RESEARCH ARTICLE

Influence of habitat complexity on the prey mortality in IGP system involving insect predators (Heteroptera) and prey (Diptera): Implications in biological control

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Abstract

Intraguild predation (IGP) is common in the freshwater insect communities, involving a top predator, intraguild prey (IG prey) and a shared prey. Influence of the habitat complexity on the prey-predator interactions is well established through several studies. In the present instance, the IGP involving the heteropteran predators and the dipteran prey were assessed in the background of the habitat complexity. The three predators Diplonychus rusticus, Ranatra filiformis, and Laccotrephes griseus, one intraguild prey Anisops bouvieri and two dipteran prey Culex quinquefasciatus and Chironomus sp. were used in different relative density against the complex habitat conditions to deduce the impact on the mortality on the prey. In comparison to the open conditions, the presence of the macrophytes and pebbles reduced the mortality of the shared prey under intraguild system as well as single predator system. The mortality of the shared prey was however dependent on the density of the predator and prey. Considering the shared prey mortality, predation on mosquito larvae was always higher in single predator system than chironomid larvae irrespective of identity and density of predators. However, for both the shared prey, complexity of habitat reduced the prey vulnerability in comparison to the simple habitat condition. Higher observed prey consumption depicts the higher risk to predation of shared prey, though the values varied with habitat conditions. Mortality of IG prey (A. bouvieri) in IGP system followed the opposite trend of the shared prey. The lower mortality in simple habitat and higher mortality in complex habitat conditions was observed for the IG prey, irrespective of shared prey and predator density. In IGP system, the shared prey mortality was influenced by the habitat conditions, with more complex habitat reducing the vulnerability of the shared prey and increased mortality of the IG prey. This implies that the regulation of the mosquitoes, in the IGP system will be impeded by the habitat conditions, with the heteropteran predators as the top predator.
Introduction

The physical elements and the macrophytes contribute to the heterogeneity of the habitat condition in freshwater community, recognized as habitat complexity. The species interactions in the freshwater habitats are influenced by the habitat complexity, inclusive of foraging, evasion of predation [1, 2], and the prey consumption by the predators [3–7]. The movement of predators is affected by the vegetation impairing the prey search efficiency. However, contrasting examples with the habitat complexity facilitating the predation for the sit and wait predators have also been observed [2, 8–10]. For the prey, the presence of vegetation and physical structures provide refuge to evade predation [2, 11]. In several instances, due to the vegetation and the physical objects, the contact between predators and prey is altered with reduced space for interaction [12]. Contrastingly, certain studies have shown that in the complex habitat condition, the number of interacting prey and predators increases and thus the predatory-prey interactions are possibly more or less the same [13]. Thus habitat complexity influences the trophic interactions particularly the prey-predator links [14]. Irrespective of the size and shape of the water bodies and the water regime, the habitat complexity influences the freshwater community assemblages in both tropical and the temperate regions [15, 16]. The food web features, the identity of the interacting species [17–19], and the traits of the species concerned [20, 21] influence the outcome of the species interactions, all in the background of the complexity of the habitat conditions [22, 23].

The effects of the habitat complexity on the prey-predator interactions involving diverse taxonomic groups have been tested in several empirical studies [5, 10, 24]. In majority instances, the prey-predator interactions are impacted by the habitat conditions and therefore appear to be strong driver of the interactions and thus the diversity and stability in the freshwater community. As observed for most of the ecological communities, the species interactions are mostly indirect [25–28], inclusive of cannibalism, omnivory, apparent competition, and intraguild predation. The extent of the outcome of these indirect interactions depends on the taxonomic identity and the relative abundance in the community [5, 29]. In view of the biological regulation of the pests [30–32] or the vector mosquitoes [33–35] the implications of the indirect interactions are significant. Biological control emphasizes the regulation for the target organism using natural enemies, which are in most instances, the generalist predators [36, 37]. Since the generalist predators are most likely to be a part of one or multiple indirect interactions, the success of the biological control would also likely to vary with the interactions. Deciphering the impact of the indirect interactions [38] in the background of the habitat complexity therefore becomes more relevant in the context of the biological control of the target organisms.

Intraguild predation is a common form of indirect interactions for most of the food webs in terrestrial and freshwater communities [39–44]. Perhaps the generalist dietary choice and abundance of similar prey and predators account for the abundance of IGP as a form of indirect interaction in food web. In IGP, the richness and the abundance of the shared prey and the intraguild prey determine the extent of variation in the predation pattern in comparison to the direct prey-predator interactions. The IGP involving beneficial and harmful insects associated with the cultivable crops is considered to be of immense importance considering the biological control as a mechanism of the regulation of the pest species [41, 45]. An alteration in the density and identity of the shared prey and the intraguild prey, as well as the alteration in the density and the identity of the predators influence the outcome of IGP interactions involving different species with taxonomic identities. This applies for regulation of both the pest species and the vector mosquitoes in a typical biological control system. In a situation where the identity and the efficacy of the IG predator are known, appropriate alteration in the predator
composition may enhance the success of the biological control. Similarly, the relative density of the prey and the predators are also essential in delivering successful prey regulation. An evaluation of the prospective IGP is therefore given priority to enhance the success rate of the biological control against a target organism.

Considering IGP system under structurally complex habitat conditions, interactions among the IGP components may vary, eventually changing the fate of shared prey. In contrast to structurally simple or open habitat conditions, the antagonistic interactions between IG predator and IG prey are less likely to be occurring in complex habitat conditions that provide prey refuge [45, 46]. As a consequence, a stable coexistence of IG predator and IG prey would be possible with increased predation pressure on shared prey. Conversely, if the habitat conditions reduce the coexistence of IG predator and IG prey, reduced risk to predation will augment shared prey density. However, the antagonistic interactions between IG predator and IG prey vary with the taxonomic identity of the interacting pairs [47]. Therefore even in presence of habitat complexity, the resultant impact on the shared prey will vary depending on the identity of the IG predator. The abundance and dispersion of IG predators are influenced by the habitat structure and vegetation that may in turn influence the outcome in IGP interactions. In addition, environmental factors like habitat permanence, water depth, dispersal ability and even the availability of the alternate prey influence the outcome of IGP interactions [7].

In case of the biological control of the mosquitoes several generalist insect predators have been promoted in different larval habitats inclusive of the rice fields and bogs. Apparently, the diversity of the macroinvertebrates and the different predators are considerably high in rice fields and similar freshwater habitats that are exploited by the mosquitoes as a breeding ground [38, 48–52]. As a result, conservation biological control for the mosquitoes engaging the generalist insect predators is highlighted in these habitats. Apart from the regulation of the mosquitoes, the conservation of the insects enable sustenance of the biodiversity in the rice fields and similar freshwater habitats, supported through empirical studies in different region of the world [34, 48, 53, 54]. In India, the rice fields and similar wetlands are rich in species diversity inclusive of the mosquitoes and the water bugs, providing ample chances of direct and indirect interactions leading to the impact on the mosquito population. Several different estimates of the direct and indirect interactions can occur in the ricefield and similar habitats that may potentially interfere with the process of the mosquito regulation by the generalist insects [34]. Empirical studies have shown that the intraguild predation involving the water bugs and the dipteran larvae as prey is influenced by the density of the predator and the prey and the identity of the prey [18, 55, 56]. As a consequence, a deviation from the expected results on the biological control of mosquitoes was observed in these instances. In case of water bugs, the predation is also affected by the light and the habitat complexity [57], which may account for a barrier to predator and prey interactions or may provide refuge to the prey. In many instances, the habitat complexity may act as a barrier for the prey searching by the predators and thus may appear to be advantageous for the prey [58–60]. On the contrary, the successful regulation of the target prey may be affected due to the habitat complexity. In the present instance, we have tested the efficacy of the water bugs engaged in the intraguild predation in the different context of the habitat complexity. The observations may aid in the understanding of the extent of the regulatory effect imposed by the water bugs on the mosquito population in the rice fields. While the habitat complexity is known to influence the species interactions including prey-predator interactions, the impact on the indirect interactions like the intraguild predation are little explored. Particularly, when the generalist predators are common in several of the freshwater wetlands, the possibility of indirect interactions are considerably high. Besides, the habitat complexity due to the weeds and the sediment conditions may lead to additional constraint for the prey-predator interaction. The results of the present
study will enable us to explore the possible role of the habitat complexity on the intraguild interactions involving mosquito as target prey and the heteropterans as the predator.

**Material and methods**

**Study organisms**

**Intraguild predators.** The three water bug species, *Diplonychus rusticus* Fabricius, 1781 (Heteroptera: Belostomatidae), the water scorpion *Laccotrephes griseus* (Guerin-Manevilli, 1844) (Heteroptera: Nepidae) and the water stick insect *Ranatra filiformis* Fabricius, 1790 (Heteroptera: Nepidae) were considered as the top predators (intraguild predator) in the present study. While the water bug *D. rusticus* was active predator and hunt prey actively, the water scorpion *L. griseus* and water stick insect *R. filiformis* were sit and wait predators [61–64]. The three species are common in the different freshwater bodies in West Bengal, India, consuming wide range of the prey species including the chironomid and the mosquito larvae. Observations [65–69] on the morphological features of these predators and the habitat preferences indicate that these predators are common in the different wetlands and share mosquito and the chironomid larvae as shared prey. The collection of these predators was made from the different water bodies around the Ballygunge Science College campus, Kolkata, India, using an insect net of 200 μm mesh size fitted with a long wooden handle [70]. The insect net was moved through the littoral zone of the water bodies with moderate vegetations and the collected specimens were brought to the laboratory for the rearing and maintenance. The average body lengths of adult *D. rusticus*, *R. filiformis*, *L. griseus* were 16.4 mm (range, 15–18 mm), 52.01 mm (range, 44.8–58 mm), 26.20 mm (range, 21–32 mm) respectively. The body length was measured from the tip of the rostrum to the end of the abdomen. For the predators the body length corresponded to the adult morphs [71].

**The intraguild prey.** The backswimmer, *Anisops bouvieri* Kirkaldy, 1904 (Heteroptera: Notonectidae) was considered as the intraguild prey in the IGP system. Also, it was considered as a predator in the single predator experiments using mosquito and chironomid larvae as the prey. The backswimmers are common in almost all type of freshwater habitats including ponds, lakes, rivers, streams, stagnant waters in peatlands, marshes or swamps, though less available in vegetated area, swimming close beneath the water surface. Empirical studies suggest that the backswimmers are generalist predators and the prey item includes both mosquito and chironomid larvae. The average body length of the adult *A. bouvieri* was 6.32 mm (range, 5.8–7.2 mm) measured from tip of the rostrum to the end of the abdomen.

**Shared prey.** The larval stage of the mosquito *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae) and the chironomid, *Chironomus* sp. (Diptera: Chironomidae) were considered as the shared prey in the present experiment. The instar IV stage larvae of mosquito and chironomid midges were considered as the prey for the experiments. Following collection of the larvae of the chironomid and the mosquito, segregation and selection of the desired size classes were done for the experiment as stated below.

**Collection and maintenance of study insects**

**The insect predators.** The collection of the predatory insects was made from different ponds and temporary pools in and around Kolkata, India. The aquatic bodies were 100–400 m² in area, 50–100 cm in depth, rectangular to oval in shape with sparse vegetation upto a level of 50 cm from the bank. The vegetations included *Pistia stratiotes*, *Jussiaea repens*, *Alternanthera philoxeroides*, * Lemma major*, *Sagittaria* sp., *Nymphoides* sp., *Ipomoea aquatica* in varying proportion. During collection small indigenous fishes like *Colisa fasciata*, *Punctius ticto* and *Aplocheilus panchax* were also encountered as representative fish species. Collected
insects were brought to the laboratory in separate jars and emptied in water filled aquarium (38 x 36 x 36 cm). The predatory insects, collected from different ponds, separated species wise and maintained in the aquarium at a density of 25 individuals per 35 L of pond water with few specimens of *Lemna minor*, *Pistia stratiotes* and *Vallisneria spiralis* as refuge. Mosquito and chironomid larvae and tubificid worms collected from the same habitats and adjacent sewage drains were provided as food *ad libitum*. The insect predators were maintained in the laboratory for at least seven days before using them for experiment. The collection was continued as per the requirements of the experiment and the insects were maintained in a similar way as stated above.

**The shared prey.** The mosquito *C. quinquefasciatus* larvae were collected from the sewage drains in and around Ballygunge Science College campus, University of Calcutta, Kolkata. The collected larvae were brought to the laboratory and placed in an enamel tray (45 x 30 x 7.5 cm) for segregation of the IVth instar larvae (5.1–6.0 mm in length, IV instar; 1.9–2.1 mg in weight) to be used in the experiments. The rest of the larvae were retained in the enamel tray and were provided with Laviest capsules (Franco-Indian Pharmaceuticals, Mumbai, India) as source of food to grow to instar IV and were subsequently used in the experiments. The pupae collected if any were killed by drying. The collections of larvae were continued time to time as per the requirement of the experimental trials. For segregation and maintenance of chironomid larvae, the sediment collected was poured within enamel trays (45 x 30 x 7.5 cm) containing sewage drain and tap water (1:1: v/v) and was placed under a light source. The set-up was left undisturbed for 3–4 hrs to allow the sediment to settle down and the chironomid larvae to emerge out from the sediment and cling to the sides of the tub. Subsequently, the larvae were separated with a pipette; the larger ones (> 20 mm in length, 3.3–5.1 mg in wet weight) sorted and placed within smaller trays with little sediment from where they were used for the experimental purposes. In course of collection of study insects, the relative numbers in each sample were recorded and length and weight were measured for selected specimens.

**Experimental design**

The experimental protocol followed a complete randomized block design using 4 levels of habitat complexity, two levels of IG predator density and two levels of shared prey density. Three different species (*D. rusticus*, *R. filiformis* and *L. griseus*) were used separately as IG predators against two different species of shared prey (chironomid larvae and instar IV larvae of *C. quinquefasciatus* larvae). To determine the effects of IGP with four different habitat conditions, two different experiments were carried out with each predatory insect species, first as single predator followed by multiple predators constituting the IGP system.

**Experiment 1.** In the laboratory, experiments were carried out in eighteen glass aquarium (38 x 36 x 36 cm), each of 35 l water capacity. Each of single predator species (*D. rusticus*, *R. filiformis*, *L. griseus*, *A. bouvieri*) of a particular density (2 or 4 for three IG predators and 10 for *A. bouvieri*) was provided with two different densities (50; low and 200; high individuals) of prey species (mosquito or chironomid larvae) under 4 levels of habitat complexity and prey consumption was noted for 24 hours. In this single predator experiment *A. bouvieri* is used as a predator without any IG predator.

**Experiment 2.** In the second set of experiments two different predators (IG predator and IG prey) were provided with either mosquito or chironomid larvae as a prey, where *A. bouvieri* was considered as IG prey. The density of IG prey remained constant (10 individuals) while the shared prey was provided with two different densities—50 (low) and 200 (high) individuals and two levels of density—2 (low) or 4 (high) of IG predators. In this instance, *A. bouvieri* is used as IG prey, where it is vulnerable to the IG predator but can consume the shared prey.
Both the single predator and multiple predator experiments were carried out with four different habitat conditions separately. Three complex habitat conditions inclusive of the pebbles, vegetation and pebbles and vegetation were considered along with open condition without pebbles and vegetation. The vegetation condition was constructed using the sticks of *Ipomoea aquatica*, floating *Pistia stratiotes* and *Vallisneria spiralis* (Fig 1), while the small stones of varied diameter (used in aquarium) were used to construct the pebble condition. Using the four habitat conditions the experiments were conducted to deduce the effects of:

1. Habitat conditions on both the single predator and the IGP system
2. Differences in IGP systems with IG predators of different taxonomic identity
3. Differences in the prey mortality under different habitat conditions, both in the single predator and IGP system.

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The prey and predator combinations and the total number of replicates considered in the experiments are mentioned in Fig 1, while the arrangement of the experimental containers is shown in Fig 2. The data on the mortality of the prey (for single predator experiments) or shared prey (for IGP system) was recorded and applied to the multiplicative risk model to test the increase or decrease in the risk in mortality of the shared prey in IGP system.

**Data analysis**

**Prey consumption.** The data on the shared prey (mosquito larvae or chironomid larvae) consumption and the data on the IG prey consumption (*Anisops* sp.) were subjected to the logistic regression analysis, separately. In case of the shared prey consumption, the predator
combination (single predator system and the IGP system), the shared prey density (low or high), the predator density (low or high), the habitat conditions (open, pebble, vegetation, and vegetation and pebbles) were considered as the explanatory variables and the prey consumption as the response variable. Likewise, the shared prey density, the predator density and the habitat conditions were considered as the explanatory variables against the IG prey as the response variable. The analyses were carried out for the three top predators separately, for the shared prey consumption and the corresponding IG prey consumption. In the logistic regression, based on the principles of the binomial generalized linear model (GLM) with logit link function, the prey consumption was assumed to comply binomial (n, p) distribution. Here, n represents the number of replicates for each combination of explanatory variables, while, p is the probability parameter representing the linear combination of the explanatory variables. Using a logit link function, the parameters of the regression equation were measured through the maximum likelihood methods using XLSTAT [72].

The logistic regression was of the form, \( y = \frac{1}{1 + \exp(-a + b_1x_1 + \ldots + b_nx_n)) \), where \( x \) represents the explanatory variable (i to n numbers), \( y \) represents the response variables (shared prey consumption or the IG prey consumption). The response variable was weighted against the respective prey density provided at the initiation of the experiment to qualify for the analysis as binary (sum) proportion values A Chi square value (Wald’s Chi-square) was used to deduce the significance of the estimated parameters of the model that included predator density, prey density, predator combination and the habitat conditions for the shared prey consumption. Similarly, for the IG prey consumption, the significance of the estimated

Fig 2. Illustration of the habitat conditions used to evaluate the influence of the habitat complexity on the IGP system. Four different habitat conditions in the glass aquaria using vegetations (V), vegetations and pebbles (V+P), pebbles (P) and open conditions (O) were created in the glass aquarium. The condition (O) is used as simple condition reflects no habitat complexity in contrast to the rest three. Glass aquarium: Experimental mesocosm Water– 35L tap: pond; (pH– 7.6 to 8.2; temperature observed 29–32°C) Observation on predation was measured for 24 h. for each replicate.
parameters was deduced for the prey density, predator density and habitat conditions. In the present regression model, the explanatory variables are considered independently, and not the interactions among them.

**Model assessment**

**The model.** To assess the independence between predator effects, the observed predation rate was compared with the predicted values following the ‘multiplicative risk model’ [73–76]. This model predicts combined risk to the prey when both IG predator and IG prey were present but their effects were independent. Specifically, this model predicts that the expected proportion of prey killed by predator species A and B together (\(p_{AB}\)) is:

\[
p_{AB} = p_A + p_B - p_A p_B
\]

where \(p_{AB}\) is the predicted combined consumption for a particular initial prey density; \(p_A\) is the probability of being consumed by predator species A in isolation, and \(p_B\) is the probability of being consumed by predator species B in isolation over a 24 hrs. period of exposure. The \(p_A p_B\) term in the model accounts for prey removal by both predators.

**Habitat effect assessment.** To compare the effects of habitat complexity on the vulnerability of the IG prey, a coefficient ‘\(k\)’ was used to represent the proportional difference between open and complex habitat conditions as shown in the following equations:

\[
k_v = \frac{PM_o}{PM_v}, \text{ for habitat conditions with vegetation only}
\]

\[
k_p = \frac{PM_o}{PM_p}, \text{ for habitat conditions with pebbles only}
\]

\[
k_{v+p} = \frac{PM_o}{PM_{v+p}}, \text{ for habitat conditions with vegetation and pebbles present together.}
\]

A value greater than 1 would indicate that the prey mortality was higher in simple conditions, than in complex conditions, while a value of less than 1 would indicate greater prey mortality in complex conditions. A two-tailed t-test [77] was applied to justify whether the values are significantly different from when compared against the simple habitat conditions.

The outline of the experiments carried out along with the number of replicates and data analysis are shown in (S1 Table 1 in S1 File).

In the present instance, to carry out the experiments, no specific permissions were required for any locations / activities for the study. Besides, the experiments in the study did not engage endangered or protected species. The collection and maintenance of the study insects followed the rules and regulations of the Institutional Animal Ethical Committee, Department of Zoology, University of Calcutta, Kolkata, India.

**Results**

**Single predator experiment**

In all the experimental conditions, the shared prey mortality was observed in varying degree with reference to the density levels of the predators and the prey. When compared for the four different habitat conditions, the prey vulnerability varied for each of the prey and predator species combinations. Apparently, the prey vulnerability was a function of the predator identity and relative abundance of the concerned predatory insect. In both the single predator and IGP systems, the prey mortality was influenced by the complexity of the habitat conditions. The general trend, irrespective of density and identity of prey and predators, was that, under simple habitat condition, the prey mortality was consistently higher than the other three levels of complexity (pebbles and vegetation alone or separately present) (S1 Tables 2 and 3 in S1 File). At a low prey density (50 individuals), the vulnerability of mosquito and chironomid larvae was higher in simple habitat conditions and oppositely lower in complex habitat conditions.
Table 1. (a) The logistic regression equations representing the variations in the shared prey consumed (preycon) by the IG predators against the prey density (preyden), predator density (predden) and predator combination (predcomb) and habitat complexity (habitat) as explanatory variables. Predator combinations were (i) only IG prey and shared prey ii) both IG predator and IG prey with shared prey iii) only IG predator and shared prey. The level of significance assumed to be 0.025. The prey and predator combinations are shown in the suffix. (b) Significant values of the parameters of the model (in bold) were deduced through the Wald’s Chi-square test represented below. Here, the prey predator combinations were, mosq–Mosquito larvae, rus–D. rusticus, Chiro–chironomid larvae, ran–R. filiformis, lacco–L. griseus.

(a)Logistic regression of prey-predator combination. Significant at least at 0.025 level.

\[
\text{preycon}_{\text{mosq-rus}} = \frac{1}{1 + \exp(-(2.964-0.547\text{preyden}+0.330\text{predden}-0.793\text{predcomb}-0.476\text{habitat}))}
\]

\[
\text{preycon}_{\text{mosq-ran}} = \frac{1}{1 + \exp(-(1.515-0.052\text{preyden}+0.269\text{predden}-0.665\text{predcomb}-0.480\text{habitat}))}
\]

\[
\text{preycon}_{\text{mosq-lacco}} = \frac{1}{1 + \exp(-(0.560-0.280\text{preyden}+0.624\text{predden}-0.543\text{predcomb}-0.361\text{habitat}))}
\]

\[
\text{preycon}_{\text{chiro-rus}} = \frac{1}{1 + \exp(-(1.964-0.334\text{preyden}+0.191\text{predden}-0.611\text{predcomb}-0.368\text{habitat}))}
\]

\[
\text{preycon}_{\text{chiro-ran}} = \frac{1}{1 + \exp(-(1.814-0.493\text{preyden}+0.400\text{predden}-0.535\text{predcomb}-0.362\text{habitat}))}
\]

(b)

| Model       | Value | SE   | Wald $\chi^2$ | Value | SE   | Wald $\chi^2$ |
|-------------|-------|------|---------------|-------|------|---------------|
| Mosq-rus    |       |      |               |       |      |               |
| Intercept   | 2.964 | 0.044| 4557.052      | 1.964 | 0.042| 2165.318      |
| Preyden     | -0.547| 0.017| 1092.543      | -0.334| 0.016| 428.433       |
| Predden     | 0.330 | 0.013| 615.448       | 0.191 | 0.013| 212.878       |
| Predcomb    | -0.793| 0.008| 8832.822      | -0.611| 0.008| 5564.313      |
| Habitat     | -0.476| 0.006| 6044.175      | -0.368| 0.006| 3815.284      |
| Mosq-ran    |       |      |               |       |      |               |
| Intercept   | 1.515 | 0.043| 1213.918      | 0.560 | 0.044| 161.380       |
| Preyden     | -0.052| 0.017| 9.539         | -0.280| 0.017| 269.000       |
| Predden     | 0.269 | 0.014| 393.983       | 0.624 | 0.014| 1934.644      |
| Predcomb    | -0.665| 0.009| 6050.559      | -0.543| 0.009| 3805.720      |
| Habitat     | -0.480| 0.006| 5887.774      | -0.361| 0.006| 3169.015      |
| Mosq-lacco  |       |      |               |       |      |               |
| Intercept   | 2.487 | 0.043| 3294.710      | 1.814 | 0.042| 1886.404      |
| Preyden     | -0.562| 0.016| 1168.354      | -0.493| 0.016| 946.564       |
| Predden     | 0.403 | 0.013| 905.213       | 0.400 | 0.013| 938.073       |
| Predcomb    | -0.592| 0.008| 5013.078      | -0.535| 0.008| 4350.008      |
| Habitat     | -0.548| 0.006| 7812.272      | -0.362| 0.006| 3750.707      |

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(both vegetation and pebbles when present in the habitats). On a comparative scale the predators consumed higher number of mosquitoes than chironomid larvae when present separately as individual predator. Although density effects were evident for chironomid and mosquito larvae, the habitat conditions appeared to be more determining factor.

For all the predators, the prey consumption was density dependent and differed significantly among the predator species. *D. rusticus* consumed at a greater amount than the other two predators when present as individual predators. For *A. bouvieri*, the trend in mosquito and chironomid larvae consumption remained similar to the rest of the heteropteran predators with highest consumption in simple conditions and lowest in complex conditions with vegetation and pebbles (S3 Table in S1 File). The results of the logistic regression indicated that the shared prey mortality was influenced significantly ($P < 0.001$) by the prey density, predator density, predator combinations (either single or in IGP) and the habitat conditions, for both mosquito and the chironomid as prey against the three heteropteran IG predators (Table 1) except for the predator density in case of *D. rusticus*, and prey density in *L. griseus*, which were insignificant. Similar observations were also made for the IG prey mortality (Table 2), with the prey density and predator density and habitat combinations significantly ($P < 0.001$) contributing to the IG prey mortality in the IGP system.
IGP system experiment

The mosquito and chironomid prey mortality were influenced by the habitat conditions in the IGP system, at both the densities of IG predator (2 and 4) (S1 Table 4 in S1 File). The associated mortality of the IG prey in the IGP system was also influenced by the habitat conditions (S1 Table 5 in S1 File). In all instances, irrespective of shared prey density, IG prey mortality was high in complex habitat conditions and low in simple habitat conditions. It was apparent that for both the shared prey, complexity of habitat reduced the risk of being consumed by the predators while simple conditions increased the risk to predation (Figs 3 and 4). The observed prey consumption was consistently higher than expected for all the instances, though the values were conversely higher for the low shared prey density. This reflects that the risk to predation increased for the shared prey though the values varied with the habitat conditions.

The effects of habitat conditions on the prey mortality are substantiated through the values of $k$ factor that were consistently greater than 1 for all the combinations of shared prey and predator species density and identity (Fig 5). In contrast the $k$ factors were consistently less than 1 for the mortality of IG prey (Figs 6 and 7) except for the high predator density and low prey density combination of $L. griseus$, and the low prey density of $D. rusticus$. For both instances the $k$ factor were significantly different from 1 (Tables 3 and 4) for all instances of prey and predator combinations. The pattern of mortality of the shared prey and the IG prey under simple and complex conditions appear to be complementary to one another. High shared prey mortality is associated with low IG prey mortality and vice versa (S1 Tables 4 and 5 in S1 File). The expected values of the models too exhibited considerable variations with

Table 2. The logistic regression on the IG prey consumed (anicon) against shared prey density (preyden), predator density (predden) and habitat complexity (habitat) as the explanatory variables. The level of significance is 0.025. (b) Significant values of the parameters of the model (in bold) were deduced through the Wald’s Chi-square test represented below. Here, the prey predator combinations were, mosq–Mosquito larvae, rus–$D. rusticus$, Chiro–chironomid larvae, ran–$R. filiformis$, lacco–$L. griseus$.

(a) Logistic regression of IGP system. Significant at least at 0.025 level.

$$anicon_{\text{mosq-rus}} = 1 / (1 + \exp((-2.023 - 0.299^{\text{preyden}} - 0.037^{\text{predden}} + 0.629^{\text{habitat}})))$$

$$anicon_{\text{mosq-ran}} = 1 / (1 + \exp((-2.614 - 0.357^{\text{preyden}} + 0.780^{\text{predden}} + 0.554^{\text{habitat}})))$$

$$anicon_{\text{mosq-lacco}} = 1 / (1 + \exp((-3.699 - 0.167^{\text{preyden}} + 0.705^{\text{predden}} + 0.739^{\text{habitat}})))$$

$$anicon_{\text{chiro-ran}} = 1 / (1 + \exp((-0.626 - 0.519^{\text{preyden}} - 0.416^{\text{predden}} + 0.460^{\text{habitat}})))$$

$$anicon_{\text{chiro-lacco}} = 1 / (1 + \exp((-1.910 - 0.227^{\text{preyden}} + 0.254^{\text{predden}} + 0.416^{\text{habitat}})))$$

(b)

|          |                | Value  | SE    | Wald χ² |          | Value  | SE    | Wald χ² |
|----------|----------------|--------|-------|--------|----------|--------|-------|--------|
| Mosq-rus | Intercept      | -2.023 | 0.214 | 89.100 | Intercept| -0.626 | 0.203 | 9.541  |
|          | preyden        | -0.299 | 0.086 | 12.191 | Preyden  | -0.519 | 0.084 | 38.007 |
|          | predden        | -0.037 | 0.086 | 0.183  | Predden  | -0.416 | 0.084 | 24.439 |
|          | habitat        | 0.629  | 0.041 | 240.357| Habitat  | 0.460  | 0.039 | 141.937|
| Mosq-ran |                   |        |       |        | Chiro-ran|        |       |        |
|          | Intercept      | -2.614 | 0.208 | 157.285| Intercept| -0.308 | 0.195 | 2.499  |
|          | preyden        | -0.357 | 0.082 | 18.913 | Preyden  | -0.478 | 0.081 | 34.889 |
|          | predden        | 0.780  | 0.083 | 89.175 | Predden  | -0.453 | 0.081 | 31.280 |
|          | habitat        | 0.554  | 0.038 | 211.930| Habitat  | 0.425  | 0.037 | 132.663|
| Mosq-lacco|                   |        |       |        | Chiro-lacco|        |       |        |
|          | Intercept      | -3.699 | 0.236 | 246.060| Intercept| -1.910 | 0.206 | 85.672 |
|          | preyden        | -0.167 | 0.088 | 3.580  | Preyden  | -0.227 | 0.082 | 7.573  |
|          | predden        | 0.705  | 0.089 | 62.392 | Predden  | 0.254  | 0.083 | 9.479  |
|          | habitat        | 0.739  | 0.043 | 298.433| Habitat  | 0.416  | 0.038 | 121.017|

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respect to the complex and simple habitat conditions (Fig 7). As shown in the figures, for all the combinations of predators and prey, the $k$-values increased with the levels of complexity. In comparison to the situation when the macrophytes were present as element of complexity, the values of $k$ increased for the conditions, when the pebbles were present as well as when macrophytes and pebbles were present as the factors of complexity (Fig 7). Pertinently, the results represent that the increase in the complexity leads to the decreased consumption of the
shared prey (chironomid or mosquito larva) and more impact on the IG prey by the top predator.

Discussion

Prey predator interactions in aquatic communities are influenced by habitat conditions, as evident from studies on insect predators with varied taxonomic identity [5, 46, 78–83]. The
vegetation and other physical structures that constitute the complexity of the habitat, influence the predation of the heteropteran bugs [5, 84, 85] and odonate larvae [46, 86] against dipteran immature. The habitat conditions either augment or reduce the vulnerability of the prey species like tadpoles [85, 87, 88] and larvae of mayfly [73, 74, 89], mosquito [5] and chironomid midge [5]. While the movement of the predators is impeded due to the habitat complexity, the presence of the pebbles and the macrophytes facilitates the evasion of predation by the prey species. As a result of the complex habitat conditions, the intensity and interactions among the predators and the prey change considerably. In the present instance, the consumption of both

![Mortality of shared prey](https://doi.org/10.1371/journal.pone.0264840.g005)

**Fig 5.** Mortality of shared prey (A—mosquito larvae, B—chironomid larvae) in IGP system in complex habitat conditions (v—vegetation, p—pebbles and v+p—vegetation and pebbles) against simple conditions expressed as a ratio (k-value, mean ± SE) for the three IG predators in two density (L—2 individuals and H—4 individuals) under low and (50 individuals) high (200 individuals) prey density. The horizontal lines in each graph represents the reference value of 1, equivalent to the value of no difference between the complex habitat condition and open condition.
mosquito and chironomid prey (the shared prey) was higher in simple habitat conditions than in the complex habitat conditions, with pebbles and macrophytes. The presence of the macrophytes and the pebbles lead to the altered interaction among the species involved resulting in reduced number of the dipteran prey mortality reflected in the $k$ values (Fig 5). In all instances, irrespective of the density levels of the predator and the prey, the pattern of the prey vulnerability remained the same. For both the mosquito and the chironomid larvae, complex habitat condition lowered the risk of predation while offering newer sites as refuge. Similarly, the
The efficacy of the heteropteran was lowered due to the presence of the complex habitat conditions, with the movements being impaired due to reduced open space available. However, species specific variation in the prey consumption was also observed among the three predator species (Tables 2 and 4, S1 File).

The mosquito consumption by the insect predators *D. rusticus* and *A. bouvieri* were significantly reduced under the complex habitat conditions contrast to the simple conditions, when present as single predator [5, 10]. In the present instance similar results were observed with the IGP system involving these predators, reinforcing the concept that the complexity of the habitat conditions influences the prey predator interactions [29, 89–96]. In a similar experimental setup, it was observed that the prey selection by the odonate larvae in an IGP system, is influenced by the habitat conditions, where smaller prey individuals were selectively vulnerable under complex habitat conditions relieving the predation pressure on the larger prey [29].
The vulnerability of the IG prey was complementary to the vulnerability of the shared prey. As shown in Fig 4, the pattern of the vulnerability reduced with the complex habitat conditions but the response was density dependent. With higher density of the shared prey, the IG prey mortality was also reduced considerably reflected in all the habitat conditions. The identity of the top predator was also an important factor due to the differences in the predatory efficacy and the intensity of the predation exhibited by the water bugs contrast to the water stick insect and the water scorpion. Nonetheless, in all instances, the predators consumed higher numbers of the IG prey in complex habitat condition, while the shared prey mortality was higher in the open or simple habitat condition.

It was apparent that the density of the shared prey and the IG prey determines the extent of the risk to predation for the shared prey as well as the IG prey. When shared prey is abundant the mortality of the IG prey was reduced but the extent of reduction varied with the habitat conditions. Although the two factors–habitat conditions, and shared prey density were not
tested individually, it is apparent that the trends in the prey mortality under simple habitat conditions contrast to the complex habitat condition are significantly different with higher size effects for density. This is a sole indicator supporting that density had a significantly higher impact on prey mortality than the habitat conditions. In the IGP system involving the heteropteran predators and dipteran prey, the density of the interacting species seems to be more important than the habitat conditions. Thus, irrespective of habitat conditions the mortality of the IG prey will be influenced by the density of the interacting species than the habitat conditions. Perhaps, the insect predators are more adapted to the complex habitat conditions as observed in the natural systems where vegetations and the physical structures seems to govern the habitat quality and arena for interactions of the prey and predator [97–100]. In contrast to the complex habitat conditions the prey consumption remained higher in the simple conditions because the predators could avail greater space for locating and charging the prey species like mosquito. Similarly for the chironomid prey, the open space provides higher chances for

| Shared prey          | Predator density | Prey density | Predator species | k-value  |
|----------------------|------------------|-------------|------------------|----------|
|                      |                  |             |                  | k<sub>v</sub> | k<sub>p</sub> | k<sub>(v+p)</sub> |
| Chironomid larvae    | 2                | 50          | AB               | 3.943    | 10.457     | 6.341          |
|                      |                  |             | DR               | 4.719    | 7.435      | 5.110          |
|                      |                  |             | RF               | 4.179    | 5.240      | 4.351          |
|                      |                  |             | LG               | 1.267    | 7.609      | 3.956          |
|                      |                  |             | AB+DR            | 5.615    | 9.637      | 10.272         |
|                      |                  |             | AB+RF            | 5.200    | 8.921      | 11.983         |
|                      |                  |             | AB+LG            | 6.483    | 7.914      | 7.874          |
|                      | 200              |             | AB               | 8.679    | 6.371      | 13.542         |
|                      |                  |             | DR               | 3.742    | 11.095     | 7.010          |
|                      |                  |             | RF               | 5.074    | 5.874      | 6.439          |
|                      |                  |             | LG               | 4.025    | 9.958      | 9.190          |
|                      |                  |             | AB+DR            | 17.390   | 29.215     | 19.825         |
|                      |                  |             | AB+RF            | 4.841    | 8.597      | 8.792          |
|                      |                  |             | AB+LG            | 2.822    | 8.680      | 10.457         |
|                      | 4                | 50          | AB               | 3.943    | 10.457     | 6.341          |
|                      |                  |             | DR               | 3.140    | 7.950      | 10.565         |
|                      |                  |             | RF               | 5.315    | 5.069      | 4.840          |
|                      |                  |             | LG               | 4.989    | 6.185      | 8.814          |
|                      |                  |             | AB+DR            | 7.752    | 8.469      | 6.964          |
|                      |                  |             | AB+RF            | 7.253    | 8.544      | 7.519          |
|                      |                  |             | AB+LG            | 7.211    | 7.939      | 7.669          |
|                      | 200              |             | AB               | 8.679    | 6.371      | 13.542         |
|                      |                  |             | DR               | 11.456   | 8.976      | 6.456          |
|                      |                  |             | RF               | 12.962   | 9.954      | 7.679          |
|                      |                  |             | LG               | 8.231    | 11.693     | 23.128         |
|                      |                  |             | AB+DR            | 16.964   | 56.244     | 16.767         |
|                      |                  |             | AB+RF            | 4.991    | 7.092      | 11.616         |
|                      |                  |             | AB+LG            | 11.763   | 13.487     | 13.080         |

All the t-values (df = 17; two-tailed) are significant at P < 0.05 level except those in bold. Here AB is the IG prey *A. bouvieri*, DR—*D. rusticus*, RF—*R. filiformis*, and LG—*L. griseus*.

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Table 4. Results of the t-test using chironomid larvae as shared prey in the IGP system with different habitat conditions (v—vegetation, p—pebbles and v+p—vegetation and pebbles) against open condition.
being conspicuous to the predators and therefore increases the risk of attack by the predators. The predator specific difference in the outcome of the mosquito and chironomid species mortality was also obvious for the three heteropteran predators both in IGP system and in situations as a single predator. The water bug *D. rusticus* is comparatively more active and hunts for the prey items than the nepid predators *R. filiformis* and *L. griseus*. As a consequence the extent of mortality of the mosquito and chironomid larvae in both single predator and IGP system was higher when *D. rusticus* was present in the system. In all instances the compliance of the observed data with the model enables predictions about the possible consequences under natural system when these predators are present together. Extending the observed results for the biological regulation of both the mosquito and chironomid populations, it may be inferred that the vulnerability of the mosquito will depend more with the density of the interacting predators and the habitat condition. Presence of vegetations will favour the mosquitoes while the simple conditions will increase the chances of success of the predators. The mortality for the IG prey will depend on the density of the interacting shared prey and the IG predator, in all habitat conditions, in a reciprocal manner complementary with the mosquito prey mortality.

The mortality of shared prey was highest in simple condition, followed by vegetation only, pebbles only and vegetation and pebbles condition in sequence. The efficacy of the IG predators was noted as *D. rusticus* $\geq$ *L. griseus* $>$ *R. filiformis* for both shared prey and IG prey mortality. The mortality of *A. bouvieri* (IG prey) increased with the increase in complexity with lowest mortality in simple condition, followed by vegetation only, pebbles only and vegetation and pebbles condition in sequence. Earlier studies have demonstrated that the predation on mosquito immature is reduced in structured conditions created by the macrophytes *Pistia stratiotes* and stems of *Jussiaea repens* in case of the water bugs *Diplonychus* sp. [10]. The water bugs *D. rusticus*, *R. filiformis* and *L. griseus* exhibited differential pattern of prey consumption and the prey preferences were significantly reduced under the complex habitat conditions contrast to the simple conditions, when present as single predator [5].

Presence of filamentous algae provides refuge and food to the larvae of the mosquitoes *Anopheles pseudopunctipennis* and reduces the vulnerability to black molly *Poecilia sphenops* in rice field conditions [93]. The prey capture success in pygmy perch *Nannoperca australis* and the damselfly nymphs *Ischnura heterosticta tasmanica*, was not affected by macrophytes, but these predators were less effective in sites with higher structural complexity of habitats [84]. Among insect predators, the prey capture of *Belostoma oxyurum* to tadpoles was reduced in complex habitats. The tadpole prey used the macrophytes as refuge and successfully evaded the attack by the predatory water bug *B. oxyurum* [85]. Similar observations were noted with the water bugs *B. fluminea* and the odonate nymphs *Anax junius* when sharing the tadpoles of *Bufo terrestris* as prey [101]. Thus it appears that the prey vulnerability is influenced by the presence of the macrophytes and physical elements that render structural complexity and act as refuge. The observations on prey mortality (both IG prey and shared prey) followed a similar trend in the long term study. Both density and habitat effects were prominent in the long term studies in compliance with the findings of earlier experiments. In the long term studies, the decrease in the shared prey mortality (both chironomid and mosquito larvae) dwindled with time as a response to interspecific interactions between the two predators as a part of IGP system. Extending the results of the long term studies on IGP system, it may be assumed that under natural conditions, the shared prey mortality will be affected by the presence of multiple heteropteran predators. In comparison to the single predator system presence of multiple predators involve the interspecific interactions among the predators affecting regulation of the target prey [74–76, 84, 101, 102]. Interspecific interactions between IG predator and IG prey benefits the coexistence of the target prey (mosquito and chironomid larvae) in natural conditions [103].
The aquatic predatory insects are promoted for the regulation of the mosquitoes since long, owing to their abundance in the mosquito larval habitats, like rice fields, temporary pools and allied water bodies [48, 104, 105]. However, being generalist in prey choice, the dietary range of the predatory insects is quite broad which raises questions about the effective regulation of the mosquitoes [5, 106–108]. Among several factors, the prey choice, relative density of the predatory insects [86, 109–112], and the environmental conditions [5, 113] are considered as key factors in accomplishing successful regulation of the mosquitoes. As is known for the mosquito larval habitats, the possibilities of the indirect interactions are considerably high [34], which may reduce the effective regulation of the mosquitoes by the predatory insects. Owing to the wide range of dietary choice and quite high appetite, the predatory insects (Heteroptera) often become a part of the intraguild predation (IGP) [39, 40, 114], which may slow down the process of the mosquito regulation. Empirical studies have shown that mosquito regulation can be affected in IGP involving the water bugs and the backswimmers [18, 55, 56]. The relative density of the shared prey (mosquito or chironomid larvae) [55, 56] and the taxonomic identity and relative density of the IG predator [18] are crucial factors determining the mortality of the target prey. Actually, the consumption of the IG prey (backswimmers) in the low-density level of the target prey reduces the risk to predation of the shared prey. Thus, the role of the IG prey was modulating the strength of interactions and ultimately the vulnerability of the shared prey. Also, the identity of the top predators in the IGP system influenced the outcome of the shared prey (mosquito or chironomid larvae) mortality. Apart from the IGP, the prospective apparent competition among the shared prey and IG prey also reduced the effective regulation of the mosquito larvae.

**Conclusion**

In the context of conservation biological control [115], the predatory insects are promoted to regulate the mosquito prey naturally. In comparison to the other modes of biological control, the conservation biological control sustains species diversity in addition to the regulation of the mosquitoes. Considering the species diversity in the rice fields, temporary pools and the allied water bodies [48], the conservation biological control [115] is a feasible option for mosquito regulation. Although the multiple predators and their dietary choice may increase the possibility of evasion by the mosquitoes, the ill effects on the non-target species and the environmental concerns increase the priority of the conservation biological control as an effective measure. However, evaluation of the indirect interactions involving the predatory insects and the mosquito is essential to understand and predict the prospective mosquito species regulation. The use of the generalist insect predators in the biological control has been promoted in several ways to ensure conservation as well as regulation of the mosquitoes [116–118]. Indirect interactions, in contrast, are constraints that impede the successful regulation of the mosquitoes. With IGP as a possibility, the effective regulation of the mosquitoes is affected, which was further reduced in the complex habitat conditions, as observed in the present instance. In complex habitat conditions, the IG prey was consumed at a greater rate than the shared prey, which reduced the vulnerability of the mosquito to the predators. In the background of the habitat complexity, the IGP system involving the insect predators and the mosquito prey provides the possibility under which the predators can reduce the target prey population as well as the situations where the shared prey can evade the predators thereby sustaining the population. Similar inferences can be made for the chironomid larvae, when considered as a shared prey in IGP system. In situations where chironomids are considered as nuisance pest, the larval regulation is recommended. Alternatively, the consumption of the chironomid larvae sustains the population of several predatory insects in the freshwater wetlands. As a result, the
population of the predatory insects is maintained in absence of mosquito prey. No doubt that the predatory insects may also consume other prey, but the chironomid larvae are one of the dipteran insects that share many of the mosquito larval habitats and constitute the food for the predatory insects. Further studies regarding preference for the chironomid and mosquito larvae may be carried out to highlight the effective regulation of both the species in natural habitats, where they occur together. Apparently, the habitat conditions determine the prey-predator interactions and therefore the possibility of the effective regulation of the mosquitoes in the rice fields and similar wetlands featured by huge extent of habitat complexity and the predator diversity.

Supporting information

S1 File. The experimental design and the selected results in brief.

(DOCX)

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References

1. Persson L, Eklöv P. Prey refuges affecting interactions between piscivorous Perch and juvenile Perch and Roach. Ecology.1995; 76: 70–81.

2. Klecka J, Boukal DS. The effect of habitat structure on prey mortality depends on predator and prey microhabitat use. Oecologia. 2014; 176: 183–191. https://doi.org/10.1007/s00442-014-3007-6 PMID: 25085443

3. Heck KL, Crowder LB. Habitat structure and predator—prey interactions in vegetated aquatic systems. 1991. In: Bell SS, McCoy ED, Mushinsky HR, editors. Habitat Structure. Population and Community Biology Series, vol 8. Dordrecht, Netherlands: Springer; 1991. pp. 281–299.

4. Thomaz SM, Dibble ED, Evangelista LR, Higuti J, Bini LM. Influence of aquatic macrophyte habitat complexity on invertebrate abundance and richness in tropical lagoons. Freshw Biol. 2008; 53: 358–367.
5. Saha N, Aditya G, Saha GK. Habitat complexity reduces prey vulnerability: An experimental analysis using aquatic insect predators and immature dipteran prey. J Asia Pac Entomol. 2009; 12:233–239.

6. Platt JB. Habitat complexity and species diversity in rivers. PhD Thesis, Cardiff University. 2011. Available from: https://orca.cardiff.ac.uk/23242/1/2011plattjbphd.pdf.

7. Kovalenko KE, Thomaz SM, Warfe DM. Habitat complexity: approaches and future directions. Hydrobiologia. 2012; 685: 1–17.

8. Savino JF, Stein RA. Behavioural interactions between fish predators and their prey: effects of plant density. Anim Behav. 1989; 37:311–321.

9. Eklöv P, van Kooten T. Facilitation among piscivorous predators: effects of prey habitat use. Ecology. 2001; 82: 2486–2494.

10. Saha N, Aditya G, Bal A, Saha GK. Light and habitat structure influences predation of Culex quinquefasciatus larvae by the water bugs (Hemiptera: Heteroptera). Insect Sci. 2008; 15: 461–469.

11. Persson L. Predator-mediated competition in prey refuges: the importance of habitat dependent prey resources. Oikos. 1993; 68: 12–22.

12. Schmitz O. Predator and prey functional traits: understanding the adaptive machinery driving predator–prey interactions. F1000Research. 2017; 6: 1767. https://doi.org/10.12688/f1000research.11813.1 PMID: 29043073

13. Canion CR, Heck KL Jr. Effect of habitat complexity on predation success: re-evaluating the current paradigm in seagrass beds. Mar Ecol Prog Ser. 2009; 393:37–46.

14. Grabowski JH, Hughes AR, Kimbro DL. Habitat complexity influences cascading effects of multiple predators. Ecology. 2008; 89: 3413–3422. https://doi.org/10.1890/07-1057.1 PMID: 19137947

15. Torner I, Sala J, Gascón S, Ávila N, Quintana XD, Boix D. Pond size effect on macrofauna community structure in a highly connected pond network. Limnética. 2016; 35: 337–354.

16. Oertly B, Parris KM. Review: Toward management of urban ponds for freshwater biodiversity. Ecosphere. 2019; 10(7): e02810.

17. Crumrine PW, Crowley PH. Partitioning components of risk reduction in a dragonfly-fish intraguild predation system. Ecology. 2003; 84: 1588–1597.

18. Crumrine PW. Size structure and substitutability in an odonate intraguild predation system. Oecologia. 2005; 145: 132–139. https://doi.org/10.1007/s00442-005-0084-6 PMID: 15968538

19. Brahma S, Sharma D, Kundu M, Saha N, Saha GK, Aditya G. Intraguild predation in Heteroptera: effects of density and predator identity on dipteran prey. Neotrop Entomol. 2015; 44: 374–384. https://doi.org/10.1007/s13744-015-0286-5 PMID: 26174962

20. Emmerson MC. Raffaelli D. Predator–prey body size, interaction strength and the stability of a real food web. J Anim Ecol. 2004; 73: 399–409.

21. Rudolf VHW, Rasmussen NL, Dibble CJ, Van Allen BG. Resolving the roles of body size and species identity in driving functional diversity. Proc Royal Soc B. 2014; 281(1781):20133203. https://doi.org/10.1098/rspb.2013.3203 PMID: 24598423

22. Petchey OL, Beckerman AP, Riede JO, Warren PH. Size, foraging, and food web structure. Proc Natl Acad Sci USA. 2008; 105: 4191–4196. https://doi.org/10.1073/pnas.0710672105 PMID: 18337512

23. Arim M, Abades SR, Lauffer G, Loureiro M, Marquet PA. Food web structure and body size: trophic position and resource acquisition. Oikos. 2010; 119:147–153.

24. Hill JM, Weisburg MJ. Habitat complexity and predator size mediate interactions between intraguild blue crab predators and mud crab prey in oyster reefs. Mar Ecol Prog Ser. 2013; 488: 209–219.

25. Schmitz RJ. Indirect interactions between prey: apparent competition, predator aggregation, and habitat segregation. Ecology. 1987; 68: 1887–1897. https://doi.org/10.2307/1939860 PMID: 29357142

26. van Veen FJF, Morris RJ, Godfray HCJ. Apparent competition, quantitative foodwebs, and the structure of phytophagous insect communities. Annu Rev Entomol. 2006; 51:187–208. https://doi.org/10.1146/annurev.ento.51.110104.151120 PMID: 16332209

27. Rudolf VHW. Consequences of stage-structured predators: cannibalism, behavioral effects, and trophic cascades. Ecology. 2007; 88:2991–3003. https://doi.org/10.1890/07-0179.1 PMID: 18239834

28. Holt RD, Bonsall MB. Apparent Competition. Annu Rev Ecol Evol Syst. 2017; 48: 447–471.

29. Flynn KE, Moon DC. Effects of habitat complexity, prey type, and abundance on intraguild predation between larval odonates. Hydrobiologia. 2011; 675: 97–104.

30. Evans E W, England S. Indirect interactions in biological control of insects: pests and natural enemies in alfalfa. Ecol Appl. 1996; 6: 920–930.
31. Holt RD, Hochberg ME. Indirect interactions, community modules and biological control: a theoretical perspective. In Evaluating Indirect Ecological Effect of Biological control. edited by Wajnberg E., Scott John K., Quimby Paul C. 2001.

32. Okamoto KW, Amarasekare P. The biological control of disease vectors. J Theor Biol. 2012; 309: 47–57. https://doi.org/10.1016/j.jtbi.2012.05.020 PMID: 22659041

33. Bence JR. Indirect effects and biological control of mosquitoes by mosquitofish. J Appl Ecol. 1988; 25:505–521.

34. Kundu M, Sharma D, Brahma S, Pramanik S, Saha GK, Aditya G. Insect predators of mosquitoes of rice fields: portrayal of indirect interactions with alternative prey. J Entomol Zool Stud. 2014; 2: 97–103.

35. Buxton M, Cuthbert RN, Dalu T, Nyamukondiwa C, Wasserman RJ. Predator density modifies mosquito regulation in increasingly complex environment. Pest Manage Sci. 2020; 76: 2079–2086.

36. Symondson WOC, Sunderland KD, Greenstone MH. Can generalist predators be effective biocontrol agents? Annu Rev Entomol. 2002; 47: 561–594. https://doi.org/10.1146/annurev.ento.47.091201.145240 PMID: 11729085

37. Hurd LE. Predation: The role of generalist predators in biodiversity and biological control. 2008. In: Capinera J.L. (eds) Encyclopedia of Entomology. Springer, Dordrecht.

38. Blaustein L, Chase JM. Interactions between mosquito larvae and species that share the same trophic level. Annu Rev Entomol. 2007; 52: 489–507. https://doi.org/10.1146/annurev.ento.52.110405.091431 PMID: 16978142

39. Polis GA, Myers CA, Holt RD. The ecology and evolution of intraguild predation: Potential competitors that eat each other. Annu Rev Ecol Syst. 1989; 20: 297–330.

40. Polis GA, Holt RD. Intraguild predation: the dynamics of complex trophic interaction. Trends Ecol Evol. 1992; 7:151–154. https://doi.org/10.1016/0169-5347(92)90208-S PMID: 21235990

41. Rosenheim JA, Kaya HK, Ehler LE, Marois JJ, Jaffee BA. Intraguild predation among biological control agents: theory and evidence. Biol Control. 1995; 5:303–335.

42. Winemiller KO, Polis GA. Food Webs: What Can They Tell Us About the World? in Food Webs: Integration of Patterns & Dynamics (eds. Polis G. A. & Winemiller K. O.). 1996; 1–22, (Springer US, 1996). https://doi.org/10.1007/978-1-4615-1001-9 PMID: 48351004

43. Woodward G, Hildrew AG. Body-Size determinants of niche overlap and intraguild predation within a complex food web. J Anim Ecol. 2002; 71: 1063–1074.

44. Woodward G, Ebenmann B, Emmerson M, Montoya JM, Olesen, JM, Valido, Warren PH. Body size in ecological networks. Trends Ecol Evol. 2005; 20: 402–409. https://doi.org/10.1016/j.tree.2005.04.005 PMID: 16701403

45. Finke DL, Denno RF. Spatial refuge from Intraguild predation: implications for prey suppression and trophic cascades. Oecologia. 2006; 149: 265–275. https://doi.org/10.1007/s00442-006-0443-y PMID: 16708227

46. Janssen A, Sabelis MW, Magalhaes S, Montserrat M, der Hammen TV. Habitat structure affects intraguild predation. Ecology. 2007; 88: 2713–2719. https://doi.org/10.1890/06-1408.1 PMID: 18051638

47. Arim M, Marquet P. Intraguild predation: a widespread interaction related to species biology. Ecol Lett. 2004; 7: 557–564.

48. Bambaradeniya CNB, Edirisinghe JP, Silva DN, Gunatilleke CVS, Ranawana KB, Wijekoon S. Biodiversity associated with rice agro-ecosystem in Sri Lanka. Biodivers Conserv. 2004; 13: 1715–1735.

49. Blaustein L, Kilfawi M, Eitam A, Mangel M, Cohen JE. Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. Oecologia. 2004; 138:300–305. https://doi.org/10.1007/s00442-003-1398-x PMID: 14684191

50. Campos RE, Fernandez LA, Sy VE. Study of the insects associated with the floodwater mosquito Ochlerotatus albifasciatus (Diptera: Culicidae) and their possible predators in Buenos Aires province. Hydrobiologia. 2004; 524, 92–102.

51. Balcombe CK, Anderson JT, Fortney RH, Kordek WS. Aquatic macroinvertebrate assemblages in mitigated and natural wetlands. Hydrobiologia. 2005; 541: 175–188.

52. Mwangangi JM, Shilulu J, Muturi EJ, Murio S, Benjamin J, Kabiru EW, et al. Anopheles larval abundance and diversity in three rice agro-village complexes; Mwea irrigation scheme, central Kenya. Malaria J. 2010; 9: 228. https://doi.org/10.1186/1475-2875-9-228 PMID: 20691120

53. Luo Y, Fu H, Traore S. Biodiversity conservation in Rice paddies in China: towards ecological sustainability. Sustainability. 2014; 6: 6107–6124.

54. Ponraman G, Anbalagan S, Dinakaran S. Diversity of aquatic insects in irrigated rice fields of South India with reference to mosquitoes (Diptera: Culicidae). J Entomol Zool Stud. 2016; 4: 252–256.
55. Brahma S, Sharma D, Kundu M, Saha N, Aditya G, Saha GK. Mosquito prey vulnerability in intraguild predation between *Ranatra filiformis* and *Anisops bouvieri*: Implications in biological control. Proc Zool Soc. 2015; 68 (1): 36–44.

56. Brahma S, Aditya G, Sharma D, Saha N, Kundu M, Saha GK. Influence of density on intraguild predation of aquatic Hemiptera (Heteroptera): Implications in biological control of mosquito. J Entomol Acarol Res. 2014b; 46:461–1977.

57. Swart CC, Taylor RT. Behavioral interactions between the giant water bug (*Belostoma lutarium*) and Tadpoles of *Bufo woodhousii*. Southeast Nat. 2004; 3: 13–24.

58. Alto BW, Lounibos LP, Higgs S, Juliano SA. Larval competition differentially affects Arbovirus infection in *Aedes* mosquitoes. Ecology. 2005; 86: 3279–3288. https://doi.org/10.1890/05-0209 PMID: 19096729

59. Humphries AT, La Peyre MK, Decossas GA. The effect of structural complexity, prey density, and “Predator-Free Space” on prey survivorship at created Oyster reef mesocosms. PLoSOne. 2011; 6. e28339.

60. Smith JA, Donadio E, Pauli JN, Sheriff MJ, Bidder OR, Middleton AD. Habitat complexity mediates the predator–prey space race. Ecology. 2019; 100(07):e02724 https://doi.org/10.1002/ecy.2724 PMID: 31157915

61. Blois C, Cloarec A. Density-dependent prey selection in the water stick insect, *Ranatra linearis* (Heteroptera). J Anim Ecol. 1983; 52:849–866.

62. Bailey PCE. ‘A prey in the hand’, multi-prey capture behaviour in a sit-and-wait predator, *Ranatra dispar* (Heteroptera:Nepidae), the water stick insect. J Ethol. 1985; 3:105–112.

63. Bailey PCE. The feeding behavior of a sit- and wait- predator *Ranatra dispar* (Heteroptera:Nepidae): optimal foraging and feeding dynamics. Oecologia. 1986; 68:291–297. https://doi.org/10.1007/BF00384802 PMID: 28310142

64. Bailey PCE. Abundance and age-specific spatial and temporal distribution in two water-bug species, (Heteroptera:Notonectidae) and *Ranatra dispar* (Heteroptera:Nepidae) in farm dams in South Australia. Oikos. 1987; 49:83–90.

65. Edmondson WT. Freshwater biology. John Wiley Sons. Inc. New York, USA, 1963;1248.

66. Bouchard RW. Guide to aquatic macroinvertebrates of upper Midwest. 2004. Water resources centre, University of Minnesota, St, Paul, Minnesota, USA. 1963.

67. Epler JH. Identification manual for the aquatic and semi-aquatic Heteroptera of Florida (Belostomatidae, Corixidae, Gelastocoridae, Gerridae, Hebridae, Hydrometridae, Mesoveliidae, Naucoridae, Nepidae, Notonectidae, Ochteridae, Pleidae, Saldidae, Velidae). Florida Department of Environmental Protection, Tallahassee, FL. 2006.

68. Nieser N, Zettel H, Chen P-p. Notes on *Laccotrephes STÅL, 1866* with the description of a new species of the *L. griseus* group (Insecta: Heteroptera: Nepidae). Ann Nat Hist Mus Wien Ser B Bot Zool.2009; 110: 11–20.

69. Polhemus JT, Polhemus DA. Global diversity of true bugs (Heteroptera; Insecta) in freshwater. 2007. In: Balian E.V., Lévêque C., Segers H., Martens K. (eds) Freshwater Animal Diversity Assessment. Developments in Hydrobiology, vol 198. Springer, Dordrecht.

70. Robert V, Goff GL, Ariey F, Duchemin JB. A possible alternative method for collecting mosquito larvae in rice fields. Malar J. 2002; 1: 4. https://doi.org/10.1186/1475-2875-1-4 PMID: 12057018

71. Thirumalai G. Aquatic and semi-aquatic Heteroptera of India. Indian Assoc Aqua Biol (IAAB). 1999; 7: 1–74.

72. Addinsoft SARL. XLSTAT software, version 9.0. 2010. Addinsoft, Paris

73. Soluk DA, Collins NC. Synergistic interactions between fish and stoneflies-facilitation and interference among stream predators. Oikos.1988; 52: 94–100.

74. Soluk DA. Multiple predator effects: predicting combined functional response of stream fish and invertebrate predators. Ecology.1993; 74: 219–225.

75. Sih A, Englund G, Wooster D. Emergent impacts of multiple predators on prey. Trends Ecol Evol. 1998; 13: 350–355. https://doi.org/10.1016/s0169-5347(98)01437-2 PMID: 21238339

76. Vance-Chalcraft HD, Soluk DA. Multiple predator effects result in risk reduction for prey across multiple prey densities. Oecologia. 2005; 144: 472–480. https://doi.org/10.1007/s00442-005-0077-5 PMID: 15891830

77. Zar JH. Biostatistical Analysis. IV ed. 1999. Pearson Education Singapore Pte. Ltd. (Indian Branch). New Delhi, India. 663 pp.

78. Finke DL, Denno RF. Intraguild predation diminished in complex habitats: implications for top-down suppression of prey populations. Ecology. 2002; 83: 643–652.
79. Langellotto GA, Denno RF. Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. Oecologia, 2004; 139: 1–10. https://doi.org/10.1007/s00442-004-1497-3 PMID: 14872336

80. Fischer S, Zanotti G, Castro A, Quiroga L, Vargas DV. Effect of habitat complexity on the predation of Buenoa fuscipennis (Heteroptera: Notonectidae) on mosquito immature stages and alternative prey. J Vector Ecol. 2013; 38: 215–223. https://doi.org/10.1111/j.1948-7134.2013.12033.x PMID: 24581348

81. Jordan F, McCreary AC. Effects of an odonate predator and habitat complexity on survival of the flagfish Jordanella flavidae. Wetlands. 1996; 16: 583–586.

82. Denno RF, Finke DL. Multiple predator interactions and food-web connectance: implications for biological control. In. Trophic and Guild Interactions in Biological Control, (Brodeur J. and Bolivin G. eds.), 2006; pp 45–70.

83. Delclos P, Rudolf VHW. Effects of size structure and habitat complexity on predator–prey interactions. Ecol Entomol. 2011; 36: 744–750.

84. Saha N, Aditya G, Bal A, Saha GK. A comparative study of predation of three aquatic hemipteran bugs on Culex quinquefasciatus larvae. Limnology. 2007a; 8: 73–80.

85. Kopp K, Wachlevski M, Eterovick PC. Environmental complexity reduces tadpole predation by water bugs. Can J Zool. 2006; 84: 136–140.

86. Warfe DM, Barmuta LA. Habitat structural complexity mediates the foraging success of multiple predator species. Oecologia. 2004; 141: 171–178. https://doi.org/10.1007/s00442-004-1644-x PMID: 15300485

87. Baber MJ, Babbitt KJ. Influence of habitat complexity on predator-prey interactions between the fish (Gambusia holbrooki) and tadpoles of Hyla squirella and Gastrophyne carolinensis. Copeia. 2004; 2004:173–177.

88. Fulan Já, Almeida SC. Effect of the spatial heterogeneity on the predation of Scinax fuscovarius and Physalaemus cyanus tadpoles by Odonata larvae. Acta Sci Biol Sci. 2010; 32: 121–124.

89. Swisher BJ, Soluk DA, Wahl DH. Non-Additive predation in littoral Habitats: influences of habitat complexity. Oikos. 1998; 81:30–37.

90. Dief H. Foraging efficiency of three freshwater fishes: effects of structural complexity and light. Oikos. 1988; 53: 207–214.

91. Nelson WG, Bonsdorff E. Fish predation and habitat complexity: Are complexity thresholds real? J Exp Mar Biol Ecol. 1990; 141: 183–194.

92. MacRae PSD, Jackson DA. The influence of smallmouth bass (Micropterus dolomieu) predation and habitat complexity on the structure of littoral zone fish assemblages. Can J Fish Aquat Sci. 2001; 58: 342–351.

93. Guillarmo-Bond J, A-Jime nez J, Rodrı ´ guez MH, Quiroz-Martı ´ nez H, Williams T. Oviposition habitat selection for a predator refuge and food source in a mosquito. Ecol Entomol. 2005; 30:255–263.

94. Carey MP, Maloney KO, Chipps SR, Wahl DH. Effects of littoral habitat complexity and sunfish composition on fish production. Ecol Freshw Fish. 2010; 19: 466–476.

95. Camp EV, Gwinn DC, PineIII WE, Frazer TK. Changes in submersed aquatic vegetation affect predation risk of a common prey fish Lucania parva (Cyprinodontiformes: Fundulidae) in a spring-fed coastal river. Fish Manage Ecol. 2010; 19: 245–251.

96. Santos AFGN, Garcia-Berthou E, Hayashi C, Santos LN. When habitat complexity increases predation risk: experiments with invasive and neotropical native fishes. Mar Fresh Wat Res. 2013; 64: 752–760.

97. Gregg WW, Rose FL. Influences of aquatic macrophytes on invertebrate community structure, guild structure, and microdistribution in streams. Hydrobiologia.1985; 128: 45–56.

98. Peiró DF, Amaral GFD, Saulino HHL. Structure community of aquatic insects associated with different macrophytes in ornamental lakes in a savanna region, Southeastern Brazil.Pan-Am J aqua sci. 2015; 10: 273–282

99. Diaz-Valenzuela J, Barva-Alvarez R, Merlo- Galiazzi A, Zambrano L. Macrophytes and metaphyton as habitats for insects in temporary and permanent tropical aquatic ecosystems. Neotrop Biodivers. 2016; 2: 171–180.

100. de Souza Rezende R, Silva Monção F, Gonçalves JF Jr, dos Santos AM. Macroinvertebrate associated with macrophyte beds in a Cerrado stream. Limnetica. 2019; 10: 38: 639–652.

101. Babbitt KJ, Jordan F. Predation on Bufo terrestis tadpoles: effects of cover and predator identity. Copeia.1996; 1996: 485–488.

102. Finke DL, Denno RF. Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. Ecol Lett. 2005; 8: 1299–1306.
103. Holt RD. Spatial heterogeneity, indirect interactions, and the coexistence of prey species. Am Nat. 1984; 124: 377–406. https://doi.org/10.1086/284280 PMID: 29519131

104. Sunish IP, Reuben R. Factors influencing the abundance of Japanese encephalitis vectors in rice fields in India—II. Biotic. Med Vet Entomol. 2002; 16:1–9. https://doi.org/10.1046/j.1365-2915.2002.00325.x PMID: 11963972

105. Das PK, Sivagnaname N, Amalraj DD. Population interactions between Culex vishnui mosquitoes and their natural enemies in Pondicherry, India. J Vector Ecol. 2006; 31: 84–88. https://doi.org/10.3376/1081-1710(2006)31[84:pibcvm]2.0.co;2 PMID: 16859094

106. Saha N, Aditya G, Saha GK, Hampton SE. Opportunistic foraging by heteropteran mosquito predators. Aquat Ecol. 2010; 44: 167–176.

107. Saha N, Aditya G, Banerjee S, Saha GK. Predation potential of odonates on mosquito larvae: Implication for biological control. Biol Control. 2012; 63: 1–8.

108. Ohba S-Y, Kawada H, Dida GO, Juma D, Sonye G, Minakawa N, et al. Predators of Anopheles gambiae sensulato (Diptera: Culicidae) larvae in Wetlands, Western Kenya: confirmation by polymerase chain reaction method. J Med Entomol. 2010; 47: 783–787. https://doi.org/10.1603/me10051 PMID: 20939371

109. Saha N, Aditya G, Bal A, Saha GK. Comparative study of functional response of common hemipteran bugs of East Calcutta wetlands, India. Int Rev Hydrobiol. 2007b; 92:242–257.

110. Dalal A, Cuthbert RN, Dick JTA. Gupta S. Prey preferences of notonectids towards larval mosquitoes across prey ontogeny and search area. Pest Manage Sci. 2019a; 76, 609–616. https://doi.org/10.1002/ps.5556 PMID: 31313450

111. Dalal A, Cuthbert RN, Dick J.T.A. & Gupta S. Water depth-dependent notonectid predatory impacts across larval mosquito ontogeny. Pest Manage Sci. 2019b; 75: 2610–2617. https://doi.org/10.1002/ps.5368 PMID: 30729643

112. Dalal A, Cuthbert RN, Dick JTA, Sentsis A, Laverty C, Barrios- O'Neill D, et al. Prey size and predator density modify impacts by natural enemies towards mosquitoes. Ecol Entomol. 2020; 45: 423–433.

113. Culler L, Lamp WO. Selective predation by larval Agabus (Coleoptera: Dytiscidae) on mosquitoes: support for conservation-based mosquito suppression in constructed wetlands. Freshw Biol. 2009; 54: 2003–2014.

114. Wooton TJ. Predicting direct and indirect effects: an integrated approach using experiments and path analysis. Ecology. 1994; 75:151–165.

115. Barbosa P. Preface. In Barbosa P.(ed) Conservation Biological Control ( San Diego: Academic Press), 1998; pp. xx±xxi.

116. Kumar R, Hwang J-S. Larvicidal efficiency of aquatic predators: a perspective for mosquito biocontrol. Zool Stud. 2006; 45: 447–466.

117. Shaalan EAS, Canyon DV. Aquatic insect predator and mosquito control. Trop Biomed. 2009; 26:223–261. PMID: 20237438

118. Dambach P. The use of aquatic predators for larval control of mosquito disease vectors: Opportunities and limitations. Biol Control. 2020; 150: 104357.