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OBSERVATIONS ON THE PREDATION OF THE SNAIL *HUTTONELLA BICOLOR* ON THE SNAIL *ALLOPEAS GRACILE*

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Abstract. The predatory snail *Huttonella bicolor* (Hutton 1834) (Gastropda: Streptaxidae) was encountered along with *Allopeas gracile* (Hutton 1834) (Gastropoda: Subulinidae) during a survey of small land snail species from several terrestrial habitats in Kolkata, India. An evaluation of the predation of *H. bicolor* as a function of prey size and predator density was carried out using *A. gracile* as a model prey snail. The predatory interactions were noted with an increasing ratio of 1, 2 and 4 *H. bicolor* against 10 *A. gracile* of varied size classes in a defined terrarium. At the end of a 48 h period of exposure, *H. bicolor* was observed to consume on an average 5.32 ± 0.50 snails depending on the size class and the predator density. The predation pattern varied significantly with the prey size class, as revealed through the logistic equation, y (prey-consumed) = 1 / (1 + exp (-(0.97–0.71*size class-prey))). In a separate experiment, it was observed that the presence of *H. bicolor* induced a reduction in the fecundity in *A. gracile*, as revealed through the logistic regression, y (egg laid) = 1 / (1 + exp(-(3.45–0.67*predator-density))). The direct effect of predation and indirect effect of oviposition reduction reflect the efficacy of *H. bicolor* on population regulation of *A. gracile*. In view of conservation biological control, the use of the snail *H. bicolor* as a biocontrol agent may prove beneficial in situations where *A. gracile* is a pest.

INTRODUCTION

Land snails are diverse in terms of their taxonomy, shape and function (Bouchet 1992; Clark and May 2002; Rosenberg 2014) with equally diverse forms among predators (Barker and Efford 2004) linked to them. Consumption of land snails by land planaria (Boll and Leal-Zanchet 2015), beetles (Balbergen et al. 2014), dipteran flies (Poinar Jr 2014), snakes (Hoso et al. 2007; Hoso 2012a, b; Danaisawadi et al. 2016) and birds (Raut and Ghose 1979; Barker and Efford 2004) are common in nature. Many predatory (carnivore) land snails qualify as "snail-eating snails", as they feed not only on several soft invertebrates (Efford 2000; Herbert 2002; Lemos et al. 2012) but also on other snails of the same or different family (Fisher et al.1980; Auffenberg and Stange1986).

In India and many of the tropical countries of the world, the snail *Huttonella bicolor* (Hutton 1834) (Gastropda: Streptaxidae) is observed as a common predatory snail (Dundee and Barewald 1984) that feeds on other co-occurring snails. In the course of surveying the land snails in gardens and green lanes of the urban areas of Kolkata, India, the co-occurrence of the graceful awlsnail *Allopeas gracile* (Hutton 1834) (Gastropda: Subulinidae) and *H. bicolor* prompted us to elucidate the predation potential of the predatory snail *H. bicolor*. Until now, no systematic record is available on the predatory potential of *H. bicolor*, excepting mention about its predatory nature on other snails, particularly on *A. gracile* (Srivastava et al. 1975; Ali 2000). An exploration on the predatory nature of the snail *H. bicolor* was made using *A. gracile* as a prey species. The purpose of the study was to decipher the regulatory effect of *H. bicolor* on the co-occurring *A. gracile,* which is considered a pest of garden plants and horticultural orchid plants (Roy and Mukherjee 1969; Raut and Ghose 1984) like *Hibiscus rosa-sinensis, Clitorea tematea, Limonia crenulata, Dolichos lab lab* and *Citrus* sp. (De and Mitra 1999). As an invasive species, *A. gracile* exhibits a pestiferous nature that impacts a wide range of prospective crops and vegetables like bok choi cabbage, French marigold, spinach, amaranth, Nappa cabbage, etc. (Capinera 2017).

Selection of prey and their consumption rate by predatory snails depends on the relative sizes of the prey and predator and also on prey density as seen in the carnivorous snails *Euglandina rosea* (Férussac, 1821) (Cook 1983, 1989; Griffiths et al. 1993; Meyer and Cowie 2010; Sugiura et al. 2011), *Oxychilus alliarius* (Miller, 1822) (Meyer and Cowie 2010) and *Haplotrema concavum* (Say, 1821) (Atkinson and Balaban 1997). Thus, the present study aimed to evaluate prey size dependent consumption of *A. gracile* by the predatory snail *H. bicolor* with varying predator density. In addition, the indirect effect of *H. bicolor* on oviposition of *A. gracile* was assessed in laboratory mesocosms. Both the direct predatory impact and indirect effects on oviposition, if any, may provide insights about the regulation of the population of the pest snail *A. gracile* by the snail *H. bicolor* following the principles of conservation through biological control.

MATERIALS AND METHODS

The predatory snail *H. bicolor* was encountered during the field collection of small land snails from several terrestrial habitats in Kolkata and adjoining areas, including Howrah, in India. In gardens, moist concrete walls and soils with sparse distribution of grasses are inhabited by both *H. bicolor* and *A. gracile. Huttonella bicolor* was collected along with *A. gracile* from 1 square meter quadrats located in several terrestrial habitats in Baranagar, Garia, and Howrah, with Ballygunge Science College campus as the focal area. Following collection, the snails were placed in a glass terrarium of 38 cm × 29.5 cm × 13 cm size, separately for acclimatization as well as rearing. Individuals of the snail *A. gracile* were provided with lettuce and sweet gourd as food, and moss laden soil was provided in the terrarium for oviposition. Individuals hatching from the culture as well as the heterogeneous size classes of *A. gracile* enabled availability of multiple size classes for the experiments. The collected *H. bicolor* were acclimatized and reared in a similar mesocosm with provision of *A. gracile* as food. The collection of the snails from the study sites was continued throughout the experiment to provide sufficient supply of predators and prey for the experiment. [Specimen snails were identified from Zoological Survey of India, Kolkata, India with letter no. F. No. 229-10/Mal./2017/9118 dated 7th August 2017]. Two experiments were carried out to decipher the prey consumption potential of *H. bicolor* and the reduction in the oviposition in *A. gracile.*

Predation rate as a function of prey size and predator density

After a few days of acclimatization *H. bicolor* was exposed to the prey snails *A. gracile* in increasing ratios of 1, 2 and 4 against 10 prey snails of different size classes (0–2.0 mm, 2.1–4.0 mm, and 4.1–6.0 mm in shell height) in a defined plastic terrarium of 10.9 cm diameter. Shell height of the prey and predator was measured using a scale and a divider (length) to the nearest 0.1 mm, and the wet weight was measured in a pan balance (Afcoset®, India) to the nearest 0.1 mg. *Huttonella bicolor* of 7.1 mm ± 0.1 (range 6.1 mm – 7.9 mm) shell height and 15.3 mg ± 0. 6 (range 12.5 mg – 19.5 mg) body weight were selected for the experiment. Since all the predator and prey snails were field collected and belonged to overlapping generations, the precise age of the snails could not be predicted and therefore shell length was considered as the marker of the size of the snails. To keep the soil moist, the terrarium was covered with a perforated sheet and water was sprayed regularly. A small patch of moss was added to each terrarium to make the habitat more akin to the snail's natural habitat. The total number of snails killed and consumed by *H. bicolor* was confirmed by the presence of shells of dead individuals, and the number of dead shells was recorded at the end of 48 hours. Observations were made for at least three different replicates (Hurlbert 1984) for each ratio (predator: prey) and size class (prey snail). In each instance, after 48 hours dead shells were replaced by living individuals of the same size to keep the number of prey constant. The experiment was continued for 13 days to obtain an ample quantity of data on the predation of *H. bicolor* and *A. gracile.* The results included data of at least 117 (54 + 54 + 9) replicates against the different predator densities and the prey size classes. Each size class and predator density combination was selected independently so as to qualify as a single replicate complying with the norms of interspersion and randomization (Hurlbert 1984). The test animals were collected from the field and acclimatized for 7 days at least prior to the use in the experiments.

Effect of predator's presence on the fecundity of prey population

To observe the difference in fecundity rate, six *A. gracile* of mean shell height of 6.6 mm ± 0.07 (range 6.1 mm – 6.9 mm) with mean body weight of 15.1 mg ± 0.4 (range 13.1 mg – 18.9 mg) were added along with one, two and four *H. bicolor* in a plastic terrarium of 10.9 cm diameter. A few small sized *A. gracile* were given as food for *H. bicolor* and slices of vegetables were added as food for *A. gracile*. Oviposition rate was recorded by counting the number of eggs at an interval of 24 hours for the duration of 14 days and observations were made for three different replicates, for each predator density. A control set was used to observe fecundity rate in the absence of predators. In ach 24 hours, shells of *A. gracile* which had been eaten were replaced with live small sized prey.

Data analysis

Linear regression was used to estimate the mean value of *A. gracile* consumed by *H. bicolor* as a function of prey and predator densities to elucidate the trend in prey-predator interaction. Furthermore, data obtained for the predation of the snail *H. bicolor* on the snail *A. gracile* were subjected to logistic regression following a binomial generalized linear model with weighted binary function logit link. The equation of the logistic regressions was: y = 1 / (1 + exp (-(-a + b_1*x_1))), where y – prey consumed, x_1 – explanatory variable 1 (Zar 1999; Addinsoft 2010). In the logistic regression, prey size class and predator density were considered explanatory variables, separately as well as together to determine the impact on the vulnerability of the prey snail *A. gracile.* In the logistic regression, the prey consumption by *H. bicolor* (at a particular prey class and predator density combination) was assumed to follow the binomial distribution (n, p) with n replicates for each level of the explanatory variables (prey size class or predator density). In this instance, the probability parameter p represents a linear combination of the explanatory variables (prey size class and predator density). The parameters of the models were tested against Wald's chi-square at p = 0.05 level. The data obtained on the eggs produced on each day were also subjected to a logistic regression model to deduce the relationship between egg production and prey density. The difference in egg production for each predator density was estimated by constructing a bar plot of the mean number of eggs produced each day at each predator density.

RESULTS

The observations on the predation of the snail *A. gracile* by the snail *H. bicolor* are provided below as a function of prey size and predator density.

Predation rate as a function of prey size and predator density

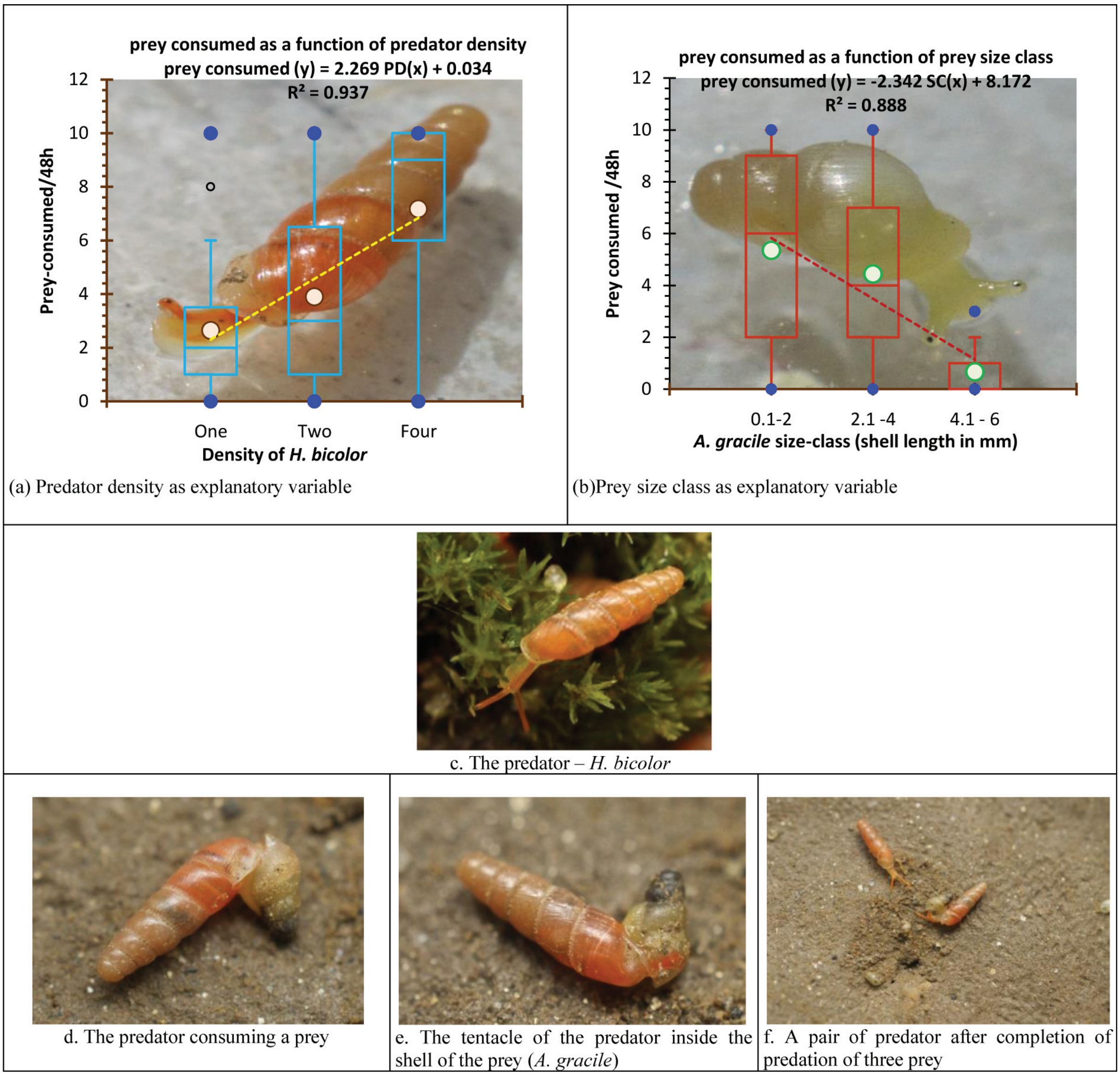
The predatory snail *H. bicolor* consumed a considerable number of *A. gracile* depending on its relative density and the size class of the prey snails, reflected through the regression lines (Figure 1a, b). On an average *H. bicolor* consumed 2.6 ± 0.4 *A. gracile* at a density of 1, 3.9 ± 0.5 at a density of 2, and 7.2 ± 0.5 at a density of 4. Vulnerability to the predator decreased with each increment in size of *A. gracile.* The predator consumed on average 5.3 ± 0.5 *A. gracile* of size 0–2.0 mm, 4.4 ± 0.4 of size 2.1–4.o mm, and 0.7 ± 0.4 of size 4.1–6.0 mm. From the graphical representation of the predation rate of *H. bicolor*, it could be inferred that predation rate decreased as a factor of prey size and increased as the density of the predator increased (Figure 1a–c). A logistic regression of prey consumption as a function of predator density could be represented as y (prey-consumed) = 1 / (1 + exp (-(-1.72 + 0.66*predator-density))), with model parameter (predator density) being significant (intercept = -1.71 ± 0.13, Wald χ^2 = 154.618; predator-density = 0.658 ± 0.052, Wald χ^2 = 157.72, p < 0.001) (Figure 1a). Consumption rate as a function of prey size class could be presented as y (prey-consumed) = 1 / (1 + exp (-(0.97-0.71*size class-prey))), with model parameter (prey size class) being significant (intercept = 0.965 ± 0.16, Wald χ^2 = 32.675; size class-prey =-0.711 ± 0.10, Wald χ^2= 50.573, p < 0.001) (Figure 1b). Similarly, an equation of the prey consumption as a combined function of predator density and prey size class could be presented as y (prey-consumed) = 1 / (1 + exp (-(-0.31–0.94*size class-prey + 0.63*predator-density + 0.04*size class-prey*predator-density))). However, the interaction was non-significant (intercept = -0.306 ± 0.39, Wald χ^2 = 0.596; size class-prey =-0.944 ± 0.25, Wald χ^2 = 14.862, p < 0.001 predator density = 0.630 ± 0.153, Wald χ^2 = 16.879, p < 0.001; size class-prey and predator-density interaction =-0.042 ± 0.089, Wald χ^2 = 0.224, p > 0.05).

Effect of predator's presence on the fecundity of prey population

The egg production in each day varied with the predator density, being high in the absence of predator and decreasing considerably with increase of predator density. Decrement of egg production followed a linear regression as y (egg produced) = 2.678 - 0.328* predator density; R^2 = 0.977. The logistic regression of the number of eggs laid as a function of predator density could be presented as y (egg laid) = 1 / (1 + exp(-(3.45 - 0.67*predator-density))) with model parameter (predator density) being significant (intercept =3.45 ± 0.48, Wald χ^2 = 50.502, predator-density = -0.67 ± 0.14, Wald χ^2 = 20.563, p < 0.001) (Figure 2).

DISCUSSION

In India, *A. gracile* is ubiquitous among different habitats, consuming various types of plant resources (De and Mitra 1999) and often qualifying as a pest species (Raut and Ghose 1984). A recent study suggests *A. gracile* was introduced into the Caribbean and some parts of North America (Capinera 2017). The predatory snail *H. bicolor* however remains associated with other prey snails in the same habitats. The predatory snail *H. bicolor* exhibited a differential predatory ability in response to the density and prey size in the experiments. Although some prey were found to move around on the soil, others concealed themselves in the moss patches as an evasive tactic against the predator. The predatory snails were able to accomplish their predation following efficient searching for prey from among the patches of moss. Following detection of the prey, *H. bicolor* grabbed the prey snail by its foot muscle, and then the prey retracted its body within the



(a) Predator density as explanatory variable

(b)Prey size class as explanatory variable

c. The predator – *H. bicolor*

d. The predator consuming a prey

e. The tentacle of the predator inside the shell of the prey (*A. gracile*)

f. A pair of predator after completion of predation of three prey

Figure 1. Box-plot representation of *A. gracile* consumed by *H. bicolor* in 48 h time period, when exposed at different predator density (a) and at different prey size classes (b). The regression equations provide the trend in the effects of density of predator (a) and prey size class (b) using the mean value of prey consumed. The predation of the snail *H. bicolor* on the snail *A. gracile* in sequence observed in the laboratory set up (c through f).

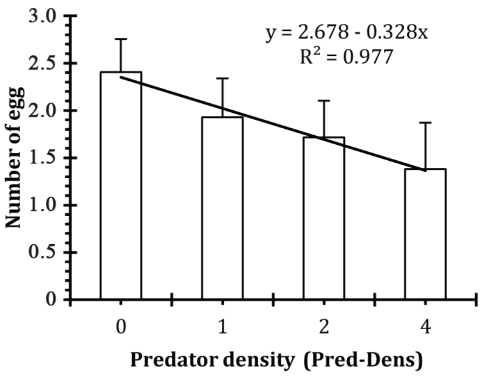


Figure 2. Oviposition (egg production) by *A. gracile* in the presence of the predator *H. bicolor* (n = 15 per predator density). A linear regression explained the decrease in the oviposition by the snail *A. gracile* with the increase in the density of the snail *H. bicolor.*

shell and began to secrete mucus. Thereafter *H. bicolor* inserted its mouth including tentacle within the shell of *A. gracile* and consumed the entire soft tissue. During the course of this consumption, the predatory snail kept the prey immobilized by holding it with its foot muscle and after consuming the prey its empty shell remained (Figure 1c–f). The predation rate of the snail *H. bicolor* was size-class dependent, which appeared to be in general parity with the predator and prey interactions. In addition, the presence of the predator appeared to influence the oviposition of the prey snail *A. gracile.* As observed in earlier studies, small snails were more vulnerable to the predatory snails *E. rosea* and *O. alliarius* (Miller, 1822) (Cook 1989; Meyer and Cowie 2010; Sugiura et al. 2011). However, on a comparative scale, *E. rosea* feed on larger prey, while *O. alliarius* consume only small sized (>3 mm) snails (Meyer and Cowie 2010). In compliance with these observations, in the present instance, the snail *H. bicolor* consumed small sized (0.1–2.0 mm) at a higher number than large sized (4.1–6.0 mm) *A. gracile* individuals. In contrast to *E. rosea*, which can consume whole prey (Cook 1989; Griffiths et al. 1993; Meyer and Cowie 2010), *H. bicolor* consumed only the flesh of the prey leaving the empty shell. The vulnerability to the predator decreased with the size-class of *A. gracile*, indicating that the *H. bicolor* is constrained in handling larger sized snails efficiently. Experimental studies suggested that total egg production of *A. gracile* decreased considerably due to the presence of *H. bicolor* in the population. The reduction in oviposition by the snails may impact population structure and cumulative population as indirect effects of the presence of the predator. However, such observations need to be assessed further in the light of the indirect nature of this predatory effect. Since both snail species exist in the same habitats, the optimal population sizes of the two snails (both *H. bicolor* and *A. gracile*) will serve to be a good indicator for prospective biological regulation. Nonetheless, *H. bicolor* appears to be able to change the age structure of *A. gracile* populations in a natural habitat through declines in newly hatched individuals and diminishing egg production. The predatory snail *H. bicolor* may be considered a biological control agent of *A. gracile* in conditions where it qualifies as a pest (Roy and Mukherjee 1969; Raut and Ghose 1984). Further studies are required to model the numerical response of the predator against a target snail. The prey species preference of predators plays a crucial role because a generalist invasive predator may be catastrophic for native species (Cowie 2001). As observed in early studies, introduction of *E. rosea* in Hawaii and other Pacific islands to control populations of the pestiferous snail *Achatina fulica* resulted in declines among other native endemic tree snails (Hadfield 1986; Murray et al. 1988; Cowie 1992). Thus, future studies should be carried out to observe prey species preferences of small and large sized (Cook 1989; Meyer and Cowie 2010; Sugiura et al. 2011; Mc Donell 2016) *H. bicolor* in order to safely promote this predatory species as an effective biocontrol agent of pest snails.

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