Predatory efficiency of Danio rerio (Cypriniformes: Cyprinidae) and Poecilia reticulata (Cyprinodontiformes: Poeciliidae) as a biocontrol agent of Aedes larvae (Diptera: Culicidae) under the laboratory condition in Bangladesh

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Research Article

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Abstract

Background

Several viral infections are transmitted by members of the genus *Aedes* (Diptera: Culicidae). This study was conducted to evaluate the predatory potential of *Danio rerio* Zebrafish (Cypriniformes: Cyprinidae) and *Poecilia reticulata* Guppy (Cyprinodontiformes: Poeciliidae) to control *Aedes* mosquito larvae and thereby manage dengue epidemics in a sustainable, and eco-friendly manner.

Methods

Under laboratory conditions, size-matched fish of each species were introduced into separate plastic containers containing 2 liters of tap water and 100 third instar larvae of *Aedes*. In addition, different prey combinations were used to assess the comparative predation potential of fish. Mosquito prey preferences were assessed using Manly's preference index.

Results

Over 24-hours of laboratory conditions, a significant difference ($p < 0.05$) between the predatory efficiency of the studied fish species was reported where the predatory efficiency of *D. rerio* was higher than that of *Po. reticulata*. *D. rerio* showed significantly ($p < 0.05$) higher predation efficiency and prey preference for 2nd instar larvae and pupae of *Aedes* than *Po. reticulata* on the presence of alternative prey.

Conclusions

Based on predation efficiency and prey preference, this study suggests that *D. rerio* can be evaluated as an alternative species to *Po. reticulata* in biological control as a more eco-friendly, low cost, and sustainable method for the management of *Aedes* mosquitoes.

Background

Mosquito-borne diseases are still a major issue in almost every tropical and subtropical country [1, 2] causing the death of over 700,000 people every year globally [3, 4]. Among these diseases, dengue is a major global public health concern, particularly in Southeast Asia, Sub-Saharan Africa, and Latin America [5]. Currently, approximately 2.5 billion people in over 128 countries are at risk of the dengue epidemic [3, 6]. Every year, globally over 390 million people are infected with the dengue virus, resulting in approximately 20,000 deaths [5]. South-East Asia and Western Pacific regions continue to be dengue hotspots, responsible for roughly 75% of the disease burden around the world in recent years [6]. Bangladesh is located in the dengue endemic area of South-East Asia [5], and is a suitable habitat for the

dengue vector and increased transmission [7]. In 2000, the country experienced its first dengue epidemic while the worst occurred in 2019, affecting over 100,000 people and resulting in 164 deaths [5, 8].

The lack of effective vaccines or drugs against the serotypes (DENV serotypes 1–4) of the dengue virus continues to be a major challenge in controlling dengue epidemics. As a result, various traditional methods for dengue control are being used, with a focus on vector control and patient management [9, 10]. Catching, applying mosquito repellent sprays, using insecticides, using bed nets, indoor residual sprays, and biocontrol agents are the most commonly used control methods [2]. The application of chemical insecticides is a widely used and effective way of mosquito management. In contrast, long-term use of chemical insecticides leads to insecticide resistance and is also dangerous to human health as well as non-target organisms and a major source of pollution [1]. As a result, biological control is an ideal eco-friendly, cost-effective, and effective vector control method in which naturally occurring predators such as amphibians, belostomatids, crustaceans, dytiscid beetles, notonectids, odonates, and larvivorous fish are employed to control the immature or adult phases of vector-borne mosquitoes [6].

However, one of the oldest and most widely used vector control methods is the use of larvivorous fish but chemical insecticides have replaced it [2]. Around the world, more than 253 fish species, both exotic and native, have been considered mosquito biocontrol agents [2, 11]. Among the most commonly used larvivorous fish species are *Gambusia affinis*, *Poecilia reticulata*, *Carassius auratus*, *Nothobranchius guentheri*, *Danio rerio*, *Danio malabaricus*, *Colisa* sp, *Rasbora daniconius*, *Trichogaster* sp, *Puntius* sp, *Oreochromis* sp, and *Aplocheilus sp* [2]. *Po. reticulata* and *Gambusia affinis* have been promoted for mosquito biological control for a long time and their effectiveness has been reported in numerous cases. However, because of their invasive nature and the inability to achieve desired results in some cases, native larvivorous fish are being promoted as a viable alternative [12]. The World Health Organization (WHO) has recommended against using exotic fish species as biological control agents [13]. Several countries use larvivorous fish directly to control mosquito vector larvae, while in others, this approach is used as part of integrated vector control programs. Although larvivorous fish are an easy way to manage vectors, native fish should be used instead to prevent any unwanted consequences from introducing new fish species into the local environment [10]. The introduction of exotic species leads to the decline and complete absence of endemic species in different parts of the world [2]. *Po. reticulata* is unable to feed on *Culex quinquefasciatus* larvae in polluted drain water and prefers to eat other available food [14].

Recent findings in Dhaka have revealed that plastic containers such as buckets, bottles, plastic bags, disposable cups, and plastic drums as well as construction materials and discarded vehicles such as battery shells, tires, and cement mixers as key containers for the production of *Aedes* [8]. Fish reared in artificial containers, such as large domestic tanks and rain-water vessels, have already been used a biological control alternative in countries such as Brazil, Nicaragua, and Mexico [15]. The purpose of the present study was to determine the predatory efficiency of native zebrafish and exotic guppies in plastic containers to determine their larvivorous potential in the presence of alternative prey. As a result, determining mosquito preference is a prerequisite before promoting these fish species for biological control [12]. The presence of alternative prey complicates the interaction, resulting in a predation pattern

that is context dependent. Because most mosquito larval habitats are heterogeneous, the predatory ability of fish must be tested in the presence of habitat structural complexity, which influences the outcome of prey-predator interactions [16].

Although chemical control is present in Bangladesh, the country has yet to establish an integrated vector control policy and lacks the necessary infrastructure, manpower, and community engagement [17]. Despite the fact that numerous studies around the world have supported the use of larvivorous fish as biological control agents for vector mosquitoes, the potential of larvivorous fish for controlling *Aedes* larvae has not been adequately evaluated in Bangladesh. Only one study used guppies to control *Culex* mosquitoes but there is no study on *Aedes* [18]. Another study used three native fish (slender rasbora, zebrafish, and banded gourami) and two exotic fish (mosquito fish and guppy) to control mosquito larvae in a waterlogged environment in Bangladesh [19]. Therefore, the current study was carried out to fill this gap by assessing and comparing the predatory efficiency of native zebrafish and exotic guppies in plastic containers to evaluate their larvivorous potential in the presence of alternative prey for a sustainable, cost-effective, and eco-friendly way to manage dengue epidemics.

Materials and methods

Experimental protocol

The experiment was conducted from November 2021 to February 2022 in the Entomology Research Laboratory and Animal Garden of the Department of Zoology, University of Dhaka, Dhaka, Bangladesh. All experiments were carried out in plastic containers and each parameter was conducted in 3 replicates.

Mosquito rearing

Collection of Aedes eggs

Eggs of *Aedes* species were collected from the Animal Garden in the Department of Zoology, University of Dhaka. Eggs were collected on moist filter paper. Plastic bowls were lined with filter paper and water was added to a depth of 2.5 cm. Several plastic bowls were then placed in the different corners of the garden. The egg-collecting bowls were left in the environment for 48 hours. The bowls were then brought to the laboratory and excess water was drained out of the bowls. A batch of eggs was placed inside larval rearing trays with 250 ml tap water. Environmental conditions were not controlled in the laboratory [20].

Rearing of larvae

The eggs hatched into first instar larvae after two days. The larvae were then transferred to small plastic bowls. The larvae were fed daily on yeast. Water was added daily to the plastic bowl to freshen up the water and replenish the water loss through evaporation. The larvae were gradually molted into second, third, and fourth instar larvae. After a few days, pupae were collected from the plastic bowl by using a dropper and placed in another small plastic bowl. The bowls were then transferred into mosquito-rearing cages. After a few days, adults emerged.

Establishment of Aedes colony

Adult mosquitoes were reared for larval production for further experiments. Adults of both sexes require carbohydrate foods in addition to blood meals for ovarian development. Carbohydrates are generally given as a form of sugar solution. In this rearing process, 10% sucrose was provided as food after the emergence of adults which was prepared by dissolving 100 g of white sugar in 1 L of water. Cotton balls were soaked in a solution of 10% sugar before being placed on top of the cage. Cotton balls were changed daily. After a week of adult emergence, a pigeon was placed in each cage to provide a blood meal for 2 hours. The thorax feathers of the pigeon were shaved before being placed in the cage. Two days following the blood feeding, oviposition cups were kept in each cage where the blood-fed females oviposited. Then the eggs were transferred to a plastic bowl for hatching. Larvae were reared up to the 4th instar by feeding them yeast. Pupae were removed from the larval-rearing bowl and placed into mosquito-rearing cages for the emergence of adults for further rearing (Fig. 1).

Identification of the using specimen

Identification of Aedes eggs

Aedes females lay one egg at a time in artificial and natural water containers by gluing the eggs to the substrate directly above the water line. The eggs were tapering anteriorly and posteriorly and broadly cigar-shaped. The color was dull or matte black or shiny jet-black color. Eggs were identified using the method outlined by Bova, Paulson & Paulson [21].

Identification of larvae

The larvae of *Aedes* have thick and short siphons, two siphon feathers, and a comb tooth with the lateral spine (*Aedes aegypti*) or without the lateral spine (*Aedes albopictus*). The details of *Aedes* larvae identification are described elsewhere [22].

Identification of adults

The adult of *Aedes* is characterized by contrasting black-and-white colouration. The scutum has silver scales in the shape of a lyre on a black background (*Ae. aegypti*) or with a median silver-scale line on a black background (*Ae. albopictus*) [23] (Fig. 2).

Collection and maintenance of fish

Two fish species namely *D. rerio* and *Po. reticulata* were collected from a local aquarium shop in Katabon, Dhaka. The fish were then brought to the laboratory and kept in 5 L plastic containers with proper aeration at a density of 8 fish per container. They were then given commercial feed after a week of acclimatization to laboratory conditions. Environmental conditions were not controlled in the laboratory. Only female fish were used in the experiment. Samples of the two fish species used in this study were identified morphologically.

Characteristic features of D. rerio

The fish has 5–7 longitudinal dark blue stripes running from behind the operculum to the caudal fin. In addition, the anal fin is striped and the upper edge of the dorsal fin is dark blue with a white border. Both males and females have similar colouration with the exception that males have larger anal fins that are more yellow in color. In gravid females, a small genital papilla presents in front of the anal fin origin and they have a more rounded body [24].

Characteristic features of Po. reticulata

The fish are small with larger females that can reach a standard length of 5 cm. The females have a uniform grey colour. The fish has 7–8 soft rays on the dorsal side and 8–10 soft rays on the anal side. Pregnant females have a black triangle between the anal and pelvic fins [25].

Collection of chironomid larvae

Chironomid larvae were collected from the animal garden, Department of Zoology, University of Dhaka. In the laboratory, chironomid larvae were maintained in transparent glass beakers. The beakers contained sewage sediment mixed with sand. In addition, as a supplement crushed fish food was added. The identification was made up to the generic level [26].

Experimental method

Predatory efficiency of fish under laboratory conditions

The predation experiment is illustrated in Fig. 3. In the first experiment, the predatory efficiency of the fish was tested under 2 L tap water and 100 prey densities. The 3rd instar larvae of *Aedes* were used separately in this experiment. Three replications were carried out to evaluate the feeding pattern of different individuals of the same fish species. The trials were carried out in transparent plastic jars containing 2 L of tap water (7.06 P^H and 22.6°C temperature) to exclude any other predators. Individual fish were placed in each container and were held without food for 24 hours prior to each experiment to standardize hunger levels. For each fish, the total length (TL) was measured using normal meter scales. Fish with sizes from 2 cm to 2.8 cm were used in the predatory potential experiment.

In the second experiment, different prey combinations such as larvae of *Aedes* (second, third, and fourth instar), pupae of *Aedes*, chironomid larvae, and fish food were used to assess comparative predation potential on larvae, pupae, and other food. This experiment was conducted under 2 L tap water (7.10 P^H and 20.7°C temperature) and 150 prey densities (25 larvae of each instar, pupae, and other food). Fish with a size from 3.5 cm to 3.8 cm were used in this experiment. Individual fish were placed in each container and were held without food for 24 hours before each experiment to standardize hunger levels. The observations were made for 24 hours. The weight of the fish was measured before and after the experiment with a Kern analytical balance and water quality was measured using a Hach HQD digital

multimeter. The consumption of the larvae in laboratory trials was calculated from the difference between the initially introduced mosquito larvae and the remaining larvae. The predatory efficiencies of zebrafish and guppies were calculated by using the following equation [6].

$$\mathrm{Predatoryefficiency} = \frac{\left(\frac{\mathrm{Numberofpreyconsumed}}{\mathrm{Numberofpredatorintroduced}}\right)}{\mathrm{Totalnumberofpreyintroduced}} * 100$$

Estimation of prey preference

The prey preference of both fish was assessed using 2nd, 3rd, and 4th instar larvae of *Aedes* as target prey, chironomid larvae and pupae of *Aedes* as live prey, and fish food as alternative prey. In comparison to other food types, a single fish was allowed to consume the food items at an equal density of mosquito larvae (25 of each food item for a total of 150). The observations were conducted for 24 hours in a plastic container and 2 L of tap water. For each of the fish species, the experiment was conducted 3 times. The predation data were recorded for each fish species, which was then applied to preference analysis. The preference for each food item was calculated by using the following equation [12]:

PP = PC/ PA

where, PC = proportion of the food item consumed, and PA = proportion of the food item available. For a specific prey type, the selectivity index is then calculated using an equation similar to Manly's α selectivity index.

MA = PP /ΣPP

A deviation from the expected value of 0.17 (for equal mosquito density) was used to determine the prey preference. As different food items were available, any value above these will indicate a relative preference whereas any value below will indicate a relative avoidance of mosquito larvae. A t test (one-tailed) was used to justify significant relative avoidance and relative preference for the food items with values less or more than expected.

Data analysis

SPSS (version 25.0) and GraphPad Prism were used to analyse the data. The results of this study were presented as mean ± standard error. An independent sample t-test was used to assess the significant difference between the larval consumption of *D. rerio* and *Po. reticulata*. The Pearson correlation coefficient was used to assess the relationship between the morphology of fish (mass and total length) and predatory efficiency. To determine the variation in the food consumption of fish one way ANOVA was used and to assess which food groups were significantly consumed Tukey's Post Hoc test was used.

Results

Total number of larvae consumption and predatory efficiency

The consumption of total *Aedes* larvae (3rd instar) and predatory efficiency of the studied fish species are shown in Table 1. *D. rerio* showed the highest predation rates within 24 hours, consuming 63.67 ± 3.17 larvae, and the predatory efficiency was 63.67 ± 3.17%, whereas *Po. reticulata* showed the lowest predation rates, consuming 33.33 ± 4.37 larvae, and the predatory efficiency was 33.33 ± 4.37%. There was a significant ($P < 0.05$) difference between the larval consumption and predatory efficiency of *D. rerio* and *Po. reticulata.* The predatory efficiency of *D. rerio* was 1.91 times higher than that of *Po. reticulata*.

Table 1
Consumption of 3rd instar larvae of *Aedes* and predatory efficiency within 24 hours

Fish species	Larvae consumed by a single fish [a]	Predatory efficiency (%) [a]
D. rerio	63.67 ± 3.17*	63.67 ± 3.17 *
Po. reticulata	33.33 ± 4.37	33.33 ± 4.37
[a] Mean ± SE		
* $p < 0.05$ in independent samples t test between *D. rerio* and *Po. reticulata* in terms of larval consumption and predatory efficiency		

Predictors of larvae consumption

Table 2 shows the total length and weight of the fish that may affect the consumption of *Aedes* larvae and predatory efficiency. The highest predatory efficiency was found in *D. rerio* (63.67 ± 3.17) which had the highest values for body weight (0.17 ± 0.12 g) and total length (2.73 ± 0.03 cm) whereas *Po. reticulata* had the smallest body weight (0.08 ± 0.003 g) and total length (2.03 ± 0.03 cm) and a predatory efficiency of 33.33 ± 4.37%.

Table 2
Morphological features of *D. rerio* and *Po. reticulata*

Fish species	Life stage	Weight in g [a]	Length in cm [a]
D. rerio	Adult	0.17 ± 0.12*	2.73 ± 0.03*
Po. reticulata	Adult	0.08 ± 0.003	2.03 ± 0.03
[a] Mean ± SE			
* $p < 0.05$ in independent samples t test between *D. rerio* and *Po. reticulata* for their weight and length			

The Pearson correlation analysis indicated that two predictors (weight and total length) significantly influenced ($P < 0.05$) the consumption of larvae and predatory efficiency of *D. rerio* and *Po. reticulata.*

There was a positive correlation between the weight of fish and predatory efficiency (Fig. 4a) and between the total length of fish and predatory efficiency (Fig. 4b).

Predatory efficiency on mosquito larvae and pupae in the presence of alternative food

The variation in the different food consumption of zebrafish ($F(5,12) = 22.786$, $p < 0.01$) and guppies ($F(5,12) = 9.65$, $p < 0.01$) differed significantly as suggested by the result of one-way ANOVA. Figure 6a shows that zebrafish consumed significantly more 2nd and 3rd instar larvae than pupae, chironomid larvae, and fish foods and the fish consumed significantly more 4th instar larvae than fish food. Figure 6b shows that guppies significantly consumed more 2nd and 3rd instar larvae than pupae and fish food and significantly consumed 4th instar and chironomid larvae over pupae. There was no significant difference between the consumption of mosquito larvae (2nd, 3rd, and 4th instars) and chironomid larvae.

The comparative predation efficiency of guppies and zebrafish is shown in Fig. 7. *D. rerio* showed significantly ($p < 0.05$) higher predation efficiency on 2nd instar larvae and pupae than *Po. reticulata*. The predation efficiency of both fish for the 3rd instar larvae was equal. *Po. reticulata* consumed more 4th instar larvae than *D. rerio* and *Po. reticulata* showed the highest predation efficiency on chironomid larvae compared to *D. rerio*.

Prey preference of D. rerio and Po. reticulata

Figure 7 depicts the different numbers of food items consumed by *D. rerio* and *Po. reticulata*. They consumed all food types but the amounts were different from each other. At equal mosquito densities, Table 3 shows that *D. rerio* and *Po. reticulata* exhibited a higher preference for all food items than expected values. *D. rerio* showed a significantly higher preference for 2nd instar larvae and pupae than *Po. reticulata*.

Table 3
Selectivity index shown by D. rerio and Po. reticulata for the different food items at an equal density of Aedes larvae (25:25)

Food items	2nd instar	3rd instar	4th instar	Pupae	Chironomid larvae	Fish food
Expected selectivity value	0.17	0.17	0.17	0.17	0.17	0.17
D. rerio						
Mean ± SE	0.33 ± 0.05*	0.33 ± 0.16	0.33 ± 0.08	0.33 ± 0.02*	0.3 ± 0.15	0.3 ± 0.08
t value	3.5	1.02	2.0	7.0	1.09	2.11
Po. reticulata						
Mean ± SE	0.34 ± 0.19	0.33 ± 0.15	0.33 ± 0.11	0.33 ± 0.23	0.3 ± 0.10	0.3 ± 0.10
t value	0.85	1.06	1.41	0.70	1.57	1.50
* $p < 0.05$ in independent samples t test between *D. rerio* and *Po. reticulata* for each food item						

Discussion

Although chemical insecticides are the most widely used method for mosquito control, the long-term use of insecticides has an adverse effect on human health, non-target organisms, and the environment and causes vector resistance [1]. As a result, a more effective, environmentally friendly, and cost-effective method is the use of natural predators to control *Aedes* mosquitoes [27]. To control mosquito larvae, several countries use larvivorous fish directly, whereas in others, this approach is used as a part of an integrated vector management program [10]. The study was conducted to evaluate the larvivorous potential of *D. rerio* and *Po. reticulata* in the presence of alternative prey. The present study provides insight into *D. rerio* and *Po. reticulata* where the fish are used as predators against the larvae of the *Aedes* mosquito. In the laboratory, both fish consumed *Aedes* larvae, although the consumption rates were significantly different.

D. rerio showed the highest larval consumption and predatory efficiency of 63.67% over *Aedes* larvae, while *Po. reticulata* consumed the least in the laboratory. Parallel to the current study results, several other studies showed that indigenous fish are more effective in controlling *Ae. aegypti* larvae than exotic *Po. reticulata* [10, 28–32]. In Sri Lanka, *Aplocheilus dayi* is more effective for controlling *Ae. aegypti* larvae with 68.4% predatory efficiency under laboratory conditions and were able to reduce 95.9% *Ae. aegypti* larvae in the field. *Puntius bimaculatus* showed 50.1% predatory efficiency in laboratory conditions and was able to reduce 80.3% of larvae in the field whereas *Po. reticulata* showed a predatory efficiency of 64.4% in laboratory conditions and was able to reduce the number of larvae by 83.1% in field conditions [10]. Five native fish, *Astyanax fasciatus, Lepisosteus tropicus, Ictalurus meridionalis, Brycon*

guatemalensis, and *Poecilia sphenops* were very effective biocontrol agents of *Ae. aegypti* larvae in a domestic cement tank in Mexico [30]. A study reported that two native fish of North Queensland, eastern rainbow fish and fly-specked hardyhead were effective in controlling *Ae. aegypti* larvae [31]. In Brazil, *Xiphophorus maculatus* had higher predatory efficiency for *Ae. aegypti* larvae than *Po. reticulata* [28]. Several other studies showed that *D. rerio* demonstrated promising predation potential against *Culex* mosquitoes and consumed an average of 62 larvae at 100 prey density [2, 33]. *D. rerio* consumed 52 fourth instar larvae of *Anopheles* per day in laboratory conditions [34]. The above study showed that the predatory potential of larvivorous fish was higher in environmental conditions than in laboratory settings. In the current study, zebrafish consumed 63.67% of larvae in laboratory settings which may increase under field conditions.

In the present study, *D. rerio* consumed 367 *Aedes* larvae per gram of weight per day and *Po. reticulata* consumed 421 *Aedes* larvae per gram per day (Fig. 5a). In terms of length, *D. rerio* consumed 23 larvae per day, and *Po. reticulata* consumed 16 larvae per day (Fig. 5b). The estimation of the number of larvae consumed per unit mass and per unit length by each fish makes it possible to compare the predatory potential of the fish species and found that female *Betta splendens* consumed 406 to 523 larvae per gram per day, female *Astyanax fasciatus* consumed 281 to 349 larvae per gram per day, and male *Trichogaster trichopteros* consumed 117 to 200 larvae per gram per day. These fish proved to be the most effective predators of the *Ae. aegypti* larvae [15]. The larval eating capacity of a fish in terms of fish size or weight is an important factor when evaluating the predation potential of fish species for controlling mosquito larvae. Control can be recognized as efficient when a particular number of fish are able to consume all the larvae that are present in a particular breeding container [15]. In the present study, the weight and size of the fish were positively correlated with larval consumption which was supported by earlier studies [19, 35, 36].

The results showed that *D. rerio* significantly consumed more 2nd instar larvae and pupae of *Aedes* than guppies. When other foods are available, *D. rerio* prefers to eat the earlier larval instar over the later instar and prefers mosquito larvae and pupae over other foods (Fig. 7). Gupta & Banerjee reported that panchax minnow consumed significantly more 3rd instar larvae of *Culex* than guppy [11]. Anyaele & Obembe showed that *Aphyosemion gularis* consumed significantly more *Anopheles* larvae than pupae and significantly more *Anopheles* larvae than *Culex* and chironomid larvae [13]. *Culex* larvae were preferred over chironomid larvae while *Anopheles* larvae were consumed significantly more than abundant ostracods [13]. Another study showed that *Aplocheilus panchax* preferred mosquito larvae over alternative prey [16]. It was found that *D. rerio* reared in rice fields filled with mosquitoes had tremendously reduced larval and pupal densities in the rice field [34]. It was also reported that zebrafish showed higher predation potential against mosquito larvae in the pond and drain water than guppies. *Trichogaster fasciata*, native fish of Bangladesh commonly known as banded gourami showed the higher predation potential against mosquito larvae than guppies and the fish can also survive in drain water [19].

For the mosquito and non-mosquito preference test involving *Aedes* larvae, pupae, chironomid larvae, and fish food combined with *D. rerio* and *Po. reticulata* showed a higher preference for all food items than expected values at equal mosquito densities. *D. rerio* showed a significantly higher preference for 2nd instar larvae and pupae than *Po. reticulata* (Table 3). From the results, it is evident that there was no significant difference between the consumption of mosquito larvae and chironomid larvae by guppies (Fig. 6b), and it consumed more chironomid larvae than *Aedes* pupae (Fig. 7). Although guppies are able to consume insect larvae, experimentally, their use as a biological control agent can be more harmful than beneficial [37]. Recent studies revealed that the gut contents of guppies consisted of a significantly higher percentage of phytoplankton in both man-made canals and natural streams [37]. Another study reported that guppies failed to feed *Cx. quinquefasciatus* larvae in polluted drain water and preferred to eat other available food [14]. Guppies showed significantly less preference for mosquito larvae over alternative food items [12]. Guppies are also regarded as invasive species, with the potential to disrupt aquatic communities and ecosystem processes [38]. Several studies have reported that the introduction of exotic larvivorous fish could have serious ecological consequences by posing a threat to native organisms such as amphibians whose populations are often in decline. Thus, indigenous larvivorous fish have been found to be more effective in vector control operations, arguing for their widespread use [2].

In the present study, zebrafish showed higher predatory efficiency than guppies which are used worldwide for mosquito control. The results of the present study indicate that zebrafish can effectively consume *Aedes* larvae and pupae. As the fish consumed chironomid larvae, they survived in the absence of mosquito larvae. Thus, the current study suggests that native fish such as zebrafish can be effectively used to control mosquitoes in different aquatic resources, either alone or in combination with other vector control programs instead of using exotic varieties because indigenous fish have higher predation efficiency than exotic fish. The findings of this study will help in determining the efficacy of indigenous fish in controlling vector mosquitoes, as well as promoting the importance of indigenous fish species conservation.

Conclusion

This study determined the predatory efficiency and feeding preference of *D. rerio* and *Po. reticulata*. The predatory efficiency of *D. rerio* is significantly higher than that of *Po. reticulata* and *D. rerio* was significantly more effective in preying upon 2nd instar larvae and pupae of *Aedes* than *Po. reticulata* when alternative prey was available. Therefore, *D. rerio* can be introduced during the start of the vector season to control the earlier stage of *Aedes* larvae and introduced at the end of the vector season to control the pupal stage of *Aedes* mosquitoes. The present study suggests that *D. rerio* can be evaluated as an alternative species to *Po. reticulata* for the management of *Aedes* mosquitoes. Further studies are recommended to evaluate the survival rate and predatory potential of *D. rerio* under different semi-environmental and environmental settings.

Declarations

Ethics approval and consent to participate

The protocols described in the manuscript were approved by the ethical review committee of the Faculty of Biological Sciences, University of Dhaka (Ref. No. 184/Biol.Scs.). All methods used in this study were performed in accordance with relevant guidelines and regulations. The study involved the species which are not endangered and were not collected in a protected area. No permissions were required to purchase and use the animals used in this study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets of the study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Tanjina Akter. The first draft of the manuscript was written by Tanjina Akter and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

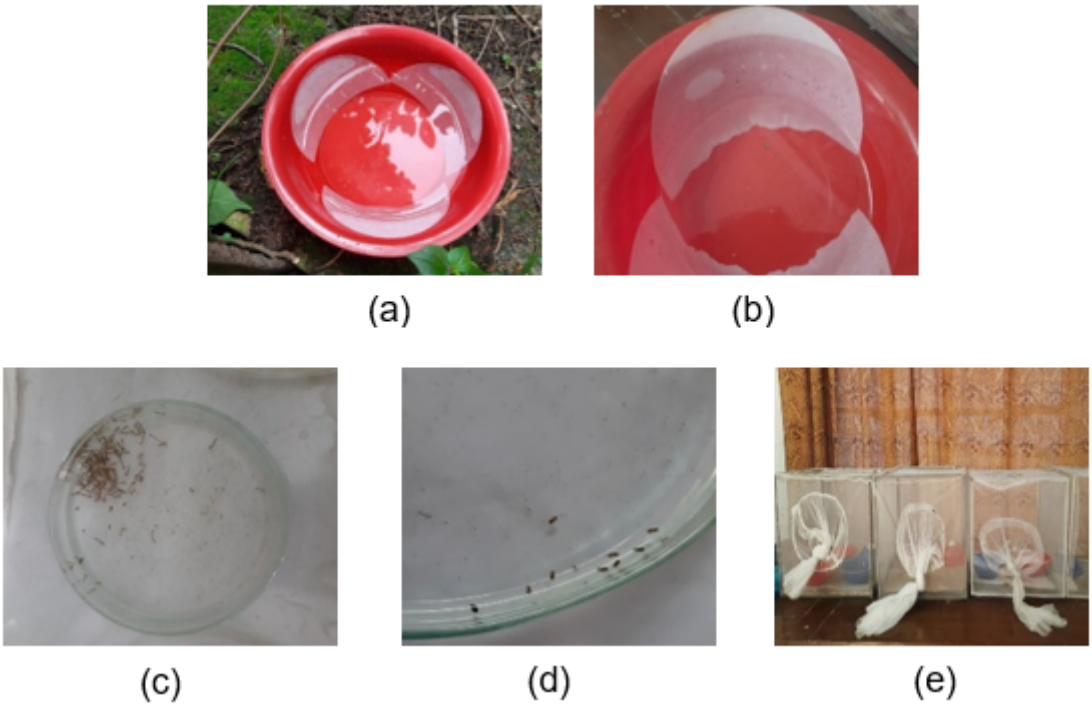

Figure 1

Establishment of Aedes colony; (a) collection of eggs (b) eggs on filter paper (c) larvae (d) pupae (e) adult mosquito in cage

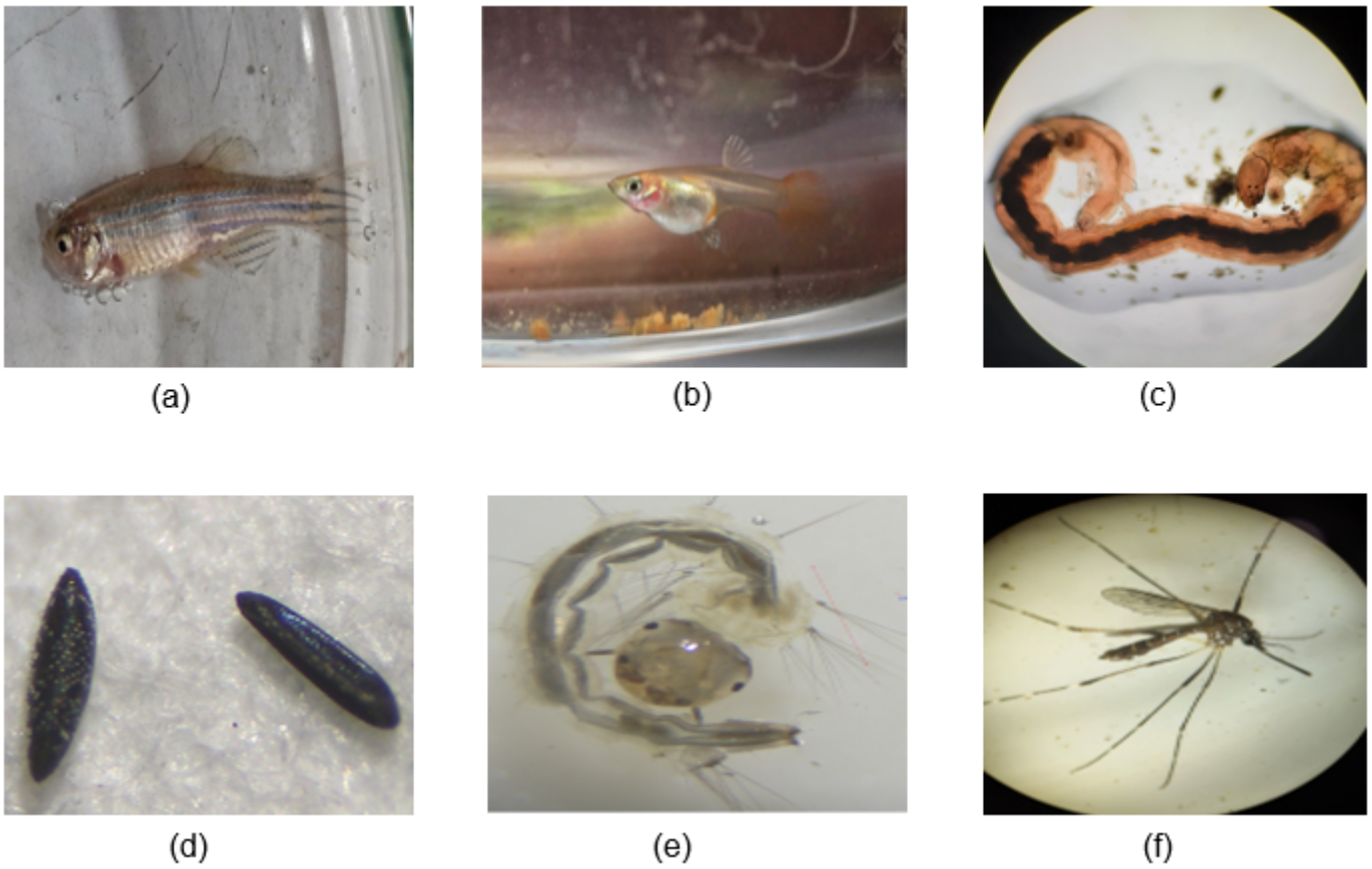

Figure 2

Identification of the species used in the experiment; (a) D. rerio (b) Po. reticulata (c) chironomid larvae (d) eggs of Aedes (e) larvae of Aedes and (f) adult of Aedes

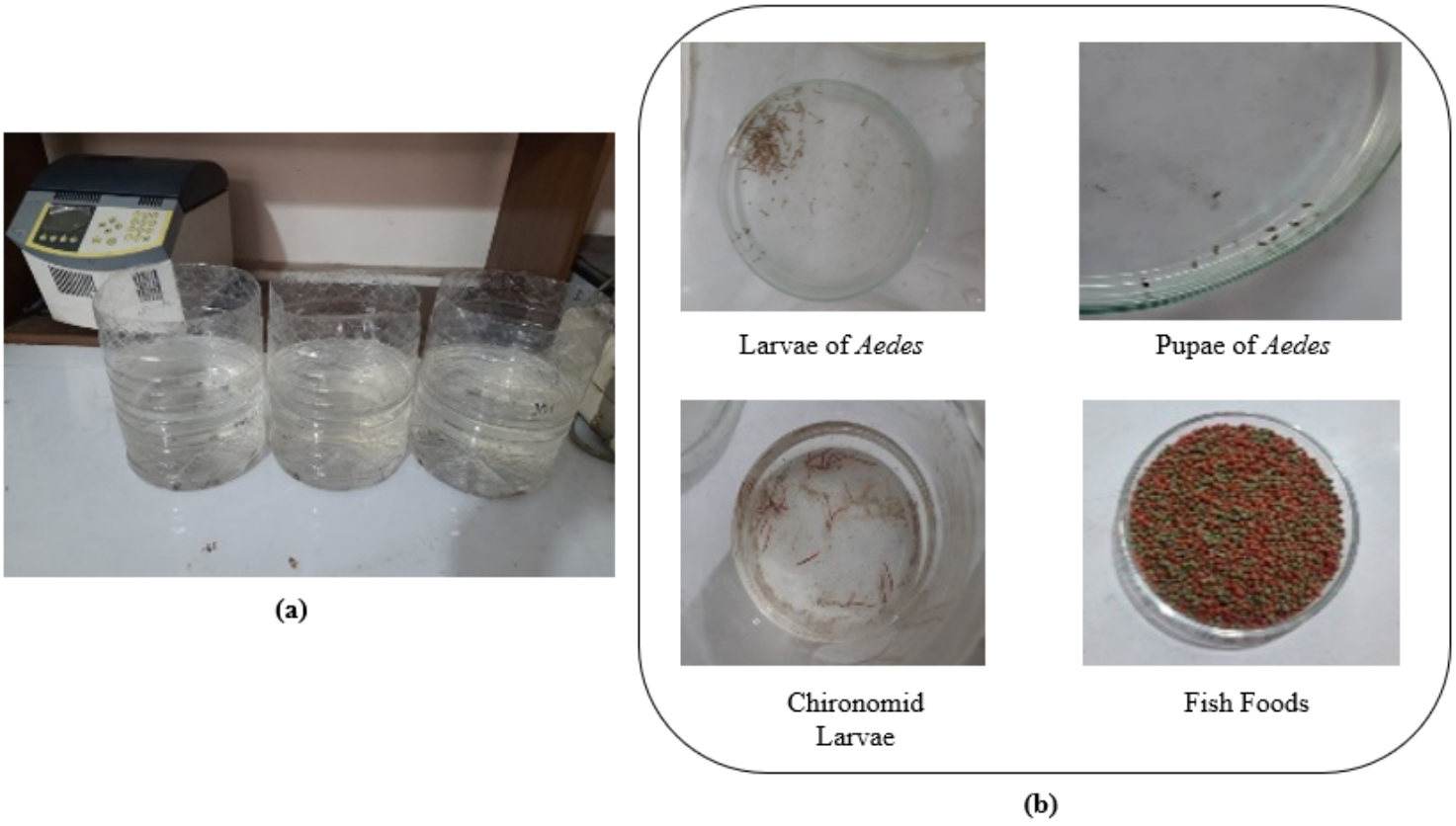

Figure 3

Predation experiment in the laboratory. (a) Experiment in the plastic containers and (b) foods used in predation experiment

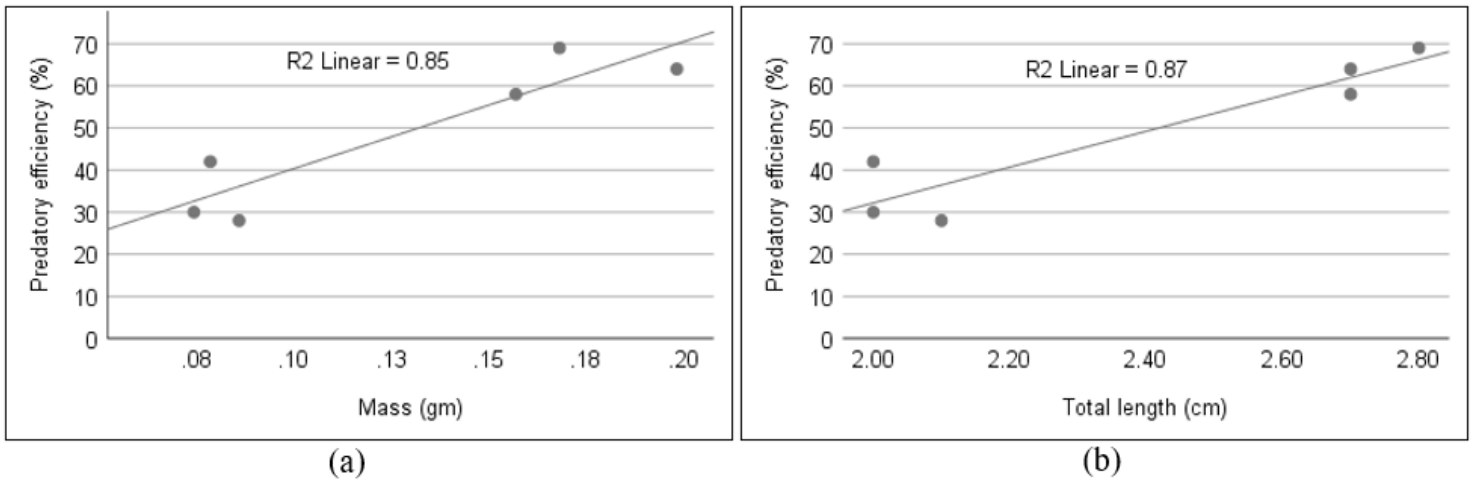

Figure 4

Correlation between predatory efficiency and morphology of D. rerio and Po. reticulata. (a) Predatory efficiency and mass and (b) predatory efficiency and total length of D. rerio and Po. reticulata

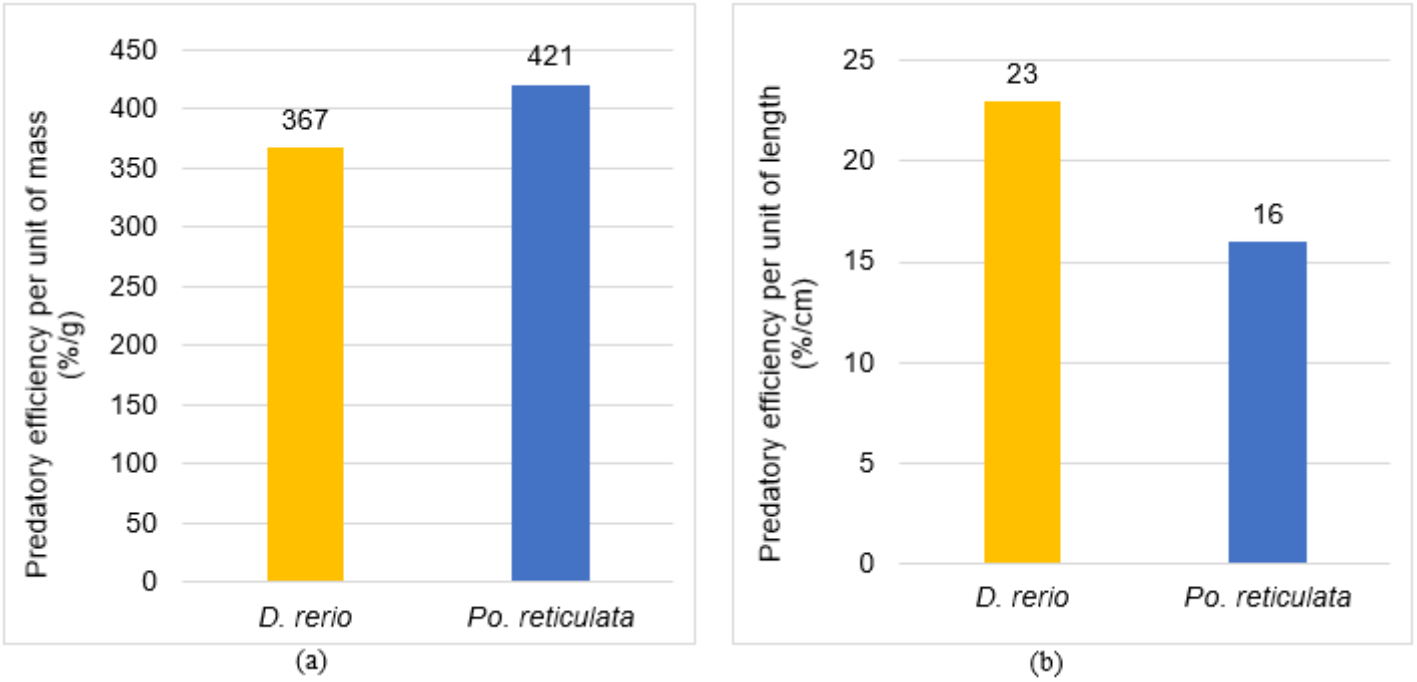

Figure 5

Predatory efficiency of D. rerio and Po. reticulata. Predatory efficiency in terms of (a) per unit of mass and (b) per unit of length. The efficiency was measured as a percentage, while mass was measured as grams and length was measured as centimeters

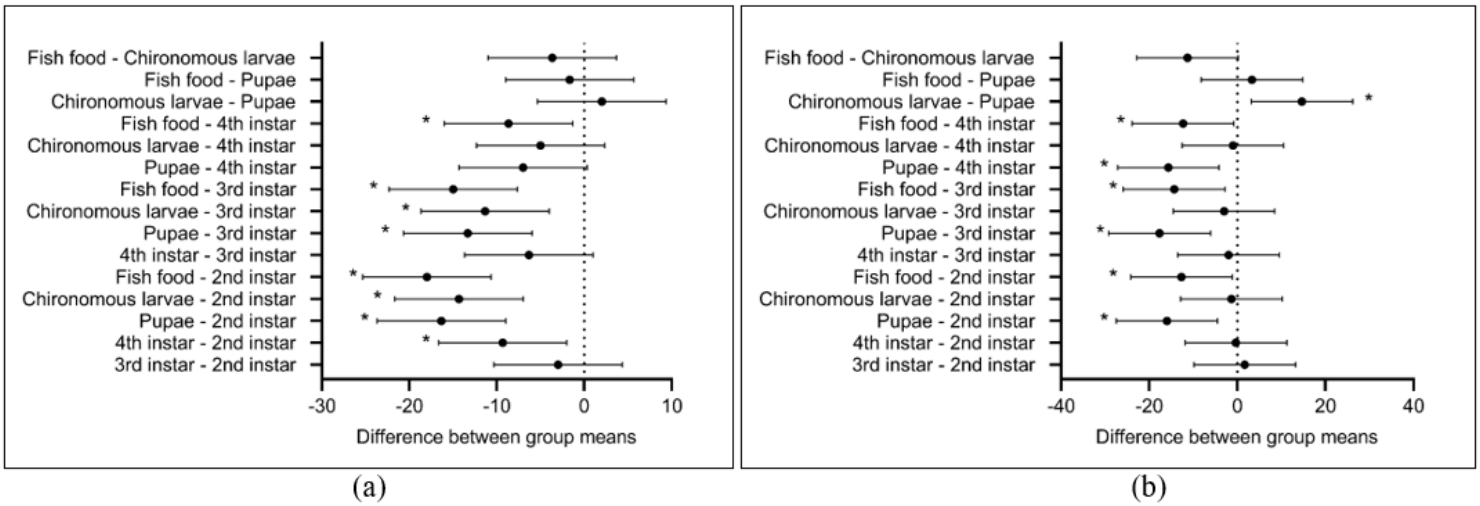

Figure 6

Comparison of different food consumption within a 24-hour period by (a) D. rerio and (b) Po. reticulata. The dot with a horizontal bar indicates the mean ± 95% confidence interval. The significant differences between food groups are indicated by asterisks (one way ANOVA $p < 0.05$ followed by Tukey's Post Hoc test)

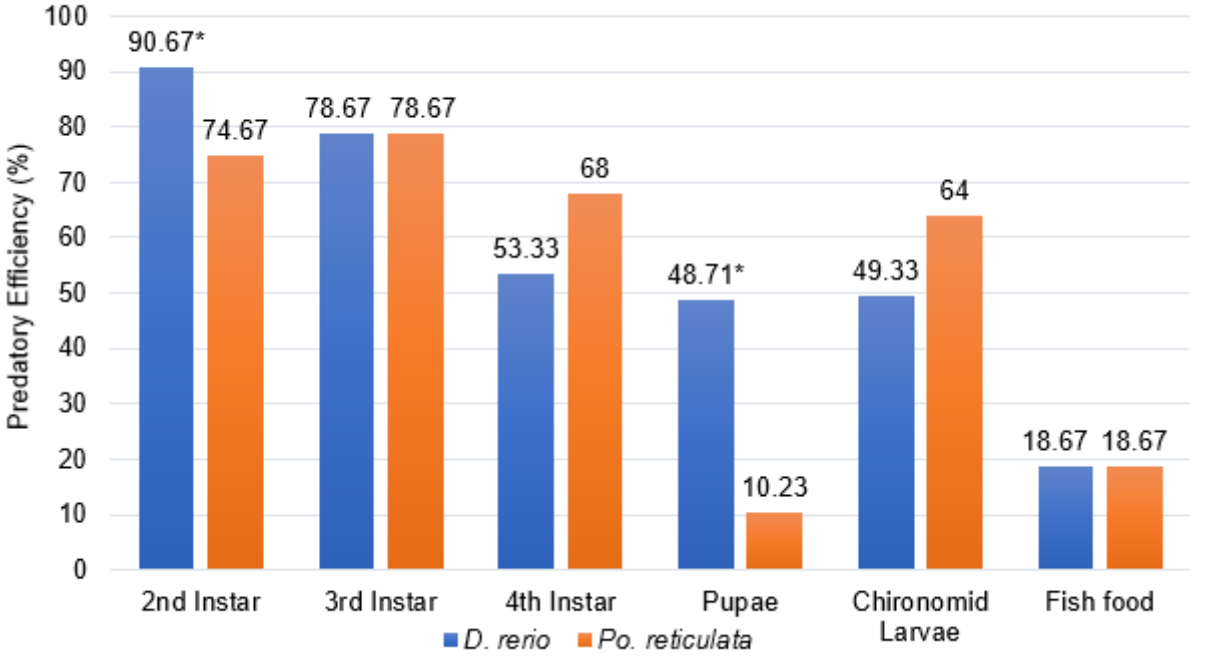

Figure 7

Predatory efficiency of *D. rerio* and *Po. reticulata* on the presence of alternative foods. A significant difference is indicated by an asterisk (independent samples t test $p < 0.05$)