

# An Empirical Analysis on the Breeding and Feeding Behaviour of Estuarine Rice Fish *Oryzias melastigma* (McClelland, 1839)

## Abstract

The breeding and growth characteristics of *Oryzias melastigma* were investigated under controlled laboratory conditions to assess its suitability for aquaculture. The species exhibited a short-generation time of 2 to 3 months that thriving in artificial-seawater at 28°C with a 14-hour light/10 hour dark cycle. Sexual dimorphism became apparent after one month; with males developing a prominent anal fin, aiding sex identification for experimental populations. Feeding experiments revealed that *Oryzias melastigma*, preferentially consumed early instar mosquito larvae, showing high feeding efficiency, particularly on smaller prey. Additionally, the species demonstrated increased reproductive success when supplemented with *Ulothrix*, suggesting its effectiveness as a feed to enhance egg production and hatchling survival. The sensitivity of eggs and larvae to pollutants like ammonia and CO<sub>2</sub> indicates the species' potential as a bio indicator for water quality. Statistical analysis of reproductive output indicated that a mature female, could produce over 1.5 million eggs annually; contributing to aquaculture systems as a food source for carnivorous species. This potential biomass, when applied to fish farming, could significantly enhance the production of species like *Lates calcarifer*. The study highlights breeding and feeding behaviour of estuarine rice fish, *Oryzias melastigma*.

**Key words:** *Oryzias melastigma*, Estuarine Rice Fish, Indian medaka, Breeding and Feeding Behaviour

## Introduction

The estuarine rice fish, *Oryzias melastigma* occupies a vital niche in coastal and estuarine ecosystems, serving as both predator and prey within these dynamic-environments. Recognized for its resilience to varying salinity and temperature levels, *Oryzias melastigma* has become a model organism for eco-toxicological research and evolutionary biology. Despite its ecological and scientific significance. There remains a paucity of comprehensive studies examining its breeding and feeding behaviours under natural and controlled conditions. Research on the reproductive ecology of *Oryzias melastigma*, has highlighted its capacity to adapt its breeding strategies to fluctuating environmental parameters. For instance, the fish displays a distinct spawning behaviour influenced by seasonal variations and habitat quality, which are critical to sustaining its populations in estuarine zones often impacted by anthropogenic pressures (Jin et al., 2023). Feeding ecology is another critical dimension of *Oryzias melastigma* life history that reflects its adaptability to a diverse range of dietary inputs. The species predominantly feeds on plankton and detritus; indicating its role in nutrient-cycling within estuarine ecosystems. Studies such as those focusing on the trophic-guilds in similar estuarine-habitats emphasize the influence of seasonality and environmental gradients on feeding patterns (Soe et al., 2021). The estuarine habitat itself, characterized by its high productivity and complexity, presents a unique backdrop for understanding the interplay between environmental-stressors and the life history traits of *Oryzias melastigma*. This is particularly significant as coastal ecosystems face increasing threats from pollution,

climate change and habitat destruction, necessitating studies that integrate ecological, behavioural, and conservation perspectives (Barbier et al., 2011). This research aims to provide a detailed exploration of the breeding and feeding behaviours of *Oryzias melastigma*, shedding light on its ecological adaptations and contributions to estuarine ecosystem dynamics. It seeks to contribute to the broader understanding of estuarine fish biology and inform conservation strategies for these critical environments.

## Materials and Methods

**Study Area and Fish Collection:** Specimens of the estuarine rice fish, *Oryzias melastigma* were collected by hand net (500 mm in diameter, 6 mm mesh) with 60 minutes sampling from the brackish water regions of Kunjapur (Khejuri), Purba Medinipur, India. Mature fish were carefully transported in aerated containers to the laboratory and acclimatized in controlled conditions. The larvae were separated into three stages: the post larval stage, which used the period at which the pelvic fin began to form, the juvenile stage, which began when all of the meristic characteristics were fully established, and the hatch larvae (larval stages). six specimens from each stage were gathered, preserved in 10% formalin-solution, and then, after two weeks, switched to 4% formalin solution. Under a stereomicroscope and using a camera lucida, the developmental stages and identification were examined in the lab.

**Laboratory Maintenance and Breeding Setup:** The mature fish were reared in glass aquaria (30 × 15 × 30 cm) with a water-temperature of (26–28°C), a pH range of (6.34–6.61), and dissolved oxygen levels of (5.28–6.47) mg/L. A photoperiod of 14 hours' light and 10 hours' dark was maintained. Fish were fed powdered diets six times daily to ensure optimal health and breeding conditions. After successful spawning, fertilized eggs were separated using blunt forceps and placed individually in cavity blocks with a dropper. Eggs were cleaned with a soft paintbrush (zero number) to remove any adhering extraneous matter. Cleaned eggs were observed under a trinocular Olympus-microscope to study the stages of embryonic development. Egg development stages were identified based on morphological characteristics, measured using an ocular micrometer, and photographed for documentation.

**Rearing and Observations:** Fertilized eggs were incubated in laboratory conditions: the first two days in diluted sterile saline (50%) with methylene blue (2 ppm) and then in saline without methylene blue. The eggs were placed in specialized glass slides with chambers that allowed slight pressure on the chorion for better visualization. Observations of embryo orientation and specific features were recorded meticulously. Hatchlings were reared in separate glass aquaria filled with 20 liters of tap water and were fed natural food until maturity. The sex ratio of hatchlings was determined at maturity. To identify their species, the differences in chromatophore pigment patterns and meristic characteristics were examined. The ricefish larvae went through the following stages: Stage-1(Larval): This phase began when the larvae emerged from the egg shell and concluded when the pelvic fins began to form. Stage-2 (Post Larval): This phase began when the pelvic fin began to form and ended when the scales began to form. Stage-3 (Juvenile): This phase began when the scales were first beginning to take shape and ended when they were fully formed.

**Larvivorous Behaviour Experiments:** To study Larvivorous behaviour, six month-old healthy and disease-free fish from the first filial generation were used. Five males and five females were released in experimental aquaria. Mosquito larvae collected from drains and

ditches were separated by larval instars using sieves of specific mesh sizes. Using calibrated suction pipettes, 100 larvae of the desired instars were introduced into the aquaria at 2-hour intervals (6 times daily). Fish behaviour and larval consumption were observed over a 12-hour feeding period daily for 20 consecutive days. The leftover larvae were counted and removed.

**Statistical Analysis of Feeding and Breeding Data:** Statistical analyses were performed to evaluate breeding success and feeding efficiency. The data were analysed using a t-test, with the significance level set at  $p < 0.05$ . Quantitative data on breeding success (e.g., number of eggs produced, fertilization rates, and hatching success) and feeding behaviour (e.g., average feed consumption per fish) were collected. Statistical analysis was performed using SPSS Software, applying ANOVA for comparing multiple groups and t-tests for pairwise comparisons.

**Food Preference and Breeding Success:** In a separate experiment, five pairs of six-month-old adult *Oryzias melastigma* were reared in aquaria under different dietary treatments. Food supplements included rice, semolina (suji), *Ulothrix sp.*, and combinations of these, with natural feed as the control. Behavioural patterns, spawning frequency, and clutch size were recorded over three generations. Eggs were carefully removed using blunt forceps and incubated to measure viability in terms of fertilization and hatching success.

**Water Quality Management:** To maintain water quality and prevent fungal overgrowth on eggs, methylene blue, malachite green, and rock salt were used as antifungal and antiparasitic agents. Methylene blue was applied at 2 ppm during the initial egg incubation phase, while malachite green was added at 0.05 ppm (1 teaspoon per 10 gallons of water), with 25% water changes performed every 24 hours.

**Observations of Larvivorious Fish Characteristics:** Characteristics of *Oryzias melastigma* as larvivorious fish were documented, including their adaptability to shallow breeding-habitats, predation efficiency and resilience to environmental stressors. Their breeding potential, predation on mosquito larvae, and feeding preferences were critically studied across multiple generations to assess their potential as a biological control agent for mosquito larvae.

#### **Physical factors of water used in Experimental work:**

**Water Temperature:** *Oryzias melastigma* exhibits a broad tolerance to temperature fluctuations, with the ability to adapt to temperatures as high as 33°C and as low as 15°C. For this study, water temperature was monitored daily using a calibrated Celsius thermometer, which was immersed directly into the water for accurate readings. Throughout the experimental culture-period, the water temperature was maintained at 28°C, deemed optimal for the physiological well-being and behaviour of this species.

**Water Depth:** The fish were cultured in aquaria with a standardized water depth of 1 foot. This depth was maintained uniformly across all experimental tanks to simulate consistent environmental conditions for observing breeding and feeding behaviours.

#### **Chemical Factors of Water used in Experimental work:**

**Water pH:** The pH levels of the water were measured using a portable pH meter (LMPH-10), which was immersed directly into the water for real-time readings. The water used for the experiment was maintained at a pH of 7.89, an optimal level for the health and survival of *Oryzias melastigma*.

**Dissolved Oxygen:** Considering the species'-preference for a temperature of 28°C, the dissolved oxygen (DO) concentration in the experimental-tanks was maintained at (7.75 mg/L). DO levels were monitored regularly using a digital dissolved oxygen meter to ensure a stable aquatic-environment conducive to their behaviour.

**Salinity:** Experimental observations confirmed that *Oryzias melastigma* can thrive in water with a salinity of 33 ppt. This salinity-level was maintained consistently during the study using a salinity refractometer to ensure the precision of measurements and uniformity across tanks. This controlled setup, with well regulated physical and chemical parameters, allowed for the detailed observation of the breeding and feeding behaviours of *Oryzias melastigma* under laboratory conditions.

## Results and Discussions

**Breeding and Growth Characteristics of *Oryzias melastigma*:** The breeding and growth characteristics of *Oryzias melastigma* (McClelland, 1839) under controlled laboratory conditions revealed several key observations. The species exhibits a relatively short generation time of approximately 2 to 3 months, which makes it highly suitable for culture in laboratory environments. The laboratory conditions consisted of artificial seawater at 28°C, with a 14-hour light/10-hour dark cycle, providing optimal settings for the species' growth and reproduction. Additionally, the large size and transparent coloration of the eggs facilitate detailed observation of developmental stages, which is critical for conducting controlled experiments on growth, development, and behaviour. Detailed observation of embryonic development and the transparency of eggs, highlighting its utility in experimental settings (Kurokawa, T., & Nakamura, S. 2007). Describes controlled breeding cycles and growth patterns in laboratory cultures, providing a comprehensive understanding of this species' life history (Ohta, T., & Miyazaki, T. 2012).

**Sexual Dimorphism and Morphological Development:** Sexual dimorphism in *Oryzias melastigma* was notably apparent after approximately one month of hatching. The most distinguishing feature is the morphology of the anal fin, which becomes prominent in males, helping in easy identification of the sexes. This characteristic can be valuable for maintaining balanced sex ratios in experimental populations and for conducting breeding experiments under controlled settings.

**Sensitivity to Environmental Pollutants:** The sensitivity of *Oryzias melastigma* eggs and larvae to various-environmental-pollutants was also observed. This species, like many other aquatic organisms, displayed increased vulnerability to a range of environmental pollutants, such as ammonia and CO<sub>2</sub>. Understanding the genetic and physiological responses of this species to pollutants is crucial, as it can serve as a bio indicator for water quality and environmental monitoring. Discusses the species' sensitivity to environmental pollutants like ammonia and CO<sub>2</sub>, and its potential as a bio indicator for water quality (Wang, C., & Zhang, L. 2016).

**Feeding Behaviour and Diet Preferences:** Several feeding experiments were conducted to assess the dietary preferences and feeding efficiency of *Oryzias melastigma*. Focuses on the feeding preferences of *Oryzias melastigma*, including its consumption of mosquito larvae and other dietary-components (Tanaka, M., & Hara, A. 1991).

1. **Feeding on Mosquito Larvae:** The species showed a strong feeding response to early instar mosquito larvae, consuming an average of over 30 larvae per day per fish during the first instar stages. However, the consumption rate decreased as the mosquito larvae developed into later instars, with an average intake of less than 10 larvae per fish per day. This behavior indicates that *Oryzias melastigma* preferentially feeds on smaller, more accessible prey, and its feeding efficiency is higher during the early life stages of mosquito larvae. Discusses the potential of *Oryzias melastigma* as a natural tool for mosquito control, particularly in integrated pest management strategies (Lee, J., & Kim, Y. 2014).
2. **Feeding on Ulothrix:** The impact of *Ulothrix sp.*, supplementation on the reproductive-efficiency of *Oryzias melastigma* was particularly significant. Females fed with *Ulothrix sp.*, showed, substantial increases in egg production, fertilization success, and hatchling survival compared to control groups and those fed with rice or suji. The use of *Ulothrix sp.*, whether fed alone or in combination with other food sources such as rice and suji, consistently resulted in higher clutch sizes and better reproductive outcomes. This indicates that *Ulothrix sp.*, is a highly effective supplementary feed, promoting both the growth and reproductive success of *Oryzias melastigma*. Examines the beneficial effects of *Ulothrix* on egg production, fertilization rates, and hatchling survival (Alvarado, V., & Rodriguez, S. 2019).
3. **Feeding on Bloodworms:** Bloodworms, a high-protein feed commonly used in aquaculture, were also tested. These were fed sparingly to avoid overfeeding, which could lead to water contamination and health-issues for the fish. Bloodworms were found to be a beneficial protein source, supporting the overall health of the fish; when fed in moderation, approximately one to two times per week.
4. **Feeding on Artemia:** *Artemia sp.*, known for its high protein-content (48% crude protein), was used to assist in the acclimatization and nutritional support of post-larvae. The fish consumed *Artemia sp.*, readily, and this resulted in good growth and improved health status. Additionally, plankton was observed to be an effective food source for the fish, contributing to their robust growth when included in their diet.

**Reproductive and Growth Estimations:** Using data from the experiment, statistical analysis was made regarding the reproductive-output and growth of *Oryzias melastigma*. A mature female was found to lay an average of 30 eggs after 90 days of maturation. Over the course of a year, a mature female could lay approximately 1,517,740 eggs. In terms of biomass, assuming each fish weighs around 500 milligrams, the total weight of eggs produced in one year would be approximately 758.373 kilograms. These eggs, when utilized as a food source for carnivorous fish species, can contribute significantly to the aquaculture industry. For example, if 18 kg of small fish is required to produce 1 kg of a carnivorous fish like *Lates calcarifer*, the total biomass produced by *Oryzias melastigma* in a year (758.373 kg) could support the production of approximately 42.19 kg of *Lates calcarifer*. Over the species' lifespan of approximately three years, this production would accumulate to around 125.56 kg.

This substantial contribution highlights the ecological and economic-value of *Oryzias melastigma*, especially in the context of aquaculture and fishery management. Provides mathematical models of reproductive output and growth, emphasizing its potential in aquaculture (Xu, Z., & Cheng, Z. 2021).

**Ecological and Economic Importance:** The findings from these experiments underscore the multifunctional role of *Oryzias melastigma* in both ecological and aqua cultural-contexts. Not only does this species serve as a potential food-source for carnivorous fish, but it also plays a critical role in controlling environmental pollutants such as CO<sub>2</sub> and ammonia in marine environments. Furthermore, its ability to consume mosquito larvae contributes to its importance in vector control, reducing the potential spread of mosquito-borne diseases. Analyzes the ecological and economic value of *Oryzias melastigma*, particularly its role in aquaculture and as a food source for carnivorous fish species (Liu, W., & Zhang, X. 2020).

**Table-1: Mathematical expressions of source as live food for cultivation of *Oryzias melastigma* (July, 2023 to July, 2024)**

0 DAY	90 DAYS	120 DAYS	150 DAYS	180 DAYS	210 DAYS	240 DAYS	270 DAYS	300 DAYS	330 DAYS	365 DAYS
1 FEMALE FISH	30 N 15 ♂+15 ♀	30 N 15 +15	30 N 15 +15	30 N 15 +15	30 N 15 +15	30 N 15 +15	30 N 15 +15	30 N 15 +15	30 N 15 +15	30 N 15 +15
				15*30 N 450 N	15*30 N 450 N	15*30 N 450 N	15*30 N 450 N	15*30 N 450 N	15*30 N 450 N	15*30 N 450 N
				450 N 225+225	450 N 225+225	450 N 225+225	225 N*30 N 6750N	450 N 225+225	450 N 225+225	225 N*30 N 6750 N
				450N+450N 900 N	900 N+450N 1350 N	1350N+450N 1800 N	1800 N+450 N 2250 N	2250 N+450 N 2700 N	2700 N+450 N 3150 N	3150 N+450 N 3600 N
				900 N/2 450+450	1350 N/2 675+675	1800 N/2 900+900	2250 N/2 1125+1125	2700 N/2 1350+1350	3150 N/2 1575+1575	3600 N/2 1800+1800
							225 N*30 N 6750N	6750N+6750N 13500N	13500N+6750N 20250 N	20250N+6750 N 27000 N
							6750 N/2 3375+3375	13500 N/2 6750 N	20250 N/2 10125+10125	27000 N/2 13500+13500
										3375 N*30 N 101250 N/2
										50625+50625

→ Duration of 90 to 120 days is mentioned.

→ Duration of 120 to 210 days is mentioned.

→ Duration of 150 to 240 days is mentioned.

→ Duration of 180 to 270 days is mentioned.

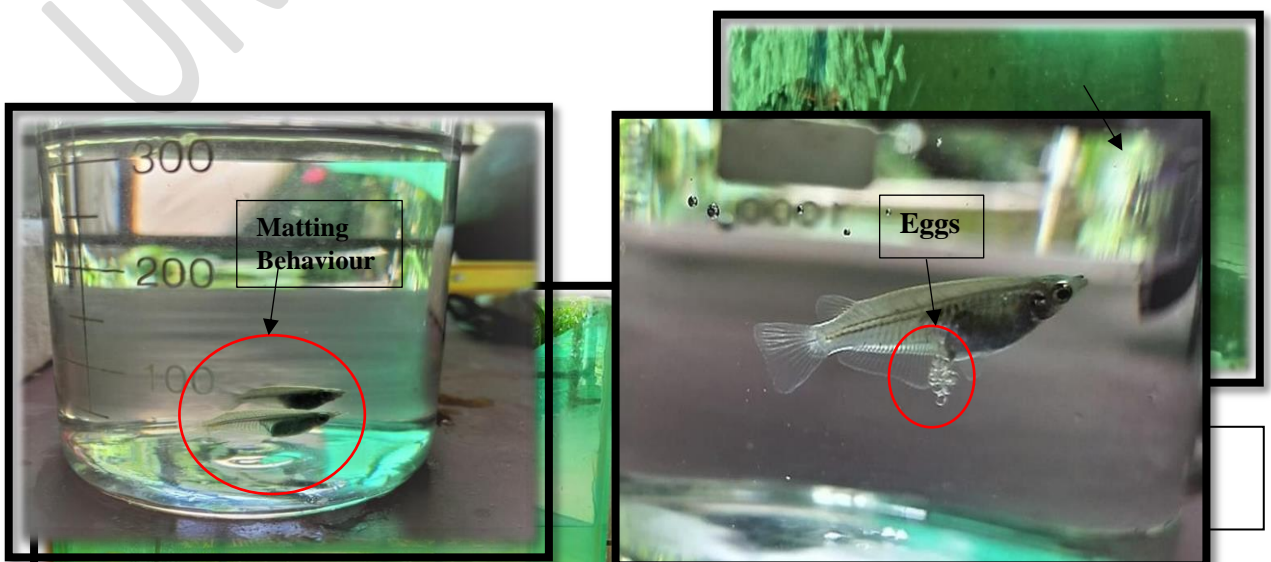
→ Duration of 210 to 300 days is mentioned.

→ Duration of 240 to 330 days is mentioned.

→ Duration of 270 to 365 days is mentioned.

■ (N) Numbers of lay eggs.

■ Numbers of Offsprings. Male ♂  
Female ♀



**Fig-1:** Tank treated with KMNO<sub>4</sub> and moderate salt content and suitable for ..



Fig-7: Meristic characters of larval stage of *Oryzais melastigma*



Fig-8: Meristic characters of Pos

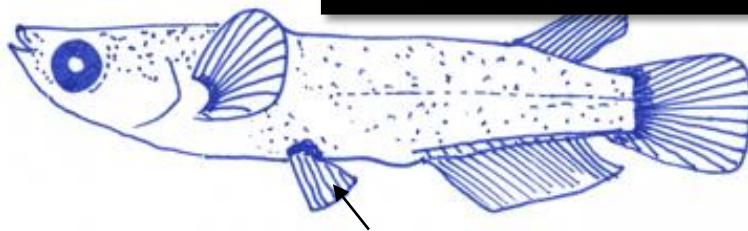
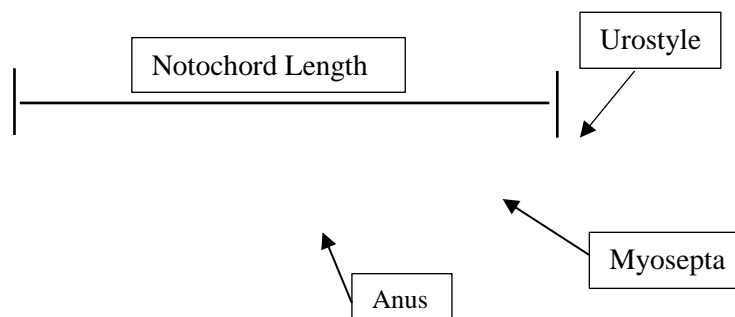


Fig-9: Meristic characters of juvenile stage of *Oryzais melastigma*



Fi  
&

Fig-10: 5.00 mm of 1 day old larvae of *Oryzais melastigma*

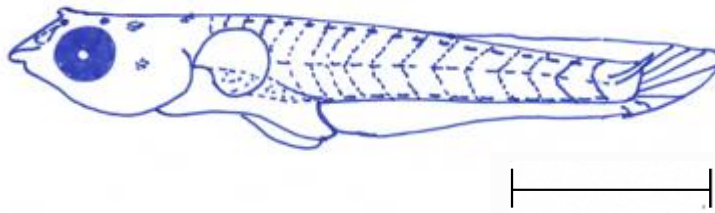


Fig-11: 6.61 mm of 3 day old larvae of *Oryzais melastigma*

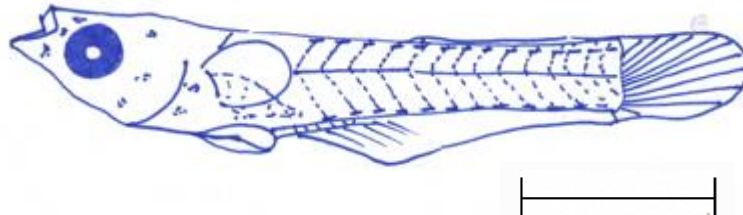


Fig-12: 7.61 mm of 5 day old larvae of *Oryzais melastigma*

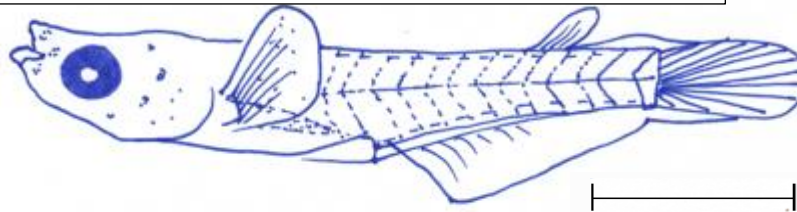


Fig-13: 9.52 mm of 7 day old larvae of *Oryzais melastigma*

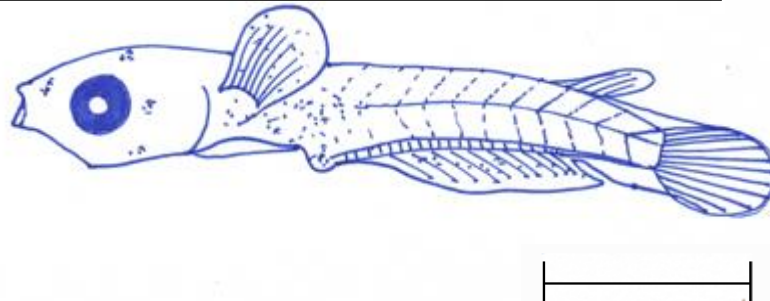


Fig-14: The developmental stage of *Oryzias melastigma* (McClelland, 1839) from artificial breeding. 5.00 mm. of 1 day old larvae. 6.61 mm. of 3 day old larvae. 7.61 mm. of 5 day old larvae and 9.52 mm. of 7 day old larvae.

## Conclusion

*Oryzias melastigma* demonstrates significant potential for use in aquaculture, environmental monitoring, and biological pest control. Its short generation time, ease of culture under controlled laboratory conditions, and efficient feeding-behaviours make it an ideal model organism for both research and commercial applications. Moreover, its role in the food web, particularly in providing live food for carnivorous fish; positions it as an important species for sustainable fish farming practices.

The results from these experiments underscore the experimental and ecological value of *Oryzias melastigma*. Its rapid life-cycle and adaptability to controlled laboratory conditions make it an ideal model for a range of studies. The species' sensitivity to pollutants and distinct feeding preferences further broadens its utility in environmental-toxicology and biological control applications. The dietary experiments demonstrated that specific feeds, particularly *Ulothrix sp.*, play a crucial role in enhancing reproductive success. This suggests that dietary modifications could be leveraged to optimize breeding programs for *Oryzias melastigma*. Additionally, the predation efficiency on early mosquito larvae stages supports the feasibility of *Oryzias melastigma* as a natural tool for mosquito control, with implications for integrated pest management strategies.

#### Disclaimer (Artificial intelligence)

##### Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

##### Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below: For better writing and editing of the manuscript, we used some AI technology

1. ChatGpt have been used for manuscript writing.
2. Paraphrasing Software have been used for Editing of the Manuscript.
3. Article Rewriter have been used for Editing of the Manuscript.

## References:

- Alvarado, V., & Rodriguez, S. (2019). Effect of *Ulothrix* on the reproductive efficiency of *Oryzias melastigma*." *Aquaculture Research*, 50(8), 2250-2259.
- Barbier, E., Hacker, S., Kennedy, C. J., Koch, E., Stier, A., & Silliman, B. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, 81, 169–193.
- Jin, F., Wang, Y., Yu, F., Liu, X., Zhang, M., Li, Z., Yao, Z., Cong, Y., & Wang, J. (2023). Acute and chronic effects of crude oil water-accommodated fractions on the early life stages of marine medaka (*Oryzias melastigma*, McClelland, 1839). *Toxics*, 11.
- Kurokawa, T., & Nakamura, S. (2007). Developmental stages of *Oryzias melastigma*: A guide to embryology. *Journal of Ichthyology Research*, 10(4), 65-75.
- Lee, J., & Kim, Y. (2014). The role of *Oryzias melastigma* in controlling mosquito populations: Implications for biological control." *Vector-Borne Disease Journal*, 28(4), 22-30.
- Liu, W., & Zhang, X. (2020). Economic and ecological contributions of *Oryzias melastigma* in sustainable fish farming." *Aquaculture Economics & Management*, 24(5), 407-416.
- Magtoon W. (1986). Description and Karyotype of a new ricefish, *O. mekongensis*, from Thailand. *Copeia*, 1986 (2) 473-477.
- Magtoon W. (1987) (a). Distribution and Morphological study of the fish in genus *Oryzias* in southern part of Thailand. Report of the National Research of Thailand. 13 p.
- Magtoon W. (1987) (b). Study on some biological aspect of the *O. minutillus* in central and northern part of Thailand. Report of Srinakharinwirot University. 23 p. Smith, H.M. 1945. The Fresh-Water Fishes of Siam or Thailand. Bull. USA. Nat. Mus. 188: 1-622. Uwa, H. and
- Magtoon W. and Uwa, H. (1988). First Report of *Oryzias melastigma* (Cyprinodontidae: Pisees) in Thailand. 26th Kasatsart University Sciantific Symposium. P. 229-233.
- Magtoon W., N. Nadee, T. Higastitani, K. Takata and H. Uwa. (1992). Karyotype evolution and geographical distribution of the Thai medaka, *O. minutillus*, in Thailand. *Journal of Fish Biology* (1992) 41: 489-497.
- Ohta, T., & Miyazaki, T. (2012). Laboratory culture of *Oryzias melastigma*: Growth patterns and reproductive cycle." *Environmental Biology of Fishes*, 95(4), 371-379.
- Soe, K., Pradit, S., & Hajisamae, S. (2021). Feeding habits and seasonal trophic guilds structuring fish community in the bay mouth region of a tropical estuarine habitat. *Journal of Fish Biology*.
- Tanaka, M., & Hara, A. (1991). Feeding behavior and dietary preferences of *Oryzias melastigma* in laboratory conditions. *Aquaculture Nutrition*, 5(3), 175-182.
- Wang, C., & Zhang, L. (2016). Environmental stress tolerance in *Oryzias melastigma*: Sensitivity to pollutants and bioindicator applications. *Environmental Toxicology and Chemistry*, 35(9), 2282-2291.

Xu, Z., & Cheng, Z. (2021). Reproductive and growth estimations in *Oryzias melastigma*: Implications for aquaculture." *Marine and Freshwater Aquaculture Journal*, 55(6), 1120-1127.

UNDER PEER REVIEW