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Predation Efficiency of Non-Blood Sucking Mosquito Larvae of *Toxorhynchites splendens* (Weidman) Determined by Kruskal–Wallis One-Way Analysis of Variance by Ranks over Immature Stages of Malaria (*Anopheles stephensi*), Filariasis (*Culex quinquefasciatus*) and Dengue (*Aedes aegypti*) Vectors

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A B S T R A C T

Introduction: Biological control is a component of the integrated vector control strategy. Its aquatic habitat, which is safe for non-target organisms and appropriate for coexistence with target organisms and predators without the presence of predator' enemies, is its limitation in terms of mosquito larval control.

Methods: A predator *Toxorhynchites splendens* second instar larva was used in this study to consume immature stages of the second and third instars of *Anopheline stephensi Culex quinquefasciatus* and *Aedes aegypti*. The immature stages were raised in a rectangular enamel-coated tray that measured 17 x 12 x 6 inches and was filled with enough chlorine-free water in a lab at the Institute of Vector Control and Zoonoses (IVCZ), Hosur. To assess the predator's effectiveness, the number of preys it consumed was noted. A nonparametric ANOVA was used to model the relationship between three distinct prey intakes and time.

Results: This led to finding that *Anopheles stephensi*, the malaria vector, was the predator's first choice in order of preference, followed by *Culex quinquefasciatus*, the vector of filaria, and *Aedes aegypti*, the vector of dengue. It became clear that the predator prioritized the *Anopheles stephensi* larvae as its primary source of food even all three of these types were present altogether.

Conclusion: The potential predator of Anopheles stephensi larvae, which is the malaria vector, is larvae *Toxorhynchites splendens*, a *non-blood sucking mosquito species.*

Keywords: Biological Control, *Toxorhinchites splendens, Anopheles stephensi, Aedes aegypti*

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Introduction

Biological control is a vector control approach and it has been widely used in controlling exotic pests and vectors.[1,2] During the late 1880s, the biological control agent vedalia beetle *Rodolia cardinalis* (Mulsant) was used against the cotton cushion scale, *Icerya purchasi* (Maskal) in California and this was the incident for extending research on predator, prey interaction. It has been reported that several prey-predator models adopt different foraging strategies in response to energy values of the prey types and their relative frequencies and this was seen as an adaptation to maximise the long-term rate of energy gain.[3–6] An individual-based model was developed that predicts the strength of interference between foraging animals from basic elements of their behaviour and the model proposed by them had key differences as responses of animals to competitors are not fixed.[7]

The present study deals with the preference of prey of immature stages (larvae) of malaria, filariasis, and dengue vectors by the predator *Toxorhynchites splendens*, a non-blood sucking mosquito, which usually habitats in the arboreal ecosystem. The model was then applied to the case when all three types were present together in the system and the predictions were compared with the experimental data for such a case.

Materials and Methods

This experimental study was carried out in Hosur, Tamil Nadu at the Institute of Vector Control and Zoonoses (IVCZ). The experiment was observed for 88 days. For this present study, immature stages (II and III instar) of *Toxorhynchites splendens* were used as predators. It was introduced in an enamel-coated tray (17 x 12 x 6 inches) filled with chlorine-free water and then ten larvae of *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* were introduced as preys as these larvae are well-known as the vectors of malaria, filariasis and dengue. These were taken from the colonies raised in the Institute of Vector Control and Zoonoses (IVCZ), Hosur owned by the Department of Public Health and Preventive Medicine, Government of Tamil Nadu, India. To avoid cannibalism, both the predator and prey were in the same stage. Till the predator became pupae, they were used. A separate tray was used for each prey. The number of larvae consumed by the predator was recorded every day from each tray and in turn, the same number of fresh larvae that had been consumed by the predator was replaced to maintain the constant population of prey forever. Non-parametric ANOVA (one way) was used to predict the preference of prey and then the model was applied for the case when all three types were present together in the system using the non-parametric ANOVA (two way).[8–10] Since all the material was obtained from the mosquito colonisation in the IVCZ, Hosur, no ethical concerns emerged.

Kruskal–Wallis One-Way Analysis of Variance by Ranks

The Kruskal–Wallis one-way analysis of variance by ranks is an extremely useful test for deciding whether k-independent samples are from different populations. Sample values almost invariably differ somewhat, and the question is whether the differences among the samples signify genuine population differences or whether they represent merely chance variations such as are to be expected among several random samples from the same population. The Kruskal–Wallis technique tests the null hypothesis that the k samples come from the same population or from identical populations with respect to averages. The test assumes that the variable under study has an underlying continuous distribution. It requires at least an ordinal measurement of that variable.

In the computation of the Kruskal–Wallis test, each of the N observations is replaced by ranks. That is all of the scores from all of the k samples combined are ranked in a single series. The smallest score is replaced by rank 1, the next to smallest by rank 2, and the largest by rank N, where N is the total number of independent observations in the k samples.

When this has been done, the sum of the ranks in each sample (column) is found. The Kruskal–Wallis test determines whether these sums of ranks are so disparate that they are not likely to have come from samples which were all drawn from the same population.

It can be shown that if the k samples actually are from the same population or from identical populations, that is if H_0 is true, then H (the statistic used in the Kruskal–Wallis test and defined in the formula given below) is distributed as chi-square with df = k-1, provided that the sizes of the various k samples are not too small, i.e.

$$H = 12/N(N+1)\sum_{J=1}^{k} Rj^2/n_j - 3(N+1)$$

Where k: number of samples

n_j: number of cases in the jth sample

$N = \Sigma n_j$: the number of cases in all samples combined

$\sum_{J=1}^{k}$: directs one to sum over the k samples (columns)

Friedman Two-Way Analysis of Variance by Ranks

For the Friedman test, the data are cast in a two-way table having N rows and k columns. The rows represent the various subjects or matched sets of subjects, and the columns represent the various conditions. If the scores

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of subjects serving under all conditions are under study, then each row gives the scores of one subject under the k conditions.

The data of the tests are ranked. The scores in each row are ranked separately. That is, with k conditions being studied, the ranks in any row range from 1 to k. The Friedman test determines whether it is likely that the different columns of ranks(samples) came from the same population.

For example, suppose we wish to study the scores of 3 groups under 4 conditions. Here k = 4 and N = 3. Each group contains 4 matched subjects, one being assigned to each of the 4 conditions. To perform the Friedman test with data, we first rank the scores in each row. We may give the lowest score in each row the rank 1, the next lowest score in each row the rank 2, etc. By doing this, we obtain the data in this study as 1 to k = 3.

Now if the null hypothesis (that all the samples, i.e. columns, came from the same population) is in fact true, then the distribution of ranks in each column would be a matter of chance, and thus we would expect the ranks of 1,2,3 to appear in all columns with about equal frequency. This would indicate that for any group it is a matter of chance under which condition the highest score occurs and under which condition the lowest occurs, which would be the case if the conditions really did not differ.

If the subjects' scores were independent of the conditions, the set of ranks in each column would represent a random sample from the discontinuous rectangular distribution of 1, 2 and 3 the rank totals for the various columns would be about equal. If the subject' score were dependent on the conditions (i.e. H_0 were false), then the rank totals would vary from one column to another. In as much as the columns all contain an equal number of cases, an equivalent statement would be that under H_0 the mean ranks of the various columns would be about equal.

The Friedman test determines whether the rank totals (R_j) differ significantly. To make this test, we compute the value of a statistic which Friedman denotes as x_r^2 is distributed approximately as chi-square with df = k-1, when

$$X_r^2 = 12/N(k+1) \sum_{J=1}^{k} (R_j^2) - 3N(k+1)$$

where N: number of rows

K: number of columns

R_j: sum of ranks in the Jth column

K

Σ: directs one to sum the squares of the sums ranks overall k conditions

J = 1

Results

Predator *Toxorhynchites splendens* is observed to be feeding on immature stages of *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes species*, based on observations made using the Kruskal–Wallis and 'Friedman' one-way and two-way analyses, respectively. It was understood that the predator preferred *Anopheles stephensi* as its first choice of food, whereas *Culex quinquefasciatus* and *Aedes* species larvae were consumed in the second and third order (Table 1). Probing the outcome of this analysis, the choice of food preference was ascertained by ranks. In this study, five sets of experiments were performed with a single species of prey for 88 days and one set of experiments was performed with all three types of preys together for the same duration. Even though the second and third sets of experiments predicted *Aedes* species was the first order of preference, this trend was not true when all these preys were put together. *Anopheles stephensi* is still the first order of prey to the predator *Toxorhynchites splendens.* From this study, it has been inferred that the predator is a potential biological control agent as it is not a blood-sucking mosquito in nature. It therefore pertains to the natural condition.

Table 1.Preference of Prey by the Predator *Toxorhynchites splendens* with Kruskal–Wallis Test

Order of Groups	Name of the Prey	N	Mean Rank
Group 1	*Anopheles stephensi*	88	133.57
	Culex quinquefasciatus	88	132.61
	Aedes aegypti	88	131.31
	Total	264	-
Group 2	*Anopheles stephensi*	88	129.39
	Culex quinquefasciatus	88	130.65
	Aedes aegypti	88	137.46
	Total	264	-
Group 3	*Anopheles stephensi*	88	126.32
	Culex quinquefasciatus	88	132.23
	Aedes aegypti	88	138.95
	Total	264	-
Group 4	*Anopheles stephensi*	86	142.03
	Culex quinquefasciatus	87	114.69
	Aedes aegypti	87	134.91
	Total	260	-
Group 5	*Anopheles stephensi*	85	142.64
	Culex quinquefasciatus	87	116.78
	Aedes aegypti	85	127.86
	Total	257	-

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Group 6	*Anopheles stephensi*	87	165.97
	Culex quinquefasciatus	88	122.56
	Aedes aegypti	88	107.86
	Total	263	-

Discussion

Predator-prey dynamics have been studied extensively using mathematical models. A common feature of many of these models is the prediction that the population can cycle for some parameter sets: i.e. the population of predators and prey do not settle to constant values, but rather, oscillate periodically in time. There is considerable data from field studies and laboratory experiments to support the existence of such population cycles and also observe the behaviour behind such invasions for cyclic populations. Further, it was stated that if a small group of predators is introduced into an otherwise spatially uniform population of prey, the predators will tend to invade the prey, leaving behind a mixture of predators and prey.[11–13] The results are somewhat surprising; the invasion leaves behind spatiotemporal oscillations which fall into one of the two categories, either a periodic travelling wave or spatiotemporal irregularity; mixed behaviour also occurs. Also stated in their research paper on predator diversity strengths trophic cascades in kelp forests by modifying herbivore abundance and positively correlated with kelp abundance. Further, it was observed that two prey species are not competing with each other and do exclude each other when they share a predator that is eating them both. Further, it was known that some new developments in statistics were also possible while formal mathematical modelling was used on predation, particularly to explain periodic travelling waves in prey populations.[14]

Conclusion

In the present study, instead of doing formal mathematical modelling, one-way and two-way non-parametric analyses were used to predict the order of prey preference by the predator *Toxorhynchites splendens* which is a non-blood-sucking mosquito species. Even though the predator preferred all prey of immature stages of *Anopheles stephensi, Culex quinquefasciatus* and *Aedes* species, the vector of malaria, filariasis and dengue respectively, it is inferred from the study that predator *Toxorhynchites splendens* is the potential biological control agent, particularly for *Anopheles stephensi*, the primary malaria vector in India.

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