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Induced spawning of harlequin rasbora (*Trigonostigma heteromorpha*) using tiger barb (*Puntius tetrazona*) stimuli: Techniques and outcomes

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Abstract
This study investigated the breeding success of harlequin rasbora (*Trigonostigma heteromorpha*) in a controlled aquaculture environment using an induced spawning technique with the tiger barb (*Puntius tetrazona*) as a stimulant. The research aimed to determine the effectiveness of this technique in enhancing the spawning response of harlequin rasbora. The experiment was conducted using four different treatments: Rasbora were exposed to one pair, three pairs, or five pairs of tiger barb, with a control group receiving no exposure. The study's findings revealed that spawning occurred in the treatment involving three pairs of tiger barb as stimulants, as well as in the control group. However, spawning success was not significantly improved by the induced technique, as the harlequin rasbora in the control group spawned more frequently than those in the induced groups. Fecundity was relatively low across all treatments, with an average of 4 to 9 eggs per spawning event, significantly below the expected range of 80-300 eggs. Fertilization and hatching rates were high in successful spawns, with rates reaching up to 100%. The survival rate of larvae at 30 days was also high, indicating good larval rearing conditions. Water quality parameters, including temperature, pH, and dissolved oxygen, remained within acceptable ranges for harlequin rasbora throughout the study, although the temperature was slightly higher than the optimal range. The results suggest that while the induced spawning technique with tiger barb as stimulants did lead to spawning in harlequin rasbora, it did not significantly enhance spawning success compared to natural spawning conditions. Further research is needed to optimize spawning conditions and improve fecundity in captive breeding programs for harlequin rasbora.

Keywords: breeding, aquaculture, stimulant, eggs, fecundity

1. Introduction
Indonesia is renowned for its high biodiversity of freshwater fish. Kottelat *et al*. (1993) [5] reported that Kalimantan alone is home to 310 species of freshwater fish, while Western Indonesia and Sulawesi collectively harbor around 900 species, 25 of which have significant economic value. This biodiversity is found in river basins and other public waters, primarily in Kalimantan and parts of Sumatra, which are characterized by slightly acidic pH levels, muddy substrates, and abundant vegetation. Currently, many of these aquatic ecosystems are under threat due to land conversion for plantations and residential areas, which endangers their ecological integrity (Ng & Kottelat, 1992) [8]. Several fish species in these regions, such as the arowana (*Scleropages* sp.), tigerfish (Datnioides sp.), sundadanio (*Sundadanio* sp.), betta (*Betta* sp.), and rasbora (*Rasbora* sp.), require focused conservation efforts to ensure their survival.
The harlequin rasbora (*Trigonostigma heteromorpha*), a small fish with a maximum length of 4-5 cm, is a high-value export commodity often used in aquascaping. Research on this species at Ornamental Fish Culture Research Center, Depok, West Java, Indonesia has focusing on breeding in a cultured environment. In the previous study, technologies for gonad adaptation and maturation were developed, considering environmental factors, nutrition, and hormonal stimulation (Permana *et al*., 2020) [9].

Although spawning was achieved, the number of eggs and larvae produced was limited, indicating a need for further investigation into the factors that stimulate optimal spawning.
In 2021, efforts are being made to induce harlequin rasbora spawning through external factors, specifically the induced spawning system. This system was developed by Fish Seed Center Cangkringan, Sleman, Yogyakarta. Technically, common carp (*Cyprinus carpio*) spawning can stimulate tawes (*Puntius javanicus*) to spawn both inside and outside the hapa, typically within 10 minutes to 1 hour 45 minutes (Lestari, 1998). Natural spawning, triggered by such stimuli, results in larvae with better survival rates compared to those produced by stripping techniques (Junior *et al*., 2005) [7]. The induced spawning method is cost-effective since the stimulating common carp can be reused multiple times. Successful induced spawning has been reported by Junior *et al*. (2005) [7], showing high fertilization rates in tawes eggs induced by Ovaprim-injected common carp. Given these successes, it is worth attempting the induced spawning system for harlequin rasbora, which has not yet successfully spawned in a cultured environment. This study will explore the potential of using tiger barb (*Puntius tetrazona*), a species within the same family as harlequin rasbora, as the stimulating fish. The objective of this research is to evaluate the spawning of harlequin rasbora in a cultured environment using the induced spawning system stimulated by tiger barb.

2 Material and Methods

This research was conducted in 2021 at the Ornamental Fish Culture Research Center in Depok, West Java, Indonesia. It adhered to Republic of Indonesia Law No. 18 of 2002 regarding the National System for Research, Development, and Application of Science and Technology. The study was approved by the Research Institute for Ornamental Fish and was carried out in accordance with ethical guidelines.

2.1 Gonad maturation of harlequin rasbora broodstock

Before implementing the induced spawning system, male and female harlequin rasbora were maintained and their gonads were matured. They were fed ad libitum with natural food, *Moina* sp. To accelerate gonad maturation, Oodev hormone (0.5 ml/L) and turmeric powder (0.003 mg/L) were added to the *Moina* sp. through a 6-hour soaking process, following the best results from previous research (Permana *et al*., 2020) [9]. The enriched Moina sp. was given once a week over a period of one to two months. Daily feeding consisted of bloodworms provided twice a day until satiation.

2.2 Preparation of stimuli fish for spawning induction

For the application of the induced spawning system, fish that are easy to spawn were used as stimuli. The chosen species was tiger barb, which belongs to the same family as harlequin rasbora.

2.3 Induced spawning treatments for harlequin rasbora

The study employed a Completely Randomized Design (CRD) consisting of three treatments and one control, as follows:
A: Harlequin rasbora induced with one pair of tiger barb.
B: Harlequin rasbora induced with three pairs of tiger barb.
C: Harlequin rasbora induced with five pairs of tiger barb.
D: Control (harlequin rasbora without induction).

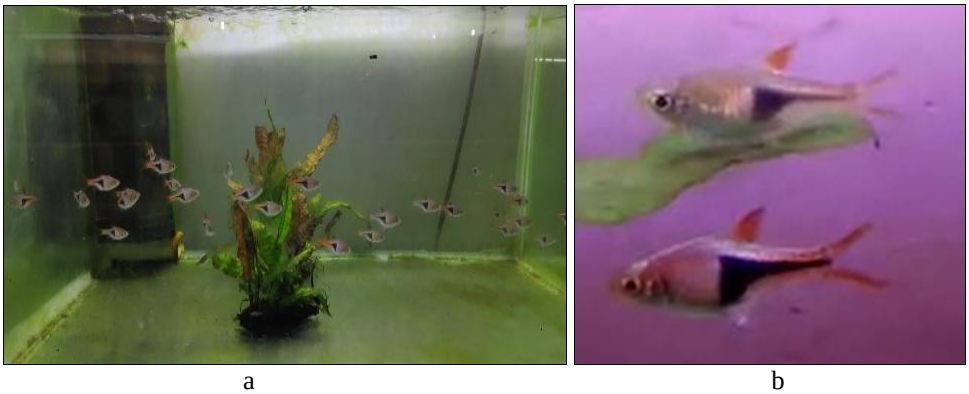
Each treatment had three replicates. The spawning tanks for harlequin rasbora were 40 × 40 × 40 cm^3 aquariums equipped with corner filters and *Anubias* sp. aquatic plants. The water height in the spawning tanks was maintained at 20 cm, and each tank contained five pairs of harlequin rasbora (3-4 cm). The stimuli fish (tiger barb) were placed in 40 × 30 × 30 cm^3 plastic boxes. After the tiger barb spawned, 2 liters of their spawning water were transferred to the harlequin rasbora spawning tanks. The number of tiger barb used in each aquarium was according to the respective treatment.
The spawning response of harlequin rasbora was observed by the presence of eggs in the spawning media. The observed parameters included fecundity, fertilization rate, hatching rate, and larval survival rate. Environmental parameters such as temperature, pH, and oxygen levels were also monitored.

3 Results and Discussions

This study consisted of several stages: gonad maturation of harlequin rasbora broodstock, preparation of stimuli fish for spawning induction, and the induced spawning of harlequin rasbora. Below is a detailed description of each stage's results.

Gonad maturation of harlequin rasbora broodstock

Gonad maturation of harlequin rasbora broodstock was conducted over two months. The broodstock were fed *Moina* sp. enriched with turmeric and gonad maturation hormones as described in the methods. This resulted in the maturation of gonads in the harlequin rasbora broodstock, making them ready for spawning. Mature gonads were identified by the distended abdomen, which remained even after fasting for at least one day. Additionally, the males exhibited brighter coloration compared to the less vivid females. Photos of the gonad maturation stage can be seen in Figure 1 below.



a b

Fig 1. A: Mass maintenance of harlequin rasbora (*Trigonostigma heteromorpha*) broodstock for gonad maturation, b) mature gonad broodstock, female (top: less vivid color) and male (bottom: brighter red color)

Preparation of stimuli fish for spawning induction

Tiger barb was used as the stimuli fish in the induced spawning of harlequin rasbora. This choice was made because it belongs to the same family, which was expected to influence the spawning of harlequin rasbora through pheromones. Photos of tiger barb maintenance for gonad maturation are shown in Figure 2 below.

a b

Fig 2: Maintenance of tiger barb (*Puntius tetrazona*) for gonad maturation to be used as stimuli fish in the spawning of harlequin rasbora (*Trigonostigma heteromorpha*) (a: male broodstock, b: female broodstock)

Induced spawning treatments for harlequin rasbora (*Trigonostigma heteromorpha*)

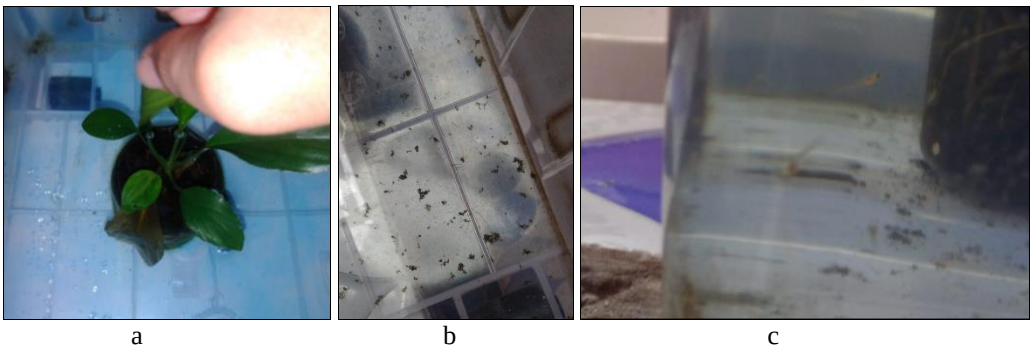
In the induced spawning treatment stage, tiger barb was first allowed to spawn. The water from their spawning media was then transferred to the harlequin rasbora spawning tanks to stimulate their spawning. The spawning process of tiger barb can be seen in Figure 3 below.

Fig 3: Spawning process of tiger barb (*Puntius tetrazona*) as stimuli fish

After the tiger barb spawned, 2 liters of their spawning water were introduced into the harlequin rasbora spawning tanks according to the treatment protocols to stimulate their spawning. The spawning behavior of harlequin rasbora is illustrated in Figure 4 below.

Fig 4: Spawning behavior of harlequin rasbora (*Trigonostigma heteromorpha*), with the male (bottom) and the female (top, attaching her abdomen to a leaf)

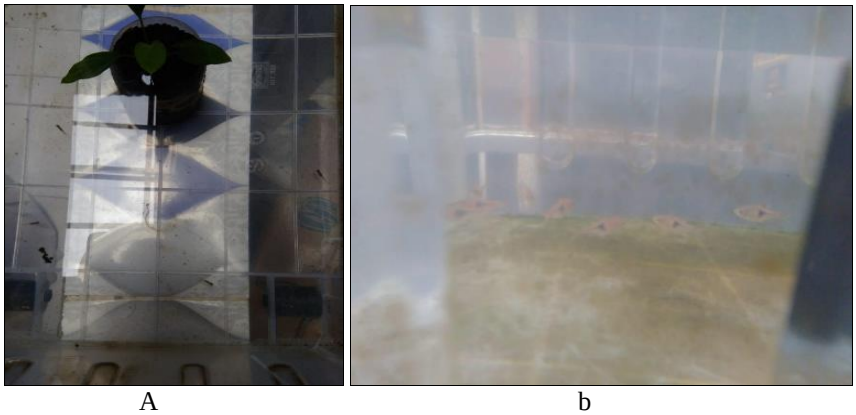
Eggs from the spawning process were observed to adhere to leaves and the tank bottom due to the adhesive nature of harlequin rasbora eggs, though their adhesion is not as strong as that of common carp eggs. The eggs hatch into larvae in approximately 24 hours (Figure 5b), and the larvae deplete their yolk sac in about 4 days, after which they can be fed *Artemia* nauplii (Figure 5c).



a b c

Fig 5: (a) Eggs, (b) newly hatched larvae, and (c) larvae during their first feeding of harlequin rasbora (*Trigonostigma heteromorpha*)

One-month-old larvae begin to take on a definitive form and can be fed water fleas due to their larger mouth size, with fry reaching about 1.5 cm in length. Three-month-old fry are capable of eating Culex larvae, bloodworms, and Tubifex, with a length of 2.5 cm (Figure 6 b).



A b

Fig 6: (a) One-month-old fry and (b) three-month-old fry of harlequin rasbora (*Trigonostigma heteromorpha*)

Spawning of harlequin rasbora (*Trigonostigma heteromorpha*) with induced spawning using tiger barb (*Puntius tetrazona*) stimuli

The spawning data of harlequin rasbora according to the treatment can be seen in Table 1 below.

Table 1: Spawning of harlequin rasbora (*Trigonostigma heteromorpha*) with induced spawning system using tiger barb (*Puntius tetrazona*) stimuli

Parameters	Treatments			
	A	B	C	D
Fecundity	Did not spawn	Spawning 1: 5 eggs	Did not spawn	Spawning 1: 4 eggs Spawning 2: 9 eggs
Fertilization rate	Did not spawn	Spawning 1: 90%	Did not spawn	Spawning 1: 0% Spawning 2: 100%
Hatching rate	Did not spawn	Spawning 1: 100%	Did not spawn	Spawning 1: 0% Spawning 2: 100%
Larval survival (30 days)	Did not spawn	Spawning 1: 100%	Did not spawn	Spawning 1: 0% Spawning 2: 100%

Note: A. Harlequin rasbora induced with one pair of tiger barb, B. Harlequin rasbora induced with three pairs of tiger barb, C. Harlequin rasbora induced with five pairs of tiger barb, D. Control (no induction)

Based on the data in Table 1, harlequin rasbora spawned under treatment B (three pairs of tiger barb) once and under the control treatment (no induction) twice. Treatments A (one pair of tiger barb) and C (five pairs of tiger barb) did not result in spawning. This indicates no significant effect of the induced spawning system on the spawning success of harlequin rasbora, as spawning also occurred in the control treatment. The fecundity in treatment B was 5 eggs, while the control treatment had 4 and 9 eggs, which is low compared to the typical fecundity range of 80-300 eggs for harlequin rasbora (Anonim, 2013) [2]. This low fecundity could be due to other broodstock eating the eggs during spawning.

Another assumption that can be drawn from this study is that the success of harlequin rasbora spawning using the induced spawning technique may depend on the precise dosage of pheromones produced to stimulate spawning. In the treatment involving a single pair of tiger barb, it is suspected that insufficient pheromones were produced to effectively trigger spawning in harlequin rasbora. Conversely, in the treatment with five pairs of tiger barb, an excess of pheromones may have been produced, which could have had an inhibitory effect. Thus, the most effective dosage of pheromones for inducing spawning in harlequin rasbora was likely achieved with the use of three pairs of tiger barb. This finding aligns with the statement Junior *et al*. (2005) [7], which suggests that the pheromone response causes an increase in neurophysin hormones, leading to egg release by the female once a certain threshold is reached. This implies that pheromones, at an optimal concentration, can indeed stimulate spawning.

Additionally, the study revealed that harlequin rasbora were able to spawn naturally in the control group, without the influence of pheromones from the tiger barb. This indicates that harlequin rasbora can spawn naturally in a cultured environment, a positive outcome for the domestication efforts of this species. The success of natural spawning is likely influenced by both internal and external factors that support reproduction, as noted by Effendie (2002) [3]. Internal factors include the maturity of the gonads, while external factors encompass environmental conditions such as physical factors (light, temperature, current), chemical factors (pH, oxygen solubility, pheromones), and biological factors (presence of a mate or opposite sex).

Water quality data for harlequin rasbora (*Trigonostigma heteromorpha*) spawning with induced spawning system

The water quality data for harlequin rasbora spawning with the induced spawning system are shown in Table 2 below.

Table 2: Water parameters of harlequin rasbora (*Trigonostigma heteromorpha*) with induced spawning system using tiger barb (*Puntius tetrazona*) stimuli.

Parameter	Value Range (for all treatments)	Tolerance Range (Fishbase, 2024)
Temperature °C	26-30°C	22-25
pH	6,5-7	5-7
Dissolved oxygen (ppm)	5-7	>5

Based on the water quality data in Table 2, the pH and dissolved oxygen levels were within the tolerance range for harlequin rasbora. However, the temperature was slightly higher than the optimal range, but it did not adversely affect the health of the harlequin rasbora.

5 Conclusion

The study on the breeding of harlequin rasbora successfully achieved gonad maturation through the administration of *Moina* sp. enriched with turmeric and the gonad maturation hormone Oodev, resulting in fully mature gonads ready for

spawning. Physical indicators, such as the fish's abdominal girth and color intensity in males, were effective in determining maturity. The research explored the impact of using an induce system with tiger barb as a breeding stimulant for harlequin rasbora, but found no significant effect on spawning success. The control group, without the induce system, produced comparable or even better results. Fecundity was lower than expected, with egg counts ranging from 4-9 per event, likely due to egg predation by other adults in the breeding tank, suggesting the need for improved breeding setups. Despite this, fertilization and hatching rates were high, reaching 90-100% in successful events, indicating good quality of eggs and sperm. Larval survival was excellent, with a 100% survival rate up to 30 days, and the larvae showed good growth, reaching 1.5 cm at one month and 2.5 cm at three months, thriving on a diet of nauplii *Artemia* followed by *Moina* sp. Water quality parameters, including pH and dissolved oxygen levels, were within acceptable ranges, though the temperature was slightly above optimal, potentially impacting health and breeding behavior. Despite this, no significant health issues were observed. In conclusion, while the induce system with tiger barb did not enhance breeding success, the study provided valuable insights into the species' breeding behavior, challenges with fecundity, and the importance of maintaining optimal conditions for successful breeding.

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