

Predation of the beetle *Rhantus sikkimensis* (Coleoptera: Dytiscidae) on the larvae of *Chironomus* Meigen (Diptera: Chironomidae) of the Darjeeling Himalayas of India

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Abstract

The dytiscid beetle *Rhantus sikkimensis*, Regimbart, 1899 (Coleoptera: Dytiscidae), a member of the freshwater insect communities of the Darjeeling Himalayas, were noted to predate on the coexisting larvae of *Chironomus* sp. Meigen. Evaluation of predation by *R. sikkimensis* on *Chironomus* sp. larvae, in the laboratory, revealed that a single adult morph of *R. sikkimensis* could kill and consume on an average 10–90 and 10–78 numbers of small and large *Chironomus* sp. larvae, respectively, per day, depending on the prey density. The attack rate ranged between 520 and 537, and the handling time ranged between 4.3 and 8.6 depending on the size of the preys. The predation varied with respect to predator density also, with a maximum of 151 larvae killed by three predators per day. Two indices of predation, ingestion rate (IR) ranging between 13.33 and 74.15 larvae/day/predator and clearance rate (CR) ranging between 19.67 and 39.99 L prey/day/predator, varied with the prey size and predator density, significantly, when the predation was observed for 9 consecutive days, at two predator densities. It was also noted that *R. sikkimensis* predated on an average 9.8 larvae of *Chironomus* sp. and 1 larva of *Culex* sp., when the larvae of both the species are present together as preys, showing a preference for the *Chironomus* sp. larvae.

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Introduction

Among the aquatic insects, *Rhantus sikkimensis* Regimbart, 1899 (Coleoptera: Dytiscidae) is common and abundant in both natural and artificial lentic ecosystems of the Darjeeling Himalayas, India, in association with the larval stages of *Chironomus* sp.

and *Culex* sp. Although little is known about the species numbers in chironomids of this region (Chaudhuri & Som 1998; Hazra 2000; Hazra, Saha, & Chaudhuri 2000, 2002, 2005), yet Darjeeling as a part of the Eastern Himalayan biodiversity hotspots of the earth, a high number of (new) chironomid species as well as other aquatic insects are expected to be found. Also, the species composition and interaction of the aquatic habitats in this regions are yet to be explored, compared to the general information of the similar habitats in different region of the world where the dytiscid beetles

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have been noted to regulate the population of the chironomid and mosquito species through predation (Campos, Fernandez, & Sy 2004; Friss, Bauer, & Betz 2003; Kehl & Dettner 2003; Kögel 1987; Lundkvist, Landin, Jackson, & Svensson 2003; Tate & Hershey 2003). Several aquatic hemipteran water bugs like *Notonecta*, *Anisops*, *Buenoa* (Blaustein 1998; Hampton 2004) are also known to play a similar role ecologically, in different lentic habitats and structure the aquatic communities. Since dytiscid beetles are known to be predator of chironomid, the role of *R. sikkimensis* as a predator in the aquatic habitats of Darjeeling Himalayas, would help to judge the level of regulation it imparts upon the population of *Chironomus* sp. in particular and on the aquatic insect communities of this region. Association of *Rhantus signatus signatus* as predators with the mosquitoes and other dipterans has been noted in the temporary pools in Argentina (Campos et al. 2004; Fischer, Marinone, Fontanarrosa, Nieves, & Schweigmann 2000). Also, *Rhantus consputus* of central and eastern European countries predate on the larvae of the mosquitoes *Aedes vexans* (Kögel 1987). Since, in several temporary aquatic bodies in Darjeeling larval stages of *Culex* mosquitoes co-occur with the chironomid larvae (Aditya, Pramanik, & Saha 2006), predation of mosquito larvae, too, by *R. sikkimensis* can be assumed.

In view of these facts, and following the observation of the beetle *R. sikkimensis* predated on the chironomid midges in nature, an assessment of predatory efficiency of *R. sikkimensis* on the larval stages of the chironomid midges was made. Also, a prey preference of the beetle was evaluated using *Chironomus* and *Culex* sp. larvae as preys. Apart from establishing *R. sikkimensis* as a predator of dipteran larvae, the results of the study are expected to reveal the species interaction in the aquatic insect communities of Darjeeling Himalayas, at least partly.

Material and methods

Chironomid and culicid larvae (all instars) and adult dytiscid beetles were collected from roadside springs as well as temporary pools in and around Darjeeling Government College campus, Darjeeling. The preys (midge and mosquito larvae) and predators (beetles) were collected using a plankton net of appropriate diameter and were placed separately in the enamel trays of $30 \times 26 \times 10 \text{ cm}^3$ capacity containing a mixture of spring water and rain water poured over autoclaved mud bottom and were allowed to acclimatize in the laboratory. After a period of 3 days, the larvae of the midges were separated depending on the body length into two size groups—small (<20 mm; corresponding to

II and early III instars) and large (>20 mm corresponding to late III and IV instars), and were placed in separate enamel trays of the same type. In the experiments, these two sizes of the prey larvae were considered. At times, chironomid and mosquito larvae from other habitats of the same area were used to feed the predator in the experiments. Identifications of collected beetles, chironomid and mosquito larvae were done from ZSI, Kolkata, India and Entomology laboratory, University of Burdwan, Burdwan, India.

The following experiments were carried out during June and July 2004, to evaluate the aspects of predation of *R. sikkimensis* on two size groups of *Chironomus* sp. larvae.

Experiment I. To each adult specimen of *R. sikkimensis*, larvae of *Chironomus* sp. were supplied at densities of 10, 20, 40, 80, and 160 per 500 ml of a glass beaker and were allowed to predate for a period of 24 h. Nine replicates for each of the prey densities as well as prey sizes were carried out for determination of the rate of predation and the functional response. The functional response was analyzed after Fox and Murdoch (1978) using the linear regression form of the Holling Disc Equation:

$$H_a = aHT/(1 + aHT_h)$$

or

$$1/H_a = (1/a)(1/HT) + T_h/T \text{ equivalent to } y = \alpha x + \beta$$

where $1/a = \alpha$ and $T_h/T = \beta$,

where a is the search rate, T_h the handling time, T the total time of predation, H_a total prey killed, and H the prey density. The values a and T_h are calculated after deducing the values of α and β from several observations of H_a against different H values.

Experiment II. In this experiment, 40, 80, and 160 *Chironomus* sp. larvae per 500 ml were provided to either a single or two or three *R. sikkimensis* for a period of 24 h in order to determine the rate of predation. Nine replicates of the experiment for each of the predator densities as well as prey sizes and densities were carried out.

Experiment III. For a long-term study on predation rate, for 9 consecutive days, 2 or 3 adult individuals of *R. sikkimensis* were kept in a plastic bucket of 20 L capacity, 40 chironomid larvae were given as food for the first 3 days, 80 for the next 3 days, and 160 for the last 3 days. Nine replicates for each of the predator densities as well as prey sizes were carried out. The rate of predation was noted and the data obtained from the experiments were used to calculate the ingestion rate (IR) and clearance rate (CR) following Gilbert and

Burns (1999), with required modifications, as stated below:

$$IR = P_C - P_E/TN$$

and

$$CR = V \ln(P_C - P_E)/TN,$$

where V is the volume of water, T the time in days/h/min, N the number of predators, P_E the prey left after T time in the experiment and P_C the prey at the start of the experiment.

Experiment IV. A total of 20 preys (10 *Chironomus* sp. larvae and 10 *Culex* sp. larvae) were provided to an adult individual of *R. sikkimensis* for predation for a period of 4 h and to find out any preference for a particular kind amongst the two kinds of preys used in the experiment. The preys and the predator were placed in a 1 L glass beaker. Murdoch's coefficient of preference C (Murdoch, Avery, & Smyth 1975) was calculated based on the following equation, with respect to the preys killed:

$$C = (R_a/R_b)(N_b/N_a),$$

where, C is the Murdoch's index of preference; R_a and R_b are proportion of preys a and b killed; N_a and N_b are proportions of prey species available.

In order to ensure hunger stability of *R. sikkimensis* individuals, they were fed to satiation followed by a period of 24 h starvation, before using them in any of the experiments. Stored spring water (pH 7.9–8.7) was used in the experiment and the water temperature ranged between 19.5 and 23.9 °C. The data obtained from the experiments on rate of predation were subjected to various statistical analyses following Zar (1999). The data obtained on the functional and numerical responses as well as IR and CI values were subjected to paired t -test and regression analysis. Two-way ANOVA was applied to the data obtained on numerical responses to find out the difference in predation rate with respect to variable prey and predator densities.

Results

The predatory beetle *R. sikkimensis* was observed to use its foreleg to catch the prey (chironomid and mosquito larvae) in one swift motion and consumes the prey wholly barring the head and the tip of the abdomen. In higher prey densities, only a part of the prey was consumed and the rest was left over. Also, the larger preys (>20 mm) were partially consumed in higher proportions compared to the smaller preys (< 20 mm). When caught by the predator, the

preys were unable to get rid of the deadly grip, yet showed lateral undulations of the body. The beetles *R. sikkimensis* were found to dive to the bottom of the containers for the preys, but once caught, the preys were consumed resting at the surface of the water with the hind pair of legs spread wide sometimes to allow it to float at an angle to the water surface. Depending on the prey type and size, the time taken for devouring a prey varied between 19 and 28 min as observed for predation of 10 random small and large larvae of *Chironomus* sp.

The rate of predation of the beetle *R. sikkimensis* on chironomid larvae was found to vary with the size and density of prey with a mean value of 10–89.78 per day. At a lower density, the rate of predation was same for the small and large prey size but at a higher density, the small preys were killed at a mean rate of 89.78 per day and for large prey size, the value was 77.67 per day. One-tailed paired t -test carried out on the rate of predation of the smaller and the larger prey sizes differed significantly, with respect to different prey densities ($t = 2.074$; $df = 4$; $p < 0.05$). The attack rate ' a ' for the small prey size and the large prey size were 520 and 537 ml respectively and did not vary significantly ($\chi^2 = 0.61$; $df = 1$; NS). The respective handling time ' T_h ' was 4 min 3 s and 8 min 6 s and did not vary significantly as well ($\chi^2 = 1.44$; $df = 1$; NS). The regression equations obtained on the predation rate and prey densities are presented in Table 1. The rate of predation was found to be increasing with the densities of the prey as well as the predator with a maximum of 151 larvae predated by three *R. sikkimensis* within a period of 24 h. In the numerical response analysis, the predation rate varied significantly with respect to the prey densities, for both the prey sizes, but did not vary with the predator densities significantly as revealed by the two-way ANOVA tests (for small prey size: between prey densities, $F_{2,4} = 24.01$, $p < 0.01$; between predator densities, $F_{2,4} = 2.53$, NS; for large prey size: between prey densities, $F_{2,4} = 14.21$, $p < 0.05$; between predator densities, $F_{2,4} = 2.28$, NS). The functional and numerical responses based on the data of experiments I and II are presented in Tables 1 and 2, respectively.

In experiment III, the rate of predation by *R. sikkimensis* was observed to vary between 40 and 144 larvae per day for predator density of 2 and between 40 and 155 larvae per day for predator density 3 in case of smaller preys. The values for large preys ranged between 40 and 140 larvae per day for predator density 2 and 40 and 155 larvae per day for predator density 3. The IR and CR were found to differ significantly (paired t -test at $df = 8$) with respect to the size of the prey and the density of predator. The t -values for IRs are: between prey sizes: for predator density 2, $t = 2.93$, $p < 0.02$; for predator density 3, $t = 3.58$, $p < 0.01$; IR between predator densities: for small preys, $t = 6.22$, $p < 0.001$; for large preys, $t = 5.63$, $p < 0.001$. The t -values for CRs are: between prey sizes: for predator

Table 1. Analysis of functional response of *Rhantus sikkimensis* against the chironomid larvae as preys ($n = 9$ replicates/prey density/prey type). $T = 24$ h

H	Total prey killed	H_a (mean \pm SD)	$1/H_a$	$1/HT$
<i>(A) Prey = small chironomid larvae, II and early III instar, < 20 mm length</i>				
10	90	10 \pm 0	0.1	0.1
20	175	19.44 \pm 0.88	0.05	0.05
40	334	37.11 \pm 2.47	0.027	0.025
80	664	73.78 \pm 3.19	0.014	0.0125
160	808	89.78 \pm 5.24	0.0111	0.00625
<i>(B) Prey = large chironomid larvae, late III and IV instar, > 20 mm length</i>				
10	90	10 \pm 0	0.1	0.1
20	179	19.89 \pm 0.33	0.05	0.05
40	290	32.22 \pm 2.47	0.031	0.025
80	557	61.89 \pm 4.86	0.016	0.0125
160	699	77.67 \pm 2.83	0.0129	0.00625

Transformation of the Holling Disc Equation into linear regression mode gives: $1/H_a = (1/a)(1/HT) + T_h/T$ equivalent to $y = \alpha x + \beta$, where $1/a = \alpha$ and $T_h/T = \beta$. So, for small prey regression equation is $y = 0.96x + 0.003$ ($r^2 = 0.99$; $F_{1,3} = 2251.86$; $P < 0.0006$) and for large prey the equation is $y = 0.93x + 0.006$ ($r^2 = 0.98$; $F_{1,3} = 1078.17$; $P < 0.0006$).

Table 2. The numerical response

Predator density	Prey number		
	40	80	160
<i>(A) Prey = small chironomid larvae, II and early III instar, < 20 mm length</i>			
1	29.78 \pm 6.47	64.89 \pm 4.81	89.78 \pm 5.24
2	40 \pm 0	72.44 \pm 0.53	146.33 \pm 1.22
3	40 \pm 0	75.78 \pm 0.67	151.67 \pm 1.00
<i>(B) Prey = large chironomid larvae, late III and IV instar, > 20 mm length</i>			
1	32.22 \pm 2.64	61.89 \pm 4.86	77.67 \pm 2.83
2	40 \pm 0	73.22 \pm 0.83	146.67 \pm 1.22
3	40 \pm 0	76.56 \pm 0.53	151.00 \pm 0.87

Mean \pm SD of prey consumed by *R. sikkimensis* in a 24 h period ($n = 9$ replicates/prey type/prey density/predator density).

density 2, $t = 2.36$, $p < 0.05$; for predator density 3, $t = 3.75$, $p < 0.01$; IR between predator densities: for small preys, $t = 24.53$, $p < 0.001$; for large preys, $t = 24.28$, $p < 0.001$ (Figs. 1 and 2).

In experiment IV it was noted that *R. sikkimensis* prefers chironomid larvae to the mosquito larvae. In unit time, the number of mosquito larvae consumed was much low compared to the chironomid larvae even though the mosquito larvae were observed to float more on the surface compared to the chironomid larvae. Paired t -test carried out on the individual values of Murdoch's coefficient of preference for the two prey species varied significantly ($t = 12.02$, $p < 0.001$, $df = 9$) (Table 3).

Discussion

In Darjeeling Himalayas, the natural lentic water bodies are intermittent with their existence restricted to

a brief period of 3–4 months per year coinciding with the monsoon. The abundance of freshwater organisms in the temporary pools and other artificial and natural water bodies reaches its peak during this period, between June and September, including the immatures of chironomid midges and mosquitoes and the beetles *R. sikkimensis* (Aditya et al. 2006). In some of these water bodies, the densities of dipteran larvae are positively correlated with the predators like *R. sikkimensis*. Information on species interactions and general bio-ecology of these annual aquatic communities are little and fragmentary, even considering western Himalayas (Bisht & Das 1988; Chakraborty & Saha 1993; Kaul 1972; Zutshi, Subla, Khan, & Wanganeo 1980). The present study reports for the first time, about a predator of the larval forms of the chironomid midges of Darjeeling Himalayas and thus establishes a food chain in part. Chironomid larvae, for the major part occupy the primary consumer level in the detritus-based food chain of the aquatic communities and play a major role

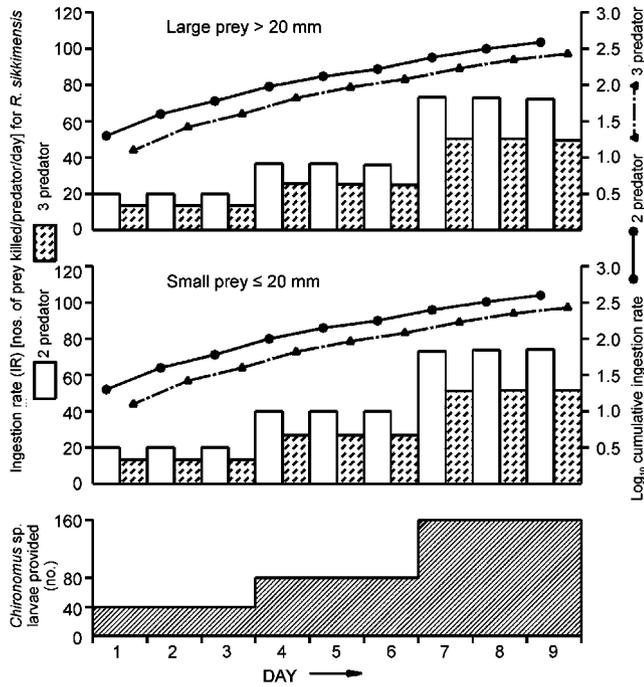


Fig. 1. Comparative account of ingestion rate (IR) and cumulative IR of adult morph of *R. sikkimensis* provided with chironomid larvae (of two sizes) for 9 consecutive days. (S.E. omitted due to values < 1).

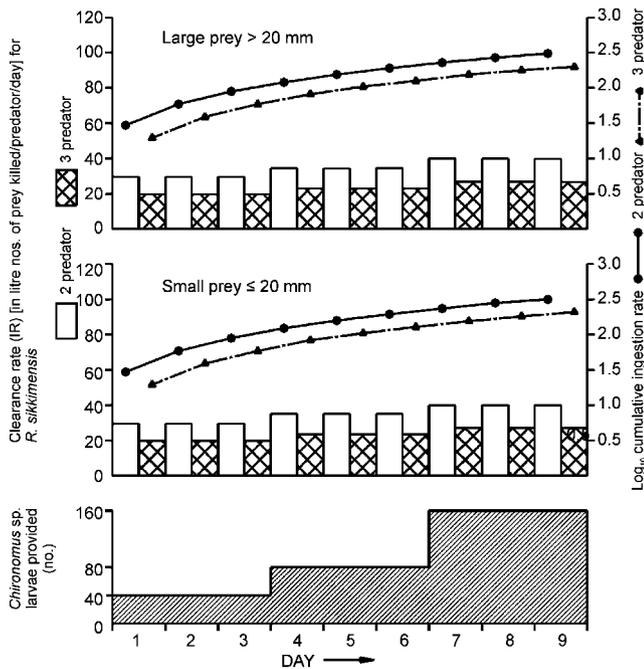


Fig. 2. Comparative account of clearance rate (CR) and cumulative CR of adult morph of *R. sikkimensis* provided with chironomid larvae (of two sizes) for 9 consecutive days. (S.E. omitted due to values < 1).

in nutrient cycling, (Lods-Crozet et al. 2001; Marian & Pandian 1985; Rasmussen 1985; Robinson, Uehlinger, & Heiber 2001) and sustenance of aquatic insect

Table 3. Rate of predation of preys provided in equal ratios to a single *R. sikkimensis* for a period of 4 h

	Prey species	
	<i>Culex</i> sp.	<i>Chironomus</i> sp.
Prey killed and consumed	1 ± 81	9.8 ± 0.42
Murdoch's coefficient (C)	0.15 ± 0.05	0.98 ± 0.42

n = 10 trials; *Culex* sp. 10: *Chironomus* sp. 10.

predators (Blaustein 1998; Blaustein, Kiflawi, Eitam, Mangel, & Cohen 2004; Campos et al. 2004; Elliot 2004; Fischer et al. 2000). It can be assumed that in the present context too, the chironomid larvae play a similar role and also help to maintain the population of *R. sikkimensis* and other predators, if any. Predation of the aquatic bugs and the beetles plays a major role in insect species interactions and abundance in aquatic communities, in general (Bisht & Das 1988; Blaustein 1998; Eitam, Blaustein, & Mangel 2002; Gilbert & Burns 1999; Giller & Mc Neill 1981; Hampton 2004; Murdoch, Scott, & Ebsworth 1984; Tate & Hershey 2003). Killing and consumption of chironomid larvae by *R. sikkimensis* is expected to impart an effect on the temporal and spatial abundance of the insect species in the aquatic communities and can play a regulatory role, keeping apart the possible roles of other coexisting aquatic insects whose ecological functions are yet to be elucidated.

Among the invertebrate predators of chironomid larvae studied in recent years, the leech *Erpobdella octoculata* (Kutschera 2003), and the larvae of the stoneflies *Dinocras cephalotes*, *Perla bipunctata*, *Isoperla grammatica*, and *Perlodes microcephalus* (Elliot 2004) are noteworthy. In both leeches and larvae of stoneflies, the preference varied with the size of the prey and presence of other prey species. The predation pattern of *R. sikkimensis* was found to be similar to these as reflected through the predation rate depending on size of chironomid larvae and the preference of larvae of *Chironomus* sp. over *Culex* sp. However, in case of the notonectid bug *Anisops sardea*, as prey the larvae of *Chironomus* sp. were less vulnerable compared to larvae of *Culiseta* sp. (Eitam et al. 2002). Also, in comparison to *Culiseta longiareolata* larvae, *Chironomus* larvae are found to be less vulnerable to the predation by *Notonecta maculata* (Blaustein 1998), and thus do not exhibit oviposition habitat selection (Blaustein et al. 2004). In natural conditions, in artificial ponds, dytiscids beetles of the genus *Hydroporus*, *Ilybius*, *Colymbetes*, and *Rhantus* were found to lower the populations of mosquito larvae. Under laboratory conditions, mosquito larvae were less preferred than other prey type like *Daphnia* by the dytiscids beetles *Ilybius ater*, and *Ilybius fuliginosus* in contrast to *Colymbetes paykulli* which

shows preference for *Culex* larvae (Lundkvist et al. 2003). In case of *R. consputus*, a species of central and eastern Europe, *Aedes vexans* larvae were preferred over cladocerans (Kögel 1987). It seems thus that the choice of prey may differ with the community representatives as far the dytiscids beetles are concerned.

Choice of a perfect habitat for reproduction is obvious for the chironomids also. In case of *Chironomus riparius*, a choice of sediments with higher level of food is being observed (DeHass, Wagner, Koelmans, Kraak, & Admiral 2006). In terms of the aquatic insect community, particularly of Darjeeling Himalayan region, this carries implications with the formation and maintenance of the insect diversity in the temporary water bodies. Thus the *Chironomus* larvae are expected to be found in aquatic bodies with high level of detritus and so is their predator *R. sikkimensis*. Preliminary surveys carried on the diversity of mosquitoes in Darjeeling are supportive of this fact and it has been observed that a positive correlation exists with the population of *Chironomus* larvae and *R. sikkimensis* in some temporary pools (Aditya et al. 2006). It was also noted that the mosquito larvae appear late compared to the chironomid larvae and dytiscids *R. sikkimensis* in these habitats.

The numbers of prey killed per day by the beetles *R. sikkimensis* varied with the size of the prey even when the foraging area remained same and thus the differences in IR and CR values indicate that the beetles hunt for the preys with higher yield in terms of energy spent and acquired. When the prey size was small, higher numbers of chironomid larvae were killed compared to situations when the larger larvae were available as preys. Also, with the increment of prey numbers through the days, the predation rate increased irrespective of prey size. In natural situations where the habitat is structured and the temporal and spatial variations of species abundance is more complex, the predation rates of *R. sikkimensis* as observed here are expected to vary, as has been noted in *Buenoa* sp. and *Notonecta* sp. (Hampton 2004) and other aquatic predators (Blaustein 1998; Eitam et al. 2002; Gilbert & Burns 1999). The present study focused on the predation of the *R. sikkimensis* on the larvae of *Chironomus* under laboratory conditions. Considering the availability of alternate preys along with the heterogeneity of the habitats in the temporary pools, deviation from the present finding on the prey preference by *R. sikkimensis* cannot be ruled out. Especially when the prey preference of dytiscids beetles are known to vary under natural and artificial conditions (Lundkvist et al. 2003). Assessment of predation of chironomid larvae by *R. sikkimensis* under such natural circumstances would help to substantiate its role in aquatic communities of Darjeeling Himalayas more appropriately.

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