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Encouraging Mosquitoes to Breed in Controlled Environments to Use them as a Sustainable Protein Source for Aquaculture

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ABSTRACT

This study aimed to identify the most effective method of producing mosquito larvae as a protein source for aquatic animals. Four attractants were tested for their ability to encourage mosquito egg-laying: aquaculture wastewater (carbon/nitrogen ratio of 10); lettuce-incubated water; mung bean sprout–incubated water; and sugarcane bagasse–incubated water. After overnight incubation to stimulate bacterial growth, the containers were opened nightly to attract mosquitoes. The larvae collected were reared under four feeding treatments: baker's yeast; rice bran; fish meal; and no feed (starvation). After six days, lettuce-incubated water showed the highest egg production (93.5 egg rafts), followed by mung bean sprout–incubated water (49 egg rafts), aquaculture wastewater (37.5 egg rafts), and sugarcane bagasse–incubated water (1 egg raft). Newly hatched larvae measured approximately 0.57mm. After five days of feeding, larval length ranged from 2.26 (starvation) to 6.17mm (fish feed), although the survival rate of the fish-feed group (46%) was lower than those of the rice-bran (74.4%) and yeast (66%) groups. These results show that lettuce juice is the most effective attractant for egg-laying, and rice bran offers a good balance between growth and survival, supporting its potential use in large-scale mosquito larvae production for aquatic animal feed

INTRODUCTION

Aquaculture is recognized as an important sector for ensuring food security for humans, providing employment and economic benefits for many countries around the world, and offering a sustainable source of aquatic food, thereby reducing pressure on wild fish stocks. Today, the per capita consumption of aquatic products (including seaweed) is 20.2kg, more than double that in the 1960s, and this demand is expected to continue increasing in the future. However, capture fisheries production has declined, from 90% in 1974 to 64.6% in 2019, due to the decrease in exploitable fish stocks **(FAO, 2022)**. Thus, aquaculture plays a crucial role in meeting the growing demand.

Like other farming industries, aquaculture depends on feed input to achieve production targets. As aquaculture expands globally, both in terms of volume and farming area, the demand for feed continues to rise. Animal protein used in aquafeed is mainly sourced from the ocean. However, the wild fish catch has not seen significant growth in recent decades. Therefore, alternative animal protein sources with high protein content, such as blood meal from slaughterhouses **(Dominy & Ako, 1988)**, mosquito larvae **(Christaki *et al*., 2022)**, black soldier fly larvae **(Mohan *et al*., 2022; Eide *et al*., 2024)**, and seaweed **(de Celente *et al*., 2023)**, are now being explored for aquafeed. Mosquitoes, in particular, are highly nutritious and are considered a potential feed candidate for both terrestrial animals and aquatic larvae. Mosquitoes consist of 54–62% protein and 16–28% fat **(Christaki *et al*., 2022)**. However, adult mosquitoes are known vectors of human diseases. During their larval stage, mosquitoes live in aquatic environments. They tend to breed in nutrient-rich, bacteria-laden water. Therefore, mosquito egg-laying can be stimulated by incubating vegetable matter to promote bacterial growth, which attracts them. It is important to find a suitable environment to lure mosquitoes, collect their eggs, and cultivate their biomass for use as feed for fish and shrimp larvae. This also contributes to reducing the presence of mosquitoes in human living environments and helps lower the transmission of mosquito-borne diseases.

MATERIALS AND METHODS

1. Experimental setup

This study consisted of two independent experiments: the first was an investigation of the effects of environment on the number of mosquitoes reproducing; and the second was a study of the effects of food type on mosquito larvae survival and growth rates.

Experiment 1: The effects of environmental attributes on mosquito egg raft production

Four types of environment stimulating concentrated mosquito breeding were studied: wastewater from red tilapia ponds; lettuce-incubated water; mung bean sprout–incubated water; and sugarcane bagasse–incubated water. These four types corresponded to four treatments, each treatment repeated three times. Rectangular black plastic tanks (60cm × 40cm × 5cm) were used for incubation of water to stimulate mosquito breeding.

The lettuce water medium and bean sprout water medium were prepared by using 0.25kg of lettuce or 0.25kg of bean sprouts pureed and mixed with 20L of water. Sugarcane bagasse water medium was prepared by using 0.35kg of sugarcane bagasse in 20L of water. Red tilapia wastewater medium was taken from intensive farming tanks. The total ammonia nitrogen (TAN) content of wastewater was measured with a Sera test kit (Germany). Based on the results for TAN in wastewater, molasses (38% carbon) was added to achieve a water carbon:nitrogen (C:N) ratio of 10 to stimulate heterotrophic bacterial growth **(Avnimilech, 2010)**.

After preparation, the tanks were sealed with plastic sheets and the media were incubated for a day to stimulate bacterial growth. The plastic cover was opened only at 17:00 p.m. every night to allow mosquitoes to lay eggs. The mosquito egg rafts in each tank were harvested at 7:00 a.m. the next morning and the number of egg rafts was counted. Mosquito egg rafts were collected for a week. The egg rafts collected on day 3 were hatched for the experiment on raising larvae on different types of food.

Experiment 2: Effects of different types of food on survival and growth of mosquito larvae

Newly hatched mosquito larvae of similar size (0.57±0.02mm) were reared and stocked in 1000mL plastic cups containing 500mL of water at a density of 600 larvae/L of water. Three types of feed—baker's yeast, rice bran, and commercial fish feed (40% protein, De Hues)—were provided twice a day at 8:30 a.m. and 17:00 p.m. The survival rate and growth in length of mosquito larvae in the treatments with supplemented food were compared with those of larvae in the control treatment. Each treatment was replicated three times. This experiment was conducted with newly hatched larvae until 10% of the larvae transformed into pupae and then stopped to determine the survival rate of mosquito larvae and larval length in the treatments. Ten to 15 larvae from each treatment were sampled and stored in 25% formalin to determine length. Then, the remaining mosquito larvae were collected for use as food for fish larvae.

2. Food preparation and feeding

Rice bran, yeast, and commercial fish feed were soaked in water for 30 minutes and then filtered through a 50µm mesh. Mosquito larvae were fed to fish larvae twice a day at 8:00 a.m. and 4:00 p.m., with a feed ration of 1000ppm.

3. Data collection and calculation

Environmental factors, pH and temperature, in experiment 2 were measured once a day at 8:00 a.m. using a pH and temperature tester (HI 98128, Hanna, Romania). The number of egg rafts in each tank was counted at 7:30 a.m. and the total number of egg rafts in each treatment was recorded on day 6.

The number of mosquito larvae still alive at the end of the experimental cycle was counted and the survival rate was calculated using the following formula:

$$\textbf{Survival}\ (\%) = \frac{\textit{Number of larvae at the end of study}}{\textit{Number of larvae at begining of study}} \times \mathbf{100}$$

Length growth was determined at hatching, after 2 days of rearing, and at the end of the experiment. Larval length was determined on the first day (newly hatched) and on days 2 and 5 after hatching. Five larvae from each culture were harvested and fixed with Lugol's solution and were then measured under a specialized stereomicroscope using the NIS-Elements software (version 1.21.00).

Larval growth under different diets was determined, with daily length gains (DLG; mm/day) calculated using the formula:

$$\textbf{DLG (mm/day)} = \textbf{(Lf − Li)/rearing period,}$$

Where, Lf was final length and Li was initial length

The specific growth rate in terms of length (SGR_L; %/day) was calculated using the formula:

$$\mathbf{SGR_L\ (\%/day) = 100 \times (LnWf - LnWi)/rearing\ period,}$$

Where, Wf was final length and Wi was initial length.

4. Statistical analysis

Data were processed with Microsoft Excel and Statistica 6.0 program, with one-way ANOVA and Tukey's HSD test used for comparing treatments. The threshold for significance was set at $P<0.05$.

RESULTS & DISCUSSION

The effects of environmental attributes on mosquito egg raft production

Fig. (1) shows that lettuce water and aquaculture water environments initially attracted the most mosquitoes to lay eggs. Eighteen egg rafts were collected from the lettuce water environment on the first day, with the number peaking at 28.5 egg rafts on the second day, and then decreasing continuously until the end of the experimental period. In the environment using aquaculture water, 11.5 egg rafts were collected on the first day; the number peaked at 14 egg rafts on the second day, and then gradually decreased to 0 egg rafts by day 6. In the cold water environment, the number of eggs collected was relatively small on the first day (2.5 egg rafts) and the second day (4 rafts), but it jumped to 13 egg rafts on the third day of collection and remained stable until the fifth day with 12 egg rafts, then decreased to 5.5 egg rafts on day 6. In contrast, almost no eggs were laid in the sugarcane bagasse environment, possibly because sugarcane bagasse did not stimulate heterotrophic bacteria to grow as quickly as they did in vegetable water and aquaculture water. The total number of mosquito egg rafts collected over 6 days from the lettuce water environment was the highest (93.5 egg rafts), followed by the mung bean sprout environment (49 egg rafts) and the aquaculture water environment (37.5 egg rafts). Only 1 egg raft was collected from the sugarcane bagasse environment.

Mosquito larvae develop in aquatic environments and those rich in food are ideal mosquito breeding sites. According to **Asahina (1964)** and **Linenberg *et al*. (2016)**, the main food of mosquito larvae is bacteria and organic debris. Nutrient-rich environments and those with bacterial growth are ideal mosquito-breeding sites **(Mosquera *et al*., 2022)**. According to **Sabila *et al*. (2013)** and **Pradani *et al*. (2023)**, water rich in organic matter and ammonia is an ideal mosquito-breeding environment because it is rich in nutrients for the development of larvae. Wastewater from aquaculture ponds, as well as vegetable waste, often contains high levels of nutrients. From 15 to 25% of total lettuce waste contains organic nutrients **(Pujiwati *et al*., 2024)**. In tilapia farms, the fish convert 23–26% of total nitrogen and 40–45% of total phosphorus in the feed into biomass **(Boyd *et al*., 2007; Osti *et al*., 2017)**. The remaining total phosphorus and nitrogen is discharged into the environment, accumulating in the sediment, settling to the bottom of the pond, dissolving in the water, or volatilizing into the atmosphere. **Savage (1990)** reported that fermented mung bean sprout water generally provides a good source of protein, fiber, and vitamins, with fermentation helping to break down antinutrients, such as phytic acid, making nutrients more readily available for absorption. In contrast, the remaining bagasse is mainly fiber. Therefore, fermented lettuce is the most attractive breeding environment for mosquitoes.

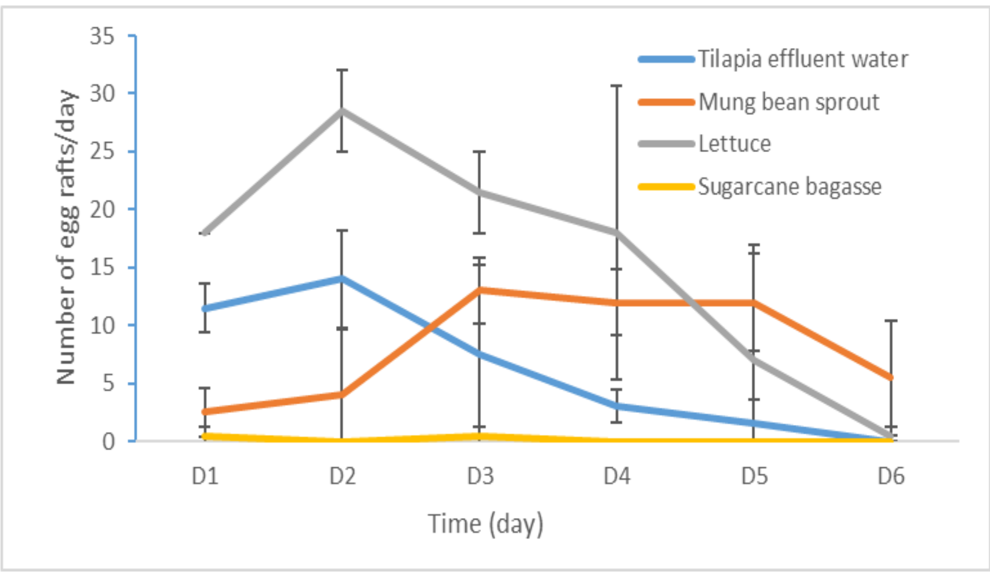

Fig. 1. The number of mosquito egg rafts produced each day over six days in different environments

6. Effects of different types of food on survival and growth of mosquito larvae

6.1. Environmental conditions

Mosquito larvae live in water but obtain oxygen from the air, so they are able to survive in polluted environments. During the rearing period, the temperature of the rearing tanks did not fluctuate much and was in the range of 27.5–27.6°C. The pH of the medium in the rearing tanks fluctuated between 7.1 and 7.7. The experiment was conducted under covered conditions; therefore, the temperature was stable and relatively similar between treatments. According to **Chaiphongpachara *et al*. (2018)**, turbidity, water depth, and water temperature are not associated with mosquito larval abundance.

6.2. Survival rate of mosquito larvae

The survival rate of mosquito larvae after 5 days of rearing fluctuated significantly from 0.7 to 74% (Fig. 2). The rice bran treatment showed the highest survival rate (74.4%), which was significantly higher than those of other treatments, except for the baker's yeast treatment (66.4%). The survival rate of larvae fed with baker's yeast was not significantly higher than that of larvae fed commercial fish feed (40.8%). The survival rate of the unfed larvae was the lowest (0.7%; $P<0.05$).

According to **Elora and Sarkar (2018)**, the survival of mosquito larvae depends greatly on the type of food present in the habitat; the rate was high when the larvae were fed with soybean meal, while it was lower when fed fish feed. The carbohydrate:protein ratio of the feed and the percentage of time the larvae reach adulthood are correlated.

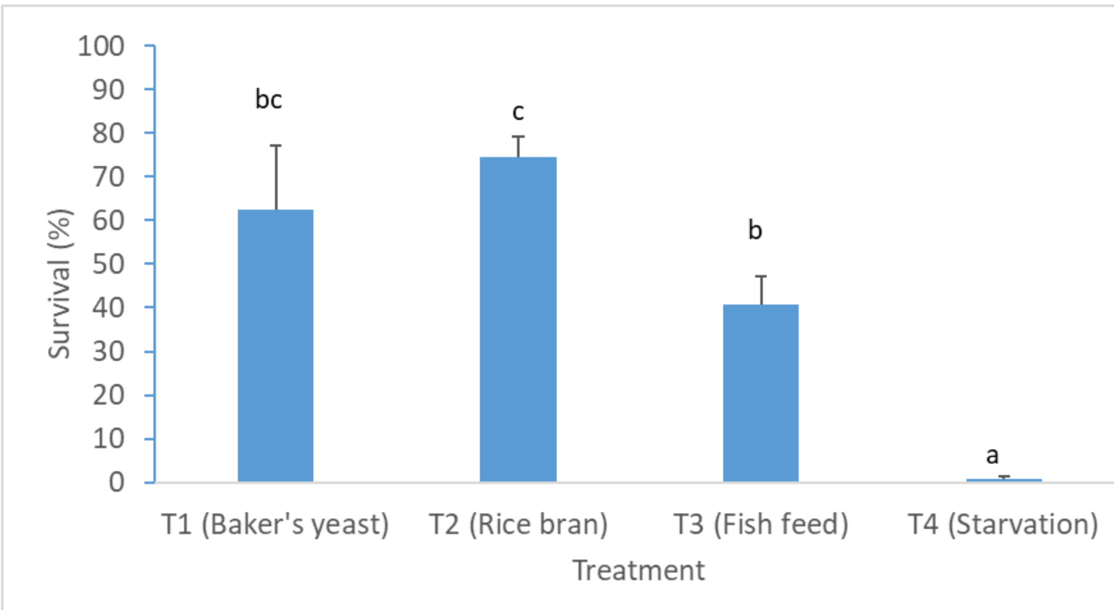

Fig. 2. Survival rates of mosquito larvae fed different types of food

6.3. Growth of mosquito larvae

The results for mosquito larvae length are presented in Table (2). The average length of newly hatched mosquito larvae was 0.57±0.02mm. On day 2, length ranged from 1.18 to 5.23mm, a 2–9 times increase compared to the length of newly hatched larvae. The larvae fed fish feed were the longest (5.23±0.21mm), followed by larvae fed rice bran (4.11±0.29mm), those fed baker's yeast (2.69±0.42mm), and the starved larvae (1.18mm) ($P<0.05$).

By day 5 of rearing, mosquito larval length reached 2.26–6.17mm/individual and the specific growth rate over 5 days of rearing was 27.47%–47.55%/day (Table 2). The larvae fed fish feed were the longest (6.17±0.33mm), with a significantly higher percentage daily length gain of 47.55±1.10%/day ($P<0.05$) compared to the larvae fed other diets. In contrast, the larvae in the no-feeding treatment were the shortest (2.26±0.16mm/individual) and their percentage daily length gain was the lowest (27.47±1.40%/day; $P<0.05$). Mosquito larvae fed baker's yeast or rice bran were relatively shorter and their growth was relatively lower than their counterparts fed commercial fish feed, but they were significantly longer than the larvae not fed. Larvae fed baker's yeast were relatively longer and grew faster than those fed rice bran, but these differences were not significant.

The fish meal treatment had the highest DLG (1.12±0.07 mm/day; $P<0.05$), followed by the baker's yeast treatment (0.95±0.07mm/ day), the commercial fish feed treatment (0.88±0.08mm/ day), and the starvation treatment (0.34±0.03mm/ day) (Table 2). These results show that fish meal helped mosquito larvae grow the fastest.

Table 2. Growth rates of mosquito larvae on the 5th day of rearing

Parameter	T1 (baker's yeast)	T2 (rice bran)	T3 (fish feed)	T4 (starvation)
L_{day1} (mm/indi.)	0.57±0.02^{a}	0.57±0.02^{a}	0.57±0.02^{a}	0.57±0.02^{a}
L_{day2} (mm/indi.)	2.69±0.42^{b}	4.11±0.29^{c}	5.23±0.21^{d}	1.18±0.10^{a}
L_{day5} (mm/indi.)	5.30±0.33^{b}	4.97±0.41^{b}	6.17±0.33^{c}	2.26±0.16^{a}
DLG (mm/day)	0.95±0.07^{b}	0.88±0.08^{b}	1.12±0.07^{c}	0.34±0.03^{a}
SGR_L (%/day)	44.50±1.23^{b}	43.19±1.75^{b}	47.55±1.10^{c}	27.47±1.40^{a}

Values in the same row indicated by different letters are significantly different ($P<0.05$).

Many mosquito's species are non-selective filter feeders **(Araújo *et al*., 2012)**. They can filter organic particles suspended in water and microorganisms, such as bacteria, viruses, protozoans, and fungi, and can live and develop in environments where only bacteria are present, such as ponds, vessels full of water, and old tires with stagnant water **(Ekedo *et al*., 2020)**. The quality and quantity of food determine the growth and development of larvae. The nutritional content of rice bran is high, with a composition of 13.4% protein and 40% carbohydrate. This is the best food for filter feeders, especially *Artemia* **(Sorgeloos *et al*., 1980)**. Mosquito larval growth is correlated with a higher microbiota load in pellet-fed larvae, in agreement with the known positive effect of microbiota on mosquito development (**Linenberg *et al*., 2016)**. Rice bran is a source of carbon for heterotrophic bacterial growth **(Avnimelech, 2012)** and bacteria are a food source for mosquito larvae. Although the protein content of rice bran is not equal to that of fish feed, the growth of rice bran-fed mosquito larvae was not significantly better than that of mosquito larvae fed fish feed, which may be due to the contribution of bacteria. In particular, mosquito larvae still grew in the no-feed treatment, which may be due to bacteria decomposing mosquito larvae carcasses in the habitat.

CONCLUSION

The lettuce water environment was the most suitable treatment for attracting mosquitoes to lay eggs. Fish meal resulted in mosquito larvae reaching the highest length, specific growth rate, and percentage of daily length gain among the diet types in the experiment. After five days of rearing, mosquito larvae fed rice bran showed the highest survival rate (74.4%).

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