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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Enhancing goldfish reproduction: Role of substrates in optimizing fertilization and hatching rates under controlled conditions

Japon balığı üremesinin iyileştirilmesi: Kontrollü koşullar altında döllenme ve yumurtadan çıkma oranlarının optimize edilmesinde substratların rolü

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Abstract: Ornamental fish production is significantly impacted by whether fish spawn naturally under controlled conditions. Therefore, goldfish (*Carassius auratus*) were allowed to breed naturally using various types of substrates to investigate their effects on ovulation, fertilization, and hatching rates in an experimental setup. The goldfish were subjected to five different substrate treatments: T1 (water hyacinth), T2 (jute rope), T3 (polythene), T4 (net), and T5 (no substrate). The optimal pH and dissolved oxygen levels for goldfish spawning were found to be 7.14 to 7.24 and 5.65 to 6.22 mg/L, respectively. Results indicated that the highest number of eggs (356.66±40) was observed in the polythene substrate (T3), while no eggs were found in the absence of substrate (T5). The polythene substrate also yielded the highest fertilization rate (93%) and hatching rate (95.01%). Notably, goldfish exhibited no spawning behavior without any substrate, suggesting that substrate may act as both a spawning substrate and an essential cue for ovulation in goldfish. Furthermore, the study's findings support the recommendation of goldfish as substrate breeders. This research offers valuable insights for small-scale fish farmers, entrepreneurs, and hatchery owners looking to enhance goldfish spawning techniques in aquarium settings.

Keywords: Goldfish, substrate, ornamental fish, fertilization, hatching

Öz: Süs balığı üretimi, balıkların kontrollü koşullar altında doğal olarak yumurtlayıp yumurtlamadıklarından önemli ölçüde etkilenir. Bu nedenle, Japon balıklarının (*Carassius auratus*) yumurtlama, döllenme ve yumurtadan çıkma oranları üzerindeki etkilerini araştırmak için çeşitli substrat türleri kullanılarak doğal yollarla üremelerine izin verilmiştir. Süs balığı üretimi, balıkların kontrollü koşullar altında doğal olarak yumurtlayıp yumurtlamadıklarından önemli ölçüde etkilenir. Bu nedenle, Japon balıklarının (*Carassius auratus*) yumurtlama, döllenme ve yumurtadan çıkma oranları üzerindeki etkilerini araştırmak için çeşitli substrat türleri kullanılarak doğal yollarla üremelerine izin verilmiştir. Sonuçlar, en yüksek yumurta sayısının (356,66±40) polietilen substratta (T3) gözlendiğini, substrat yokluğunda (T5) ise hiç yumurta bulunmadığını göstermiştir. Polietilen substrat aynı zamanda en yüksek döllenme oranını (%93) ve kuluçka oranını (%95,01) vermiştir. Özellikle, Japon balıkları herhangi bir substrat olmadan yumurtlama davranışı sergilememiştir, bu da substratın Japon balıklarında hem yumurtlama substratı hem de yumurtlama için önemli bir ipucu olarak işlev görebileceğini düşündürmektedir. Ayrıca, çalışmanın bulguları Japon balıklarının substrat yetiştiricileri olarak önerilmesini desteklemektedir. Bu araştırma, akvaryum ortamlarında Japon balığı yumurtlama tekniklerini geliştirmek isteyen küçük ölçekli balık yetiştiricileri, girişimciler ve kuluçkahane sahipleri için değerli bilgiler sunmaktadır.

Anahtar kelimeler: Japon balığı, substrat, süs balığı, döllenme, kuluçka

INTRODUCTION

In recent times, a growing number of people across various societal segments have embraced the practice of maintaining aquariums in commercial, public, and residential settings. This trend highlights the potential of domestic ornamental fish production to contribute significantly to export revenues and foster cost savings (Rahaman et al., 2011). Bangladesh is renowned for its extensive inland water bodies and diverse indigenous fish species (Ali et al., 2017; Mia et al., 2017), ranging from large to small. Some indigenous species like *Trichogaster fasciata*, *T. lalia*, *Badis badis*, *Esomus danricus*, and *Ompok bimaculatus* have been popular choices for aquariums or ornamental purposes. Additionally, several exotic species have been imported from other countries. The goldfish (*Carassius auratus*) stands out as the most widespread cyprinid fish in freshwater aquariums globally.

In contrast, both common carp and goldfish have become invasive on a global scale (Chan et al., 2019; Halas et al., 2018). Their life history strategies, including broad feeding habits, high reproductive rates (Tang et al., 2020), early maturation, rapid growth compared to native species (Jones and Stuart, 2009; Morgan and Beatty, 2007), and tolerance to extreme environmental conditions (Tang et al., 2020), contribute to their success in unfamiliar habitats. These fish are generally hardy, peaceful towards other tank inhabitants, and well-suited for aquarium environments. Originating from the Prussian gibel carp (*Carassius gibelio*), goldfish were first domesticated in China around 1000 AD and are native to China (Komiyama et al., 2009; Vasil'eva and Vasil'ev, 2000). Breeders have developed a variety of ornamental goldfish breeds with distinctive features such as fringed, veil, or finned tails, double or triple fins, and bulging "telescope" eyes. Many of these varieties, known as scaled goldfish, exhibit metallic hues ranging from scarlet, gold, and white to silver or black.

The physiological and behavioral responses of certain aquarium fish can be significantly influenced by the presence of substrate. When deprived of substrate, some species may exhibit immobility, indicating unmet behavioral needs (Smith and Gray, 2011; Galhardo et al., 2008; Stenberg and Persson, 2005). Substrate plays a crucial role in facilitating egg adhesion during spawning in aquarium environments. Haniffa et al. (2007) demonstrated the use of substrate for breeding koi carp, where it served both as a hiding place and a surface for egg attachment. Certain fish species naturally deposit their eggs on the tank floor; without substrate, these eggs are vulnerable to predation by mature fish. The introduction of variegated substrate helps to camouflage the eggs, and larger substrate sizes can provide protective gaps where eggs can settle securely. Goldfish eggs, known for their transparency and adhesive properties, typically adhere to aquatic vegetation (Battle, 1940).

In Bangladesh, the number of well-established goldfish hatcheries is quite limited, with most breeding operations conducted on a small scale. Goldfish are valued as experimental subjects due to their ability to adapt well to various environmental conditions (Battle, 1940). This study aims to explore specific aspects of natural reproduction within Bangladesh's ecological context. While artificial breeding methods for goldfish are established, understanding substrate preferences for their natural reproduction remains a new area of investigation. The use of substrate may enhance ovulation in goldfish, providing a simpler and cost-effective alternative to induced reproduction methods. Therefore, this study aims to develop a protocol for the controlled production of goldfish larvae under confined conditions, focusing on identifying an optimal substrate that promotes successful ovulation, fertilization, and hatching of goldfish.

MATERIALS AND METHODS

Study area

The three-month study took place in the wet laboratory of the Faculty of Fisheries, BAU, Mymensingh, Bangladesh, spanning from February to April.

Accumulation of specimens

Adult goldfish were sourced from various pet shops in the Katabon market, Dhaka city. Males and females were collected in groups of twenty pairs for breeding purposes. Two oxygenated polythene containers were used to transport these pairs, with each container accommodating ten pairs of fish. The broods exhibited colors ranging from red, orange, yellow, to black. Fish were acclimatized by submerging them in water within poly sacks for two hours. After conditioning, the fish were transferred to a glass aquarium for further rearing, where they were fed twice daily and had their water changed once daily. After seven days of acclimatization, the brood fish were moved to the breeding aquarium.

Determination of broods

Male and female goldfish were distinguished based on specific physical characteristics, such as abdominal condition, pigmentation of genital organs, and their ability to release sperm or eggs when gently pressed on the lower abdomen. Females could be identified by the presence of abdominal edema (43.35±2.2g), while mature males typically exhibited a slender physique and flattened abdomens (36.52±1.8g) (Figure 1).

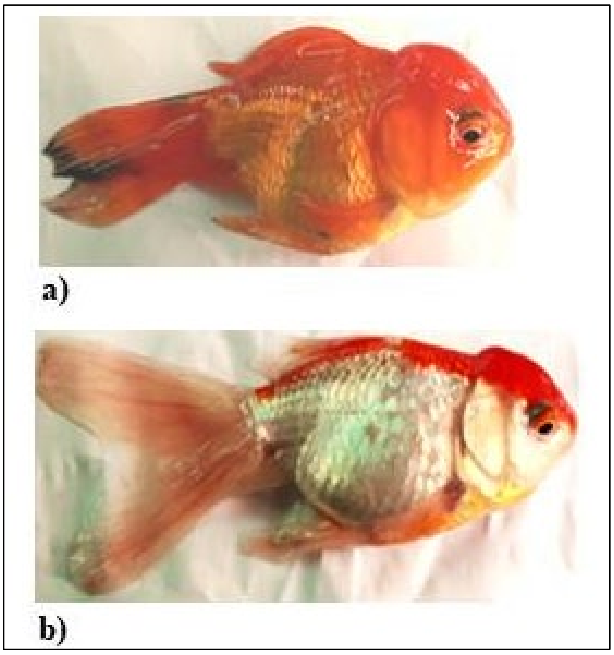

Figure 1. Broods of goldfish (a) Mature male and (b) Gravid female

Selection of substrates for spawning

Four types of floating substrates were employed in the study, namely water hyacinth, polythene, net, and jute rope (Figure 2). Goldfish naturally deposited their eggs near these floating substrates, which effectively adhered to their surfaces due to their sticky and adhesive nature. The presence of substrate induced ovulation without the need for external stimulation. The substrates were inspected twice daily to monitor egg deposition. To prevent filial cannibalism, brood fish were promptly removed from the breeding tank after spawning.

The breeding tank preparation

The spawning tanks were assembled using fifteen plastic drums. Prior to each drum being used in the experiment, they underwent a thorough cleaning process involving washing with detergent, rinsing with tap water, and air-drying. Each drum had a water capacity of 80 liters. To simulate natural conditions, a plastic hose was installed in each breeding tank for water circulation. At the base of each container, an outlet was created to facilitate drainage of excess water. The drums were arranged in a row and filled with ground water. Additionally, an aerator was affixed to each drum to ensure adequate oxygen supply.

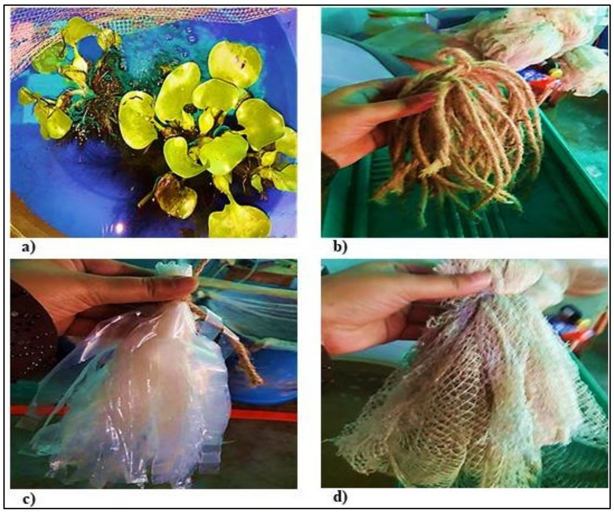

Figure 2. Photographs showing substrates used for breeding (a) water hyacinth, (b) jute rope, (c) polythene and (d) net

Experimental design

Goldfish spawning was evaluated across five different experimental conditions using paired adult broods at a 1:1 ratio. The five conditions were considered as different treatments. Fish were assigned to substrates such as water hyacinth, jute rope, polythene, net, or no substrate (control), and were labeled as T1, T2, T3, T4, and T5, respectively. Three replicates were conducted for each treatment.

Observation of spawning behavior

The male and female goldfish were placed together in a spawning drum. During this period, the female laid a large number of eggs, which adhered to the substrates. The male released sperm to fertilize the eggs. The substrates of each treatment were inspected daily, and after spawning, the breeders were promptly removed. The eggs were then left to hatch in the breeding drum. Both male and female fish were housed together in the spawning tank, allowing them to naturally release eggs and sperm for fertilization in the plastic drums.

Determination of fertilization rate

To assess the fertilization rate, the eggs were examined approximately 1 to 2 hours after collection. Water samples from the base of the plastic drums were transferred onto a small steel plate for inspection. Using a microscope, we distinguished between fertilized and unfertilized eggs based on their appearance. Fertilized eggs typically displayed a transparent shell with a grey or black patch inside, whereas unfertilized eggs appeared opaque. We calculated the fertilization rate using the following formula.

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total no.of egg}} \times 100$$

Collection of fertilized eggs and incubation

The fertilized eggs were transferred to a container designed for hatching, ensuring continuous water flow. After 48 hours of fertilization, the eggs were removed from the incubator and left in the container for three days without feeding, allowing absorption of the yolk sac. To promote optimal larval growth, the hatchlings were subsequently transferred to a circular tank with gentle water circulation. Water temperatures were kept between 20–23ºC using a NETONDA Aquarium Heater 50 W Heating Rod, while air temperatures were ranged around 23–26ºC throughout the period. The water circulation rate was maintained slowly using an aerator, ensuring even distribution of heat and consistent temperature throughout the water body. The hatchlings were fed commercial powder feed (Nova, Osaka, turtle) twice daily.

Determination of hatching rate

The hatching rate was calculated by visually counting the number of fertilized eggs in the samples and the resulting hatchlings. After counting, the hatchlings were removed from the hatching jar. The hatching rate was determined using the following formula:

$$\text{Hatching rate (\%)} = \frac{\text{No.of Hatchlings}}{\text{Total no.of fertilized eggs}} \times 100$$

Measurement of water quality parameter

Twice daily, we monitored water temperature, dissolved oxygen (DO), and pH levels to maintain water quality. Data were reported as mean averages (Mean±SD) for consistency. A pH meter (Hanna ISO 9001) was used to measure pH levels, while temperatures were recorded using a mercury thermometer and dissolved oxygen levels with a meter (Lutron DO-5510). Water quality parameters in all spawning tanks were carefully maintained within the optimal ranges outlined in Table 1.

Table 1. Water quality parameters in the different treatments throughout the experimental period

Months	Treatments	Temperature (ºC)	pH	Dissolved oxygen (mg/l)
February	T1	22.0±1.10	7.16±0.6	5.65±0.33
	T2	22.0±0.70	7.24±0.5	6.03±0.56
	T3	22.5±0.51	7.17±0.4	5.95±0.30
	T4	21.5±0.8	7.18±0.27	5.99±0.65
	T5	22.2±0.5	7.20±0.1	6.23±0.23
March	T1	22.0±1.10	7.11±0.09	5.85±0.27
	T2	22.0±0.70	7.04±0.7	6.05±0.65
	T3	22.5±0.5	7.07±0.19	5.89±0.32
	T4	21.5±0.8	7.14±0.17	5.92±0.63
	T5	22.2±0.5	7.11±0.21	6.20±0.29
April	T1	22.0±1.10	7.12±0.16	5.62±0.34
	T2	22.0±0.70	7.29±0.05	6.08±0.58
	T3	22.5±0.5	7.13±0.14	5.90±0.30
	T4	21.5±0.8	7.12±0.21	5.90±0.71
	T5	22.2±0.5	7.20±0.13	6.26±0.20

Data analysis

The data were analyzed using SPSS software (IBM® SPSS® Inc., IL, USA, version 20). Shapiro-Wilk's and Levene's tests were employed to assess variance normality and homogeneity. The results are presented as mean ± standard deviation. Differences among treatments were evaluated using one-way analysis of variance (ANOVA) at a significance level of $p<0.05$, with subsequent comparisons made using Duncan's post hoc test.

RESULTS

Courtship and spawning behavior

At the bottom of the spawning tank, courtship behaviors were observed from both male and female goldfish. Males frequently followed and gently nudged the females. During courtship, males displayed a distinctive behavior of circling around the female to keep her in place. The spawning process commenced a few days after initial courtship attempts. During spawning, males continued to follow females around the tank. After a period of two or three hours, females released their eggs. Males then nudged the females to position them over the substrate where the eggs were laid. Males subsequently released their milt to externally fertilize the adhesive eggs. Figure 3 illustrates the process where males fertilized each batch of eggs immediately upon release.

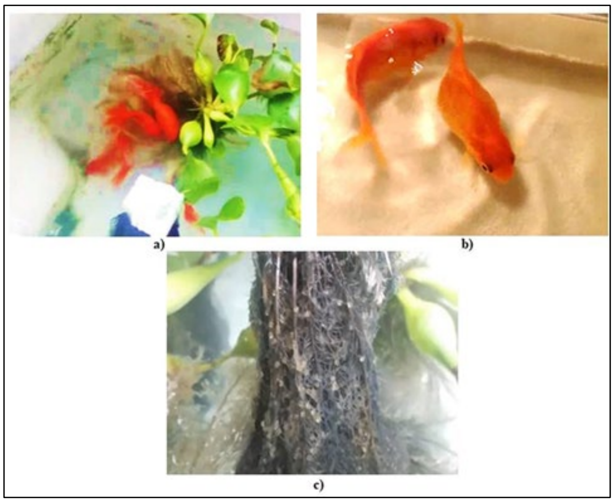

Figure 3. Photographs showing breeding behavior of goldfish; (a) male chase the female, (b) male hitting the vent of female and (c) adhesive eggs at the root of water hyacinth

Fertilization and hatching

Introduction of different substrates in treatments T1, T2, T3, and T4 markedly enhanced the fertilization rate of goldfish (Figure 4). In contrast, goldfish in the absence of substrate (T5) did not ovulate. Among the treatments, the highest number of eggs was observed in treatment T3 (356.67±40.41), while treatment T5 recorded no eggs (0±0). The mean number of eggs in treatment T3 was significantly ($p<0.05$) higher than in other treatments.

Hatching, the process of emerging from the egg envelopes (chorion), marks a critical environmental change in a fish's life. This transition is typically considered the boundary between the embryonic and larval stages. The mean numbers of hatchlings observed were 92.33±20.52, 193.33±14.15, 316.33±51.63, and 112.33±17.78 in treatments T1, T2, T3, and T4, respectively. Treatment T3 recorded the highest number of hatchlings, whereas no hatching was recorded in treatment T5 (Figure 5). Significant differences were found among treatments ($p<0.05$).

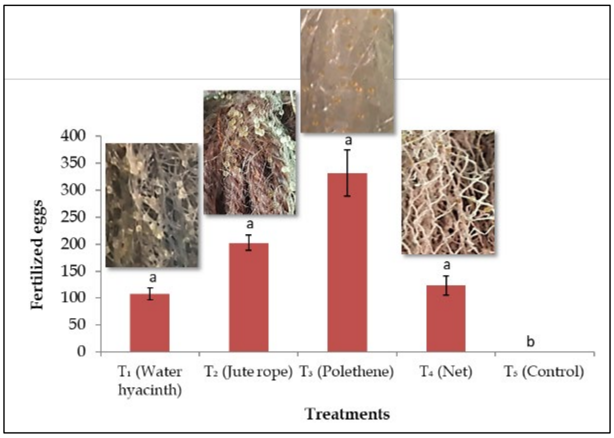

Figure 4. Effect of substrates on fertilized eggs in different treatments. Different superscripts of letters showed significant differences ($p<0.05$)

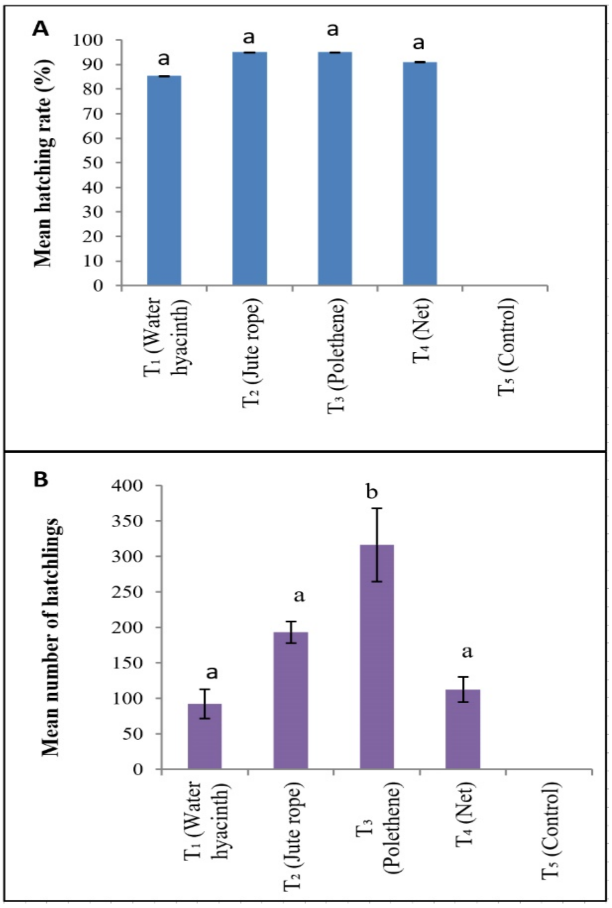

Figure 5. Effect of substrates on mean hatching rate (A) and mean number of hatchling (B) in different treatments. Different superscript letters (a and b) on the bars showed significant differences among treatments ($p<0.05$).

DISCUSSION

This study elucidates the impact of different substrates on goldfish reproduction under controlled conditions, highlighting their significant role in enhancing ovulation, fertilization, and hatching rates. The findings demonstrate that the choice of substrate is crucial for optimizing goldfish breeding, which is essential for ornamental fish production, especially in small-scale operations and hatcheries.

Courtship behavior encompasses the various interactions between male and female goldfish leading up to fertilization. In this study, males displayed a range of courtship behaviors, such as following, nudging, and circling females to position them for spawning. These behaviors, including the patterns of chasing and nudging observed, align with findings from Sharma et al. (2011); Kobayashi et al. (2002); and DeFraipont and Sorensen (1993). The courtship ultimately led to spawning, where females released eggs that were immediately fertilized by the males as they were deposited on substrates. The males' role in nudging females to ensure eggs were deposited on suitable substrates, such as the roots of water hyacinth, and the immediate fertilization of these eggs, underscore the critical interaction between behavior and substrate in goldfish reproduction. This behavior not only facilitates egg adhesion but also ensures that fertilization occurs efficiently, emphasizing the importance of substrate presence in optimizing breeding success.

The results underscore that substrates play a pivotal role in stimulating ovulation in goldfish. Specifically, the polythene substrate proved to be the most effective, resulting in the highest number of eggs (356.67±40.41), a fertilization rate of 93%, and a hatching rate of 95.01%. These outcomes are significantly higher compared to other substrates used and the control group with no substrate. The absence of a substrate led to a complete lack of egg deposition, indicating that substrates are essential not only for the physical attachment of eggs but also as a necessary cue for ovulation. The role of substrates in facilitating egg adhesion and subsequent fertilization aligns with previous research. Myriam et al. (2022) and Haniffa et al. (2007) highlighted the importance of substrates in koi carp breeding, where substrates provided both a physical surface for eggs and camouflage to protect them from predation. Similarly, our findings suggest that the goldfish's reproductive success is highly dependent on the presence of suitable substrates, which facilitate the deposition of eggs and enhance their fertilization.

Among the substrates tested, polythene emerged as the most effective, followed by jute rope, water hyacinth, and net. The high performance of polythene could be attributed to its smooth, non-absorbent surface, which likely provided an ideal environment for eggs to adhere and be fertilized. Jute rope, water hyacinth, and net substrates also supported successful reproduction, though to a lesser extent. The differences in effectiveness among substrates could be due to variations in surface texture, buoyancy, and how well these materials simulate natural conditions for egg attachment. These findings align with the observations of Hawkins et al. (2021), and Smith and Gray (2011), who highlighted that substrate characteristics play a crucial role in determining spawning behavior and success. The smooth and consistent surface of polythene might have provided a more stable and secure environment for eggs compared to the more variable surfaces of jute rope, water hyacinth, and net.

The hatching rate was notably high for the polythene substrate, which could be a result of both effective fertilization and optimal egg conditions provided by the substrate (Smith and Gray, 2011). In contrast, the absence of substrate resulted in no hatching, further emphasizing the necessity of substrates for successful egg development and hatching. This result confirms that substrates not only influence ovulation and fertilization but also play a crucial role in the early stages of egg development. Maintaining optimal water quality parameters such as pH, dissolved oxygen, and temperature was essential for successful spawning and hatching (Arindam et al., 2018; Myriam et al., 2022; Mottaa et al., 2023). Our study adhered to the optimal ranges of these parameters, ensuring a conducive environment for goldfish reproduction. Variations in pH and dissolved oxygen among treatments were statistically significant but did not impact the overall reproductive success when substrates were present.

The results provide valuable insights for ornamental fish hatcheries and small-scale fish farmers. Selecting the appropriate substrate can significantly enhance the efficiency of goldfish breeding programs. Polythene, due to its superior performance, could be recommended for use in breeding setups aiming to maximize egg production, fertilization, and hatching rates. However, it is important for hatcheries to consider the cost and availability of substrates, as well as their suitability for specific breeding environments.

CONCLUSION

In conclusion, this study emphasizes the pivotal role that substrates play in enhancing the reproductive success of goldfish in controlled environments. The research revealed that various substrates—such as polythene, jute rope, and water hyacinth—significantly improved ovulation, fertilization, and hatching rates compared to conditions without substrates. This underscores the importance of incorporating substrates to better replicate natural spawning conditions, which is crucial for successful goldfish breeding. Among the substrates tested, transparent polythene was found to be the most effective. Its ability to camouflage eggs and facilitate fertilization likely contributed to its superior performance. These findings not only advance our understanding of goldfish reproduction but also offer practical insights for optimizing breeding protocols.

The advantages of incorporating suitable substrates are clear: they can enhance reproductive outcomes and create a more natural environment for the fish, potentially leading to higher survival rates and healthier offspring. However, there are some considerations to keep in mind. The choice of substrate can affect maintenance and cleaning routines, as well as the overall management of the aquarium. Moreover, while substrates like polythene and jute rope proved beneficial, the long-term impacts of their use on water quality and fish health warrant further investigation. Overall, this study provides valuable guidance for ornamental fish production, highlighting sustainable practices that can improve breeding success. It also suggests potential applications in aquaculture where optimizing spawning conditions can lead to more efficient and effective fish production systems.

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AUTHORSHIP CONTRIBUTIONS

Asma Jaman: Conceptualization, methodology, writing original draft preparation. Umme Ohida Rahman: Data analysis, validation, writing- review and editing. Nahid Sultana Lucky: Reviewing and editing. Md. Sadiqul Islam: Conceptualization; supervision; writing; reviewing & editing.

ETHICAL APPROVAL

All procedures for experiments involving humans and animals (fish) adhered to the ethical standards set by the Ethical Committee of Bangladesh Agricultural University, Mymensingh. Additionally, all survey participants provided informed consent.

STATEMENTS AND DECLARATIONS

The authors declare of no competing interests. The authors alone are responsible for the content and writing of the paper.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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