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Influence of Probiotics on Early Maturation and Reproduction of the Goldfish (*Carassius auratus*) in Hatchery Condition in Tropical Region

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ABSTRACT

The goldfish (*Carassius auratus*) is a highly valued ornamental species in the global aquaculture industry. However, optimizing early maturation and reproductive performance remains challenging, especially in tropical regions. Probiotics, which are live microorganisms that promote gut health, immune function, and overall growth, have emerged as a potential tool for enhancing reproductive outcomes in aquaculture. This study explored the influence of probiotics on the early maturation and reproductive success of goldfish under tropical hatchery conditions. Probiotics were incorporated into the diet at levels of 1, 2, and 3 grams per kilogram of formulated feed. Eight-month-old goldfish fry were fed these probiotic-supplemented diets for 2 months before being subjected to natural breeding in controlled environments. Natural breeding was observed in 10-month-old goldfish fed probiotics. The results indicated that higher temperatures (28°C) combined with probiotic supplementation significantly reduced maturation time and improved breeding performance compared to the control group. Among the different probiotic levels tested, the diet containing 2 grams per kilogram produced the best outcomes, with increased relative fecundity (31.15%), fertilization rates (85.68%), and hatching rates (82.35%). This finding aims to offer new insights into probiotic applications for boosting breeding efficiency and contributing to more sustainable ornamental fish production in tropical regions. The enhanced early maturation and breeding performance offer valuable opportunities for entrepreneurship, foreign currency earnings, and financial security in fish culture sector of Bangladesh.

INTRODUCTION

The goldfish (*Carassius auratus*), a popular ornamental species, holds significant value in the global aquaculture and aquarium industries due to its aesthetic appeal, adaptability, and ease of breeding in controlled environments **(Kestemont, 1995; Gouveia *et al*., 2003; Kaiser *et al*., 2003)**. In Bangladesh, the high demand for the goldfish has created a pressing need for large-scale breeding. With a significant portion of fry being exported **(Faruk, 2012)**, it is essential to establish a reliable and consistent supply to meet both domestic and international demands. By refining commercial breeding practices, Bangladesh could significantly boost goldfish production, meeting market demands, fostering entrepreneurship, securing financial benefits, and conserving foreign currency. As the demand for high-quality, well-bred goldfish increases, breeders face the challenge of optimizing growth, maturation, and reproductive outcomes, particularly in tropical regions like Bangladesh where environmental conditions can differ significantly from temperate zones.

One promising approach to enhance the early maturation and reproductive success of goldfish in hatchery conditions is the use of probiotics. Probiotics, defined as live microorganisms that confer health benefits to the host when administered in adequate amounts, have gained attention for their role in improving overall fish health, growth, immune function, and stress resistance **(Balcazar *et al*., 2007; Aly *et al*., 2008; Eissa *et al*., 2014)**. By modulating gut microbiota, boosting immune responses, and reducing stress, probiotics can contribute to healthier broodstock and improved breeding results **(Mehdinajad *et al*., 2018)**. They have the potential to increase fertility and stimulate higher egg production rates, leading to more successful breeding outcomes in species like zebrafish **(Carnevali *et al*., 2017)**. Moreover, probiotics can be passed from parent fish to their offspring, fostering the development of a healthy gut microbiota in larvae and potentially enhancing survival rates and growth in juvenile goldfish **(Adorian *et al*., 2019)**. Despite these promising benefits, more research is needed to understand the specific effects of probiotics on the goldfish broodstock and their reproductive efficiency, particularly in the stressful context of captive breeding environments **(Tang & Affandy, 2000)**.

In tropical regions, the warm water temperatures characteristic of hatchery environments can accelerate the growth and metabolic rates of the goldfish **(Volkoff & Rønnestad, 2020; Muziri *et al*., 2022)**. The use of probiotics in hatchery diets offers the potential to mitigate some of these challenges by enhancing gut health, promoting efficient feed utilization, and supporting robust immune responses.

While the use of probiotics in aquaculture is well-documented for various species **(El-Haroun *et al*., 2006; Yin *et al*., 2011; Zapata & Lara-Flores, 2013; Cienfuegos *et al*., 2018; Mehdinejad *et al*., 2018; Aydin & Cek-Yalnniz, 2019)**, limited research has focused specifically on ornamental fish like goldfish, particularly in the context of early maturation and reproduction under tropical conditions. Understanding the influence of probiotics on reproductive performance is crucial for hatchery operators aiming to maximize the production of high-quality broodstock and fry. Moreover, enhancing the reproductive efficiency of the goldfish through probiotic supplementation could lead to more sustainable aquaculture practices. By promoting early maturation, probiotics could also shorten the breeding cycle, allowing for more frequent spawning and higher fry output in commercial hatcheries.

This study aimed to investigate the effects of probiotic supplementation to evaluate the influence of different probiotic formulations on early maturation, reproductive success, larval survival and quality, and health and growth performance in the goldfish. Specifically, the study aimed to assess the onset of sexual maturity in the goldfish fed with probiotic-enriched diets, measure key reproductive parameters such as spawning frequency, fecundity, and egg viability, and evaluate the survival rate and overall quality of larvae produced from probiotic-fed broodstock. Additionally, the research monitored the overall health, immune response, and growth performance of goldfish during the reproductive cycle under tropical hatchery conditions. By focusing on these key aspects, the study will contribute to a deeper understanding of how probiotics can enhance breeding efficiency and support the sustainable production of ornamental fish in tropical climates.

MATERIALS AND METHODS

Collection and rearing of experimental fishes

A total of 250 healthy and homogenous size (7.5 months, 8-9cm) juvenile goldfish were collected from a fish hatchery located at Kubuddhi, Munshiganj and transferred to Fish Hatchery of Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. The hatchery was facilitated with roof, good water supply, outlet system and natural air. Fish were allowed to acclimatize to the hatchery conditions for two weeks. Fishes were fed supplementary feed (38% protein) at 5% of body weight twice a day during acclimatization period. Circular plastic tanks (300L water capacity) were used for fish acclimatization and rearing.

Preparation of control and probiotic treated feeds

The probiotic powder was prepared from Pharma & Farm Co. (Dhaka, Bangladesh) with 35×10^{10} CFU mixture of *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus polymyxa* and *Lactobacillus* sp. A commercial diet from Mega Feed (38% crude protein, 11% lipid, 1.16% phosphorus, 11% moisture, 10% ash, and 3% fiber) was used as a control diet. Diets were prepared by adding the mixture of 50ml saline serum with 1, 2, and 3g probiotic powder per kg diet to make the concentrations 1×10^{10} (T1), 2×10^{10} (T2), and 3×10^{10} (T3) CFU g^{-1} of probiotic bacteria in diet, except for the control diet T0 (without probiotic).

Feed management

Fish were separated in two treatments with 3 replications. In treatment I, fish were fed with probiotics; whereas in Treatment II, fish fed without probiotics. The fishes were fed the probiotic treated feed at the body weight of 5% for 60 days. Feed was applied twice in a day at 9:00 and 17:00 under normal light regime.

Natural breeding of goldfish

A rectangular-shaped aquarium was utilized for the natural breeding of fish, with continuous aeration maintained throughout. Artificial polyethylene strips, known as breeding mops, were employed as a substrate for egg attachment. Mature male and female broodfish were distinguished based on secondary sexual characteristics and housed separately. Prior to being placed in the breeding tank, the selected broodfish were conditioned in separate tanks for approximately 6 hours. They were introduced into the breeding tank at a ratio of 2:1 (male to female). Once both males and females were placed in the breeding tank, egg release occurred within 48 hours.

Monitoring of water quality parameters

Water quality parameters like dissolved oxygen (mg/l), water temperature (°C), pH, and ammonia (NH_3) were monitored fortnightly during the study period as per standard **(APHA, 1989)**.

Breeding performance analysis

Relative fecundity (%), fertilization rate (%), hatching rate (%) and survival rate (%) were calculated following the method of **Sharma *et al*. (2011)**.

Data analysis

Data of relative fecundity, fertilization rate, and hatching success were collected during the study period and statistically analyzed using one-way analysis of variance (ANOVA) to test the significance of results ($P< 0.05$) between means, and the mean values were separated by LSD (least significant difference) post hoc statistic. The standard deviation (±SD) was calculated to identify the range of means by IBM SPSS 26 software.

RESULTS

Water quality parameters

During the investigation period, the water temperature in the treatment tanks ranged from 27.06 to 28.76°C (Table 1), showing no significant difference ($P> 0.05$) over the experimental duration. Throughout this period, pH levels remained stable without significant fluctuations ($P< 0.05$) (Table 1). No hypoxic or anoxic conditions were observed in the morning, with dissolved oxygen (DO) concentrations consistently between 5.2-5.4mg/ l, showing no significant variability ($P< 0.05$) (Table 1). Ammonia levels in the water across different treatments ranged from 0.25 ± 0.00 to 0.50 ± 0.00mg/l (Table 1), with no significant differences ($P> 0.05$) observed among treatments throughout the experimental period.

Table 1. Water quality parameters recorded in different treatments during rearing period

Parameter	Treatment	No. of samplings				
		Initial	1st	2nd	3rd	4th
Temp (°C)	T0	28.14±0.15	28.25±0.20	27.45±0.24	27.06±0.34	27.28±0.31
	T1	28.35±0.15	28.35±0.4	28.18±0.34	27.26±0.24	27.06±0.32
	T2	28.35±0.20	29.25±0.24	28.34±0.5	27.42±0.15	27.57±0.5
	T3	28.76±0.25	28.54±0.49	28.48±0.5	27.14±0.17	27.25±0.55
DO (mg/ml)	T0	5.52±0.06	5.67±0.2	5.30±0.04	5.19±0.11	5.67±0.26
	T1	5.28±0.14	5.8±0.19	5.53±0.16	5.89±0.07	5.92±0.02
	T2	5.37±0.28	5.08±0.24	5.19±0.06	5.88±0.15	5.19±0.16
	T3	5.86±0.18	5.00±0.09	5.24±0.08	5.9±0.17	5.79±0.26
pH	T0	7.6 ± 0.35	7.2 ± 0.23	7.8 ± 0.23	7.5 ± 0.16	7.4 ± 0.17
	T1	7.6 ± 0.35	7.4 ± 0.16	7.4 ± 0.17	7.5 ± 0.16	7.5 ± 0.16
	T2	7.5 ± 0.16	7.6 ± 0.16	7.7 ± 0.23	7.6 ± 0.35	7.6 ± 0.35
	T3	7.6 ± 0.35	7.3± 0.35	7.4 ± 0.17	7.6 ± 0.35	7.6 ± 0.35
Ammonia (mg/ml)	T0	0.32±0.14	0.41±0.14	0.50±0.00	0.33±0.14	0.41 ± 0.14
	T1	0.41±0.14	0.32±0.14	0.25±0.00	0.41±0.14	0.33 ± 0.14
	T2	0.37±0.14	0.50±0.00	0.53±0.00	0.33±0.14	0.41 ± 0.14
	T3	0.34±0.14	0.25±0.00	0.33±0.14	0.41±0.14	0.33 ± 0.14

Effects of dietary probiotic on breeding performances of goldfish

After stripping, the eggs were directly counted, yielding totals of 254, 445, 778, and 615 in treatments T0, T1, T2, and T3, respectively. The highest number of stripped eggs, 778, was observed in T2, while the lowest count, 254, was recorded in T0, indicating that the treatment without probiotics resulted in reduced egg production. The highest relative fecundity of 31.15% was also observed in T2, whereas the lowest, 12.07%, occurred in T0. There was a significant variation among the treatments, with T2 significantly outperforming both T0 and T3 ($P < 0.05$) (Table 2).

Fertilization rates varied across treatments, with T2 achieving the highest rate of 85.68%, while T0 had the lowest at 33.55%. A significant difference was observed among the treatments, with T2 performing significantly better than T0 and T3 ($P < 0.05$) (Table 2). Hatching rates for goldfish eggs were recorded as 30.05, 50.25, 82.35, and 70.58% for the 0, 1, 2, and 3mg/ kg probiotic doses, respectively. T2 exhibited the highest hatching rate at 82.35%, significantly surpassing T0, T1, and T3 (Table 2). Survival rates followed a similar trend, with T0, T1, T2, and T3 showing survival rates of 30.05, 50.25, 82.35, and 70.58%, respectively. Significant differences were observed among the treatments, with T2 demonstrating significantly higher survival rates compared to the other treatments ($P < 0.05$).

Table 2. Performance of stripped eggs, relative fecundity, fertilization rate, hatching rate and larval survival rate corresponding to the different treatments in goldfish

Treatment	Stripped eggs	Relative fecundity (%)	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)
T0	254.52±34.27^{a}	12.07±0.54^{a}	33.55±2.14^{a}	30.05±0.75^{a}	33.35±2.17^{a}
T1	445.32±35.65^{a}	20.22±0.62^{b}	55.45±2.85^{b}	50.25±1.44^{b}	55.44±2.66^{b}
T2	778.54±55.15^{a}	31.15±0.25^{c}	85.68±2.14^{c}	82.35±1.35^{c}	85.55±1.36^{c}
T3	615.45±32.15^{a}	24.05±0.92^{d}	72.45±2.35^{d}	70.58±1.24^{d}	75.55±1.34^{d}

The same superscript for the mean values did not indicate a significant difference (P>0.05).

DISCUSSION

The optimal temperature range for the goldfish is generally between 18 and 22°C **(Mahmud *et al*., 2011; Targońska *et al*., 2012)**, which is lower than the temperatures observed in our current study. However, **Sharma *et al*. (2011)** documented successful goldfish breeding at temperatures ranging from 23 to 28°C under laboratory conditions in Haryana, India. Additionally, **Krejszeff *et al*. (2008)** demonstrated that gradually increasing the water temperature from 22 to 27°C after hormone injection also led to successful spawning. These findings are consistent with our study and suggest that the goldfish can be effectively bred in tropical regions such as Bangladesh. Moreover, warmer water temperatures generally accelerate metabolic processes, potentially leading to earlier sexual maturation **(Barton & Morgan, 1993)**. **Krejszeff *et al*. (2008)** explored the impact of temperature changes on the goldfish breeding and maturation, emphasizing that gradual increases in temperature can induce earlier spawning. Similarly, **Sharma *et al*. (2011)** observed varying water temperatures affect the reproductive performance of goldfish, including the timing of sexual maturation.

The pH levels recorded during the experiment fell within the acceptable range which supports fish survival, growth and natural productivity **(Popma & Masser, 1999)**. The ideal pH range for freshwater fish is typically between 6.8 and 7.2 **(Cooper, 2004)**, though the goldfish generally thrive in pH ranges from 6.5 to 8.5. **Prema Latha and Lipton (2007)** successfully reared goldfish within pH ranges of 6.0 to 8.3 across various filter systems, consistent with the findings of this study. No hypoxic or anoxic conditions were observed during the morning of the experiment. The DO concentration ranged from 5.2 to 5.4mg/ l, with no significant fluctuations (P< 0.05). This DO range is considered suitable for goldfish, as ideal levels for warm water fish are typically above 5mg/ l, with 6.5mg/ l or higher being optimal **(Francis-Floyd, 1997)**. According to **Krejszeff *et al*. (2008)**, the DO levels in this study were adequate for the goldfish throughout the experimental period. There were no significant differences (*P*> 0.05) in ammonia levels among the different experimental aquaria. Ammonia, a key nitrogenous waste product excreted by fish through their gills and feces, typically ranges from 0.01 to 1.15mg/ l in tank culture systems, which supports the findings of this study **(Nyanti *et al*., 2012; Boyd *et al*., 2016; Devi *et al*., 2017)**.

The number of stripped eggs, relative fecundity (%), fertilization rate (%), hatching rate (%) and survival rate (%) all increased in goldfish treated with probiotics compared to the control group. Among the different treatments, the diet with a probiotic dose of 2g/ kg feed yielded the highest values across all parameters. In this study, the amount of stripped eggs was higher than what was previously observed in induced breeding of goldfish **(Targońska *et al*., 2012)**. The higher egg count in probiotic-treated fish suggests that probiotics may positively influence ovulation and egg quality. The increase in relative fecundity with probiotic supplementation aligns with findings by **Zadmajid *et al*. (2012)**. Similarly, **Gioacchini *et al*. (2010)** investigated the impact of the probiotic *Lactobacillus rhamnosus* on zebrafish fecundity, revealing that ten days of probiotic application altered gene expression and led to an increased number of ovulated eggs, indicating enhanced fecundity.

Abasali and Mohamad (2010) also demonstrated that incorporating the commercial probiotic Primalac into artificial feeds for the swordtail (*Xiphophorus helleri*) over 26 weeks significantly increased gonadosomatic indices, fecundity, and larval quality of female broodstock. **Carnevali *et al*. (2013)** reported that prolonged administration of *L. rhamnosus* positively influenced the reproductive system's physiology, supporting the findings of this study. Additionally, **Ghosh *et al*. (2007)** found that *Bacillus subtilis* supplementation in female ornamental fish could enhance fecundity, egg viability, and larval quality.

The hatching rate in goldfish reached 82% in treatment T2, which aligns with the results observed in telescopic-eyed goldfish by **Mahadevi *et al*. (2018)**. Although hatching rates of up to 96% were reported by **Zadmajid *et al*. (2012)** when goldfish were subjected to induced breeding, the probiotic treatments in this study suggest a strong hatching rate under reduced reproduction time and natural breeding conditions. The inclusion of probiotics in the diet also tended to increase the survival rate of the goldfish compared to fish that did not receive probiotics. This finding is consistent with **Rohani *et al*. (2022)**, who reported that probiotics could enhance the immune system, ultimately improving the survival rate of fish. Furthermore, probiotics can improve water quality by degrading organic compounds and inhibiting pathogenic bacteria in both the water and the fish, which may also contribute to the higher survival rates observed.

The relationship between probiotics and early maturation in the goldfish is still a developing area of study, though some research in other fish species has shown positive effects on growth, immune function, and reproductive health, which could be extrapolated to suggest similar outcomes in the goldfish. **Merrifield *et al*. (2010)** argued that the use of probiotics in aquaculture effects growth, immune function, and overall health, which can influence reproductive outcomes in fish. Although focused on tilapia, **Naiel *et al*. (2022)** demonstrates how probiotics can positively affect growth performance and immune response, which could be relevant to understanding similar effects in goldfish. **Balcázar *et al*. (2007)** and **Gatesoupe (2008)** explored the role of probiotics in improving fish health and growth, which indirectly supports reproductive health and could influence maturation timing. However, more specific research is needed to fully understand the direct impact of probiotics on goldfish maturation.

CONCLUSION

Research on the effects of probiotics on the maturation and breeding performance of the goldfish is still in its early stages but is gaining attention. The study has provided valuable insights into the potential benefits of probiotic supplementation. The findings suggest that incorporating probiotics into the diets of the goldfish can promote early sexual maturity, enhance reproductive performance, and improve the overall health and growth of broodstock. Probiotic-fed fish demonstrated increased spawning frequency, higher fecundity, and improved egg viability, leading to better larval survival and quality. Additionally, the positive impact on immune response and growth performance highlights the role of probiotics in supporting both the breeding efficiency and the sustainable production of ornamental fish in tropical regions. This research underscores the potential of probiotics to enhance aquaculture practices, making it possible to meet the growing demand for goldfish in both domestic and international markets, especially in tropical climates like Bangladesh where hatchery conditions may pose unique challenges.

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Data availability statement

The datasets used and analyzed during the current study will be provided on request to the corresponding author.

Conflicts of interest

The authors declare that they have no conflict of interest.

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