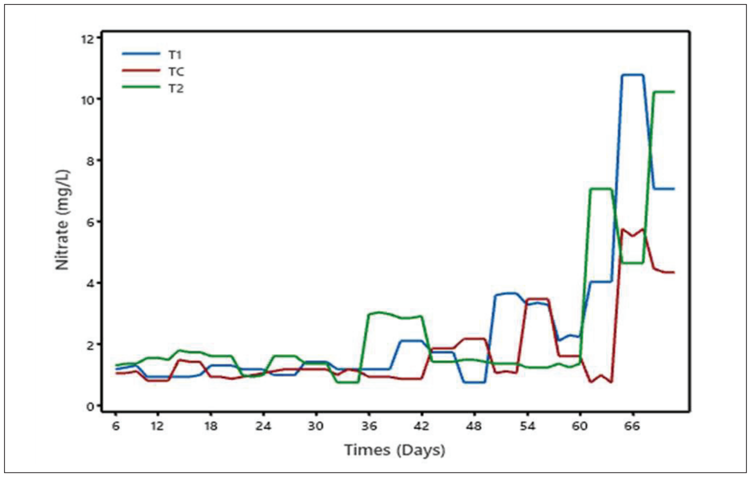

Figure 09: Variation of Nitrate (NO_3^-) concentration during the experimental period.

The study findings showed that probiotic direct supplementation significantly impacted the water quality parameters in the rearing water. The probiotic-treated tanks showed significantly greater dissolved oxygen levels than the control groups. Therefore, providing probiotic supplements containing *Bacillus* sp. can increase DO concentration in aquaculture (Mishra & Sharma, 2021; Nayak, 2020; Olmos, 2014). According to some earlier studies, adding *Bacillus* sp. improved the dissolved oxygen concentrations in waterways (Sahu *et al.*, 2008; Shah *et al.*, 2021; Tabassum *et al.*, 2021). The bacterial spores, such as *B. subtilis*, *B. licheniformis*, *P. acidilactici*, *Bacillus* sp. coagulants, *Nitrosomonas* sp., and *Nitrobacter* sp., were consistent in the bacterial mixture used in this experiment. These specific bacteria are referred to as probiotic bacteria from the genera *Pseudomonas* sp., *Rhodopseudomonas* sp., *Bacillus* sp., *Nitrosomonas* sp., and *Nitrobacter* sp. as potential bioremediation for aquaculture waste (Shah *et al.*, 2021; Tabassum *et al.*, 2021). These bacterial species inhibit pathogenic activities and expedite the aquaculture water degradation, which was undetectable in the present research.

The study showed a significant difference in TAN and NO_3^- concentrations between the treatment and control groups. Tanks treated with probiotics had lower TAN concentrations. Previous research has recently shown how *Bacillus* sp. can survive in aerobic, anaerobic, and facultative aerobic environments; this unique characteristic permits a change in the metabolism of nitrogen species, boosting the nitrification and denitrification processes (Kumar *et al.*, 2015; Wang *et al.*, 2008). This could be due to the probiotic bacteria's ability for bioremediation and the approach to using probiotic supplements (Jahangiri, 2018; Samarawardane *et al.*, 2021). When the bacteria are present in tanks, they efficiently transform the organic matter into simple, non-toxic substances, which the bacteria need to initiate their metabolism, having the organic matter in the environment as the sole carbon source (Zhou *et al.*, 2009). This might account for probiotic-treated tanks having lower ammonia concentrations than control tanks. In addition, *Nitrobacter* sp. and *Nitrosomonas* sp. were used as probiotics and effectively broke down organic matter in water. This may also be the reason for the low NH_4^+ levels and high-water transparency in probiotic-treated tanks. However, the average TDS, pH, EC, and temperature values were not affected by the addition of probiotics to rearing water and were within acceptable levels. Similar results have been reported by Zhou *et al*. (2009) and Yang *et al*. (2021) also. Probiotic tank treatments might be a valuable approach in rearing *Catla catla* fry. More comparison studies are needed to determine the impact of probiotic supplements on the water quality of tanks used for livestock farming.

Growth performance and feed utilization

The results of this study revealed that the direct addition of the probiotic combination to the culture water had no impact on the growth parameters. This might be because the fish may not have taken in enough probiotics through this method of administration to stimulate their digestive mechanisms effectively (El-kady *et al.*, 2022). Similar trends were found (Hassan *et al.*, 2022; Jahangiri, 2018). Nevertheless, *C. catla* survival in ponds treated with probiotics was significantly higher than in control ponds. Previous studies have demonstrated that probiotic administration significantly increases host species survival rates (Tabassum *et al.*, 2021). When added directly to the water, the optimal dosage of probiotics should be determined based on the weight of the fish being treated and the volume of water (Hai, 2015). It is crucial to utilize probiotics properly since the manufacturer-recommended dosage also significantly increases the host species survival. The capacity of a species to fight invasions decides how long it can survive (El-Kady *et al.*, 2022). Probiotic supplementation has improved survival rates, particularly in earlier studies (Dawood & Koshio, 2016; Jahangiri, 2018). This could be caused by the administration method of the probiotic supplement.

Length-weight relationships (LWRs) and condition factors are generally used to evaluate the health and growth of fish (Froese *et al.*, 2013; Hossain *et al*., 2015). The LWR of each fish is influenced by its behaviour, maturity, sex, and diet (Kadhar *et al.*, 2014; Bag *et al.*, 2016). In this study, all groups including those who received probiotics displayed excellent growth. LWRs are important for comparing life histories of fish and play a significant role in fisheries management (Froese *et al.*, 2013). LWRs are important for conservation and to prevent the exploitation of young fish and reduce the impact on spawning stocks (Tabassum *et al.*, 2021). Present study found no relationship between probiotic administration and LWR of *Catla catla* fry. Thus, the findings indicated that addition of probiotics into water improved only a few water quality parameters and had no effect on the growth of the *Catla catla* fry. Their survival rates also increased probably due to changing of the water quality to desirable ranges. However, more studies on the direct administration of probiotics is needed to develop effective and affordable therapies for the species used in aquaculture.

CONCLUSION

Findings of this study revealed that probiotic supplementation added to culture water could significantly reduce the NH_4^+ concentration in the water while increasing the survival rate of young *C. catla* fry. However, this method of administration did not enhance their growth performance of *C. catla*. Therefore, it is recommended that more comprehensive studies be conducted on applying probiotics as a water additive.

Acknowledgement

This study was conducted at the Fish Nutrition Research, Development, and Training Section, Udawalawe, Sri Lanka, and Centre for Water Quality and Algae Research, University of Sri Jayewardenepura, Sri Lanka. The authors thank Mr. P.M. Withanage and the farm workers for collaborating during the experiment.

Ethics Statement

The researchers adhered to all institutional and national guidelines for the care and use of laboratory animals.

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