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Role of anuran tadpoles as biological control for dengue larvae

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Abstract
Dengue is a viral disease caused by Aedes mosquitoes (Diptera: Culicidae) and cause serious illness in people community living in tropics. It is able to cause bundles of symptoms and may cause bleeding internally leading to death of the patient. Currently there is no immunization against dengue disorder but the most broadly utilized techniques for control of mosquitoes are usage of insecticides and biological control. Present study has been designed to observe and compare the consumption of dengue mosquito larvae by two anuran species, Common Skittering Frog (*Euphlyctis cyanophlyctis*) and South-east Asian Toad (*Duttaphrynus melanostictus*) tadpoles inhabiting Rawalpindi and Islamabad. The tadpoles and dengue larvae were collected from ponds and pools using dip nets. Six trails were carried with anuran species of common skittering frog and South-east Asian toad. Each trail was replicated thrice. The Kruskal-Wallis test present that the total number of consumed dengue larvae among various trials between two species (Common Skittering Frog and South-east Asian Toad) did not differ-significantly (P=0.26) and (P=0.08), while the Wilcoxon test showed that the number of consumed dengue larvae by tadpoles of the two anuran species was P=0.08 which also did not differ significantly. Therefore it was concluded that the South-east Asian toad is better in the consumption of dengue mosquito larvae as compared to common skittering frog.

Keywords: anuran, dengue, rawalpindi, islamabad tadpoles

1. Introduction
The crops, livestock and human contract diseases spread through various vectors such as mosquitoes. By using natural competitors we can control the density of vectors and disease transmission as biological control methods. However, the indirect interactions in host-vector disease system make it very problematic to practice the out-of-date nuisance control system [1].
By the health potential and natural risks of chemicals, mosquitoes have more resistant for traditional chemical sprays and pesticides for example Methoprene, Piperonyl, Butoxide etc. [2]. Due to the environmental pollution risks, an environmental protection agency (EPA) has also banned the use of many pesticides used by human [3].
For the control of mosquito-borne arboviruses, use of frogs is important, because normally there is not any specific antiviral rehabilitation. The tadpoles of frogs were usually considered to prey upon mosquitoes larvae. To reduce density of mosquitoes population which serve as disease spreading vectors, people use different predatory species and other microscopic organisms under the biological control method in the past [4].
Adults as well as tadpoles of amphibians have been employed for the control of disease vectors. Frogs showed very significant character in the ecosystems with a prodigious importance for insect and pest control system [5]. The transmission of dengue in the society can only be decreased when controlling mosquito vectors. The tropical and subtropical countries have dengue fever (brain-hemorrhagic fever) as an endemic disease [6].
In biological control of mosquitoes, only fish has been mentioned. Larvivorous fish like Guppy (*Poecilia reticulate*) and Mosquito fish (*Gambusia affinis*) has familiarized in different chunks of the universe [7]. An aspect that they reduced the efficacy by means of fish for control of mosquitoes is that, maximum fishes (particularly carnivorous fish) frequently needs related watercourses for swimming, while frogs did not need this because they can traffic over this earth, the mosquitoes hypothetically are free to produce eggs in any water body at their will [8].

The common anurans of Rawalpindi-Islamabad area were common skittering frog (*Euphlyctis cyanophlyctis*), Indus Valley toad (*Bufo Stomaticus*), Asian common toad (*Bufo melanostictus*), Indian bullfrog (*Hoplobatrachus Tigerinus*), ornamented pygmy frog (*Microhyla ornata*), Indian burrowing frog (*Sphaerotheca breviceps*) and Indian cricket frog (*Fejervarya limnocharis*) [9]. They breed mostly during summer and monsoon seasons. Hence, during these seasons the wetlands of the current areas have full of tadpoles [10].

Study on the need of anuran tadpoles in the process of biological control of *Aedes* mosquito in Pakistan are lacking. The present study has therefore, been designed to study the consumption of dengue mosquito larvae by anurans tadpoles inhabiting Rawalpindi and Islamabad, Pakistan. And to compare the consumption of dengue mosquito larvae by tadpole of anuran species.

2. Materials and Methods

2.1 Study area

The sampling of tadpoles carried out from chosen areas of Rawalpindi, Islamabad (Ghori town, Shaheen town, Fazal town and Yousaf colony). The district Rawalpindi (32° 56′ 0″ N, and 72° 52′ 0″ E) has an area of about 5282 km^2 and situated on the southern slopes north-western edges of Himalayas, also contains big tracks and rich valleys. The district comprise of seven tehsils including Kahuta, Gujar Khan, Kotli Sattian, Murree, Kallar Syedan, Taxila and Rawalpindi. Common anurans species of Rawalpindi Islamabad area are Indus Valley toad (*Bufostomaticus*), Asian common toad (*Bufo melanostictus*), ornamented pygmy frog (*Microhyla ornate),* skittering frog (*Euphlyctis cyanophlyctis*), Asian bullfrog (*Hoplobatrachus tigerinus*), Indian burrowing frog (*Sphaerotheca breviceps*) and Rice field frog (*Fejervarya limnocharis*) [11, 6].

2.2 Methodology

Dengue larvae were collected by using dipping net method, and were identify with the help of identification key [12], while the anuran tadpoles were collected from ponds and pools using dip nets from Ghori town, Shaheen town, Fazal town, Yousaf colony, Rawalpindi and Islamabad. The tadpoles were transferred to buckets containing mixture of tap water and pond/pool water, and brought to University laboratory. The tadpoles were kept in glass aquaria (tadpole bank), and fed with boiled cabbage/lettuce initially and boiled chicken after external gill stage. From tadpole bank, they were transferred to experimental beaker (1000 ml). Six trails were carried with anuran species of Common Skittering frog and South-east Asian Toad. Each trail was replicated thrice. Six anuran tadpoles were transferred to the beakers placed under glass cage and provided with the following combinations:

Trial I: Tadpoles fed with dengue larvae only.
Trial II: Tadpoles fed with dengue larvae and snail meat.
Trial III: Tadpoles fed with dengue larvae and earthworm meat.
Trial IV: Tadpoles fed with dengue larvae and beetles.
Trial V: Tadpoles fed with dengue larvae and flies.
Trial VI: Tadpoles fed with dengue larvae, beetles and snail meat.

The observations were made after 24 hours, and the number of consumed dengue larvae was noted. The comparison of consumption of dengue larvae among different trial was made using Kruskal-Wallis test. The comparison of consumption of dengue larvae between the both anuran species was made using Wilcoxon test (Field, 2009).

3. Results and Discussion

3.1 Consumption of dengue larvae by common skittering frog in various trails

The results of trial I showed 50, 100 and 75%, trial II showed 75, 100 and 50%, trial III showed 50, 100 and 75%, trial IV showed 100, 100 and 75%, trial V showed 50, 100 and 75% and trial VI showed 100, 100 and 100% consumption of dengue larvae by tadpoles of common skittering frog (*E. cyanophlyctus*) in all three replications respectively (Table 1 and figure 1). The Kruskal-Wallis test present that the total number of consumed dengue larvae among various trials did not differ significantly (P=0.48).

Table 1: Consumption of dengue larvae by Common Skittering Frog (*Euphlyctis cyanophlyctis*) in various trials

Trial	Replication	No. of tadpoles	No. of dengue larvae	Combination	Quantity of food	No. and %age of consumed dengue larvae
1	1	2	4	Dengue larvae only	1 individual	2 (50%)
	2	2	4	Dengue larvae only	1 individual	4 (100%)
	3	2	4	Dengue larvae only	1 individual	3 (75%)
2	1	2	4	Dengue larvae + Snail meat	2 individuals	3 (75%)
	2	2	4	Dengue larvae + Snail meat	2 individuals	4 (100%)
	3	2	4	Dengue larvae + Snail meat	2 individuals	2 (50%)
3	1	2	4	Dengue larvae + earthworm	2 individuals	2 (50%)
	2	2	4	Dengue larvae + earthworm	2 individuals	4 (100%)
	3	2	4	Dengue larvae + earthworm	2 individuals	3 (75%)
4	1	2	4	Dengue larvae + brown beetle	2 individuals	4 (100%)
	2	2	4	Dengue larvae + brown beetle	2 individuals	4 (100%)
	3	2	4	Dengue larvae + brown beetle	2 individuals	3 (75%)
5	1	2	4	Dengue larvae + water flies	2 individuals	2 (50%)
	2	2	4	Dengue larvae + water flies	2 individuals	4 (100%)
	3	2	4	Dengue larvae + water flies	2 individuals	3 (75%)
6	1	2	4	Dengue larvae + water flies + brown beetle	3 individuals	4 (100%)
	2	2	4	Dengue larvae + water flies + brown beetle	3 individuals	4 (100%)
	3	2	4	Dengue larvae + water flies + brown beetle	3 individuals	4 (100%)

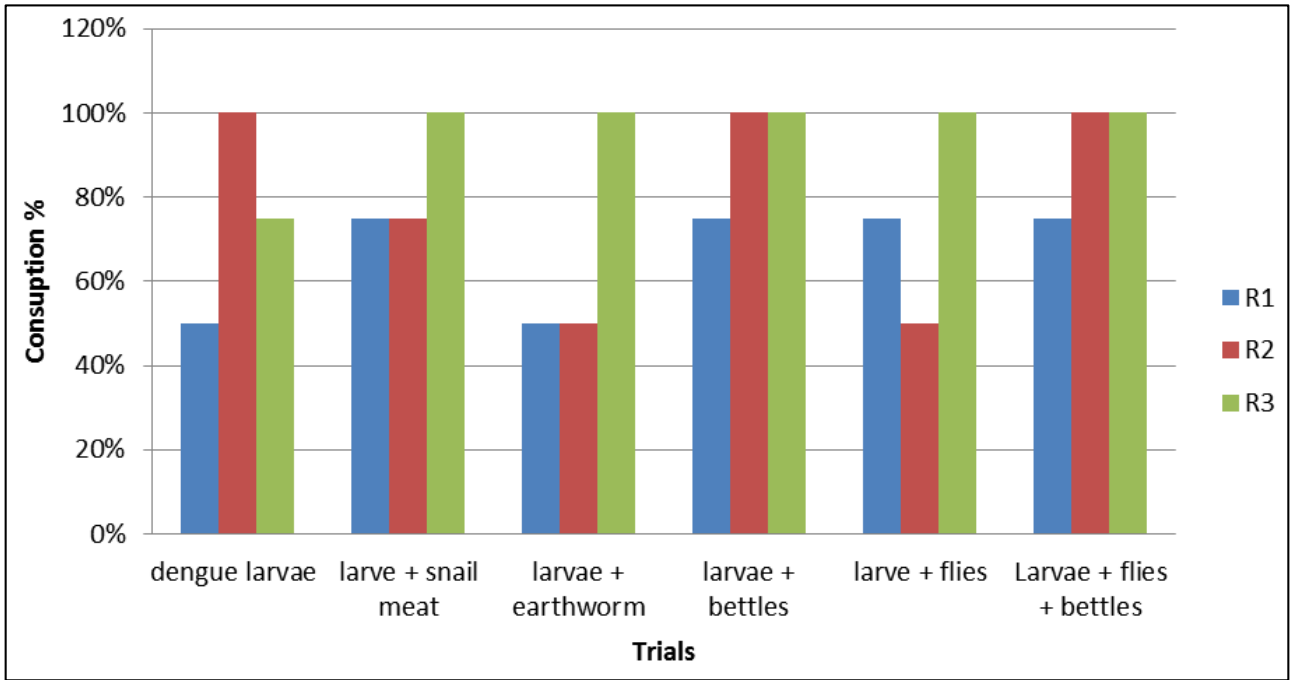

Fig 1: Consumption of dengue larvae by Common Skittering Frog (*Euphlyctis cyanophlyctis*) in various trials

3.2 Consumption of dengue larvae by South East Asian toad in various trials

The results of trial I showed 100, 100 and 100%, trial II showed 75, 75 and 100%, trial III showed 75, 100 and 75%, trial IV showed 100, 100 and 75%, trial V showed 75, 100 and 75% and trial VI showed 100, 100 and 100% consumption of dengue larvae by tadpoles of South-east Asian toad (*D. melanostictus)* in replication 1, 2 and 3 respectively (Table 2 and Figure 2). The Kruskal-Wallis test present that the total number of consumed dengue larvae among various trials did not differ significantly (P=0.26). The Wilcoxon test present that the total number of consumed dengue larvae in various trials by tadpoles of the two anuran species did not differ significantly (P=0.08).

Table 2: Consumption of dengue larvae by south-east Asian toad (*Duttaphrynus melanostictus*) in various trials

Trial	Replication	No. of tadpoles	No. of dengue larvae	Combination	Quantity of food	No. and %age of consumed dengue larvae
1	1	2	4	Dengue larvae only	1 individual	4 (100%)
	2	2	4	Dengue larvae only	1 individual	4 (100%)
	3	2	4	Dengue larvae only	1 individual	4(100%)
2	1	2	4	Dengue larvae + Snail meat	2 individuals	3 (75%)
	2	2	4	Dengue larvae + Snail meat	2 individuals	3 (75%)
	3	2	4	Dengue larvae + Snail meat	2 individuals	4 (100%)
3	1	2	4	Dengue larvae + earthworm	2 individuals	3 (75%)
	2	2	4	Dengue larvae + earthworm	2 individuals	4 (100%)
	3	2	4	Dengue larvae + earthworm	2 individuals	3 (75%)
4	1	2	4	Dengue larvae + brown beetle	2 individuals	4 (100%)
	2	2	4	Dengue larvae + brown beetle	2 individuals	4 (100%)
	3	2	4	Dengue larvae + brown beetle	2 individuals	3 (75%)
5	1	2	4	Dengue larvae + water flies	2 individuals	3 (75%)
	2	2	4	Dengue larvae + water flies	2 individuals	4 (100%)
	3	2	4	Dengue larvae + water flies	2 individuals	3 (75%)
6	1	2	4	Dengue larvae + water flies + brown beetle	3 individuals	4 (100%)
	2	2	4	Dengue larvae + water flies + brown beetle	3 individuals	4 (100%)
	3	2	4	Dengue larvae + water flies + brown beetle	3 individuals	4 (100%)

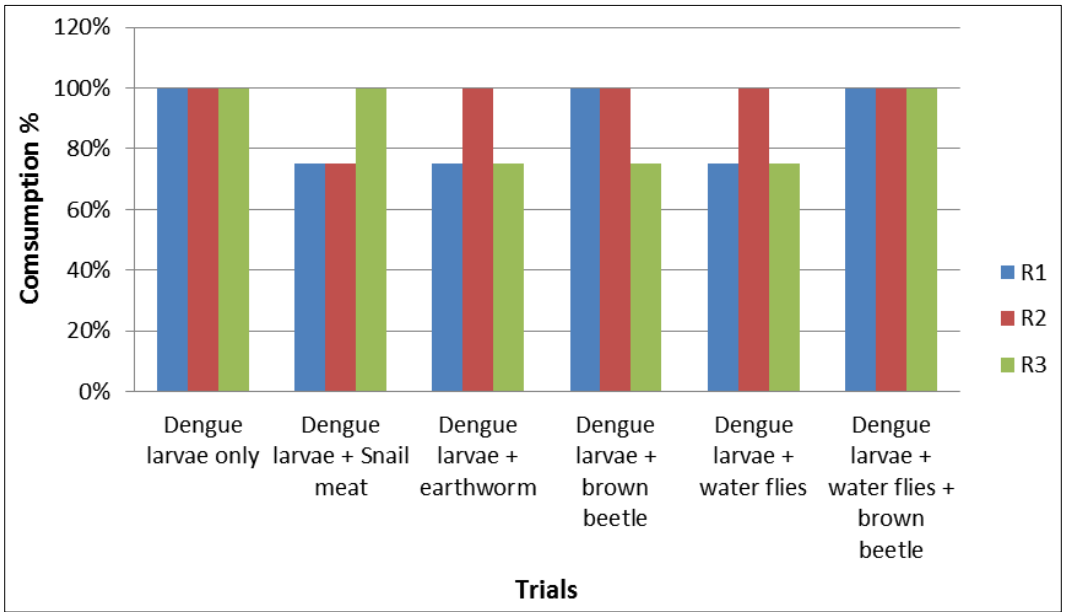

Fig 2: Consumption of dengue larvare by South-east Asian toad (*Duttaphrynus melanostictus*) in various trials.

The role of different anuran species included adult and tadpoles both serve as biological control of disease holding mosquitos are unappreciated [13]. Most of the anuran species feed is composed mainly by insects, but they also consuming other invertebrates. The Unscrupulous behavior of many anuran species, they are usually regarded as generalist predators [14]. The larvae have also significance as earlier consumers in different wetlands, and mostly compete with other aquatic herbivores like mosquito larvae. Along with, as adult and rarely in larval stages, order anurans are pillagers of particularly some other invertebrates and also arthropods [15]. It was reported that adults and tadpoles of anuran species could be used in control of *Aedes* vector mosquito techniques [16]. In another study it was reported that amphibians also participate to regulate the amenities of dropping mosquito staff from transient wetlands, actually monitoring other groups of pest, and circuitously by hunting of insects pollinators [17]. It was suggested that tadpoles of *Bufo, Ramanella, Euphlycti* and *Hoplobatrachus* could destroy mosquito eggs while the adult frogs consume only adult mosquitoes [18]. The use of anuran adult and tadpoles for disease vector control [5]. It was observed that tadpoles as the great pillagers of mosquito larvae and it are prospective that all nourishing stages of amphibians are significant predators in an amount of permanent and semi-permanent aquatic habitats [19].

A decline in *A. aegypti* infestation rate of tanks by Siamese fighting (*Betta splendens*) fish from the concentration of 70.4% of the tanks to the concentration of 7.4% after one year and reducing to 0.2% after eleven months later [20]. It was reported that in city areas 43.7% of without fish containers had larvae of *A. aegypti* in comparison to containers of 7.0% with fish [21]. Chinese catfish (*C. fuscusis*) is not just an eatable fish, although extremely larvivorous as well a merciful of rough environment hazards, fish keeping could also be highly cost-operative than other methods which are used in control purposes such as insecticide spraying [22].

4. Conclusion

From the current study it was concluded that the South-east Asian toad is better in the consumption of dengue mosquito larvae as compared to common skittering frog. So, it is recommended that the production of South-east Asian toad should be improved on large scale at dengue breeding sites. So the dengue infection can be controlled by this less expensive anuran species of South-east Asian toad.

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