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Predation and prey preference of the exotic snail *Anentome helena* on two freshwater snails: implications for species invasion

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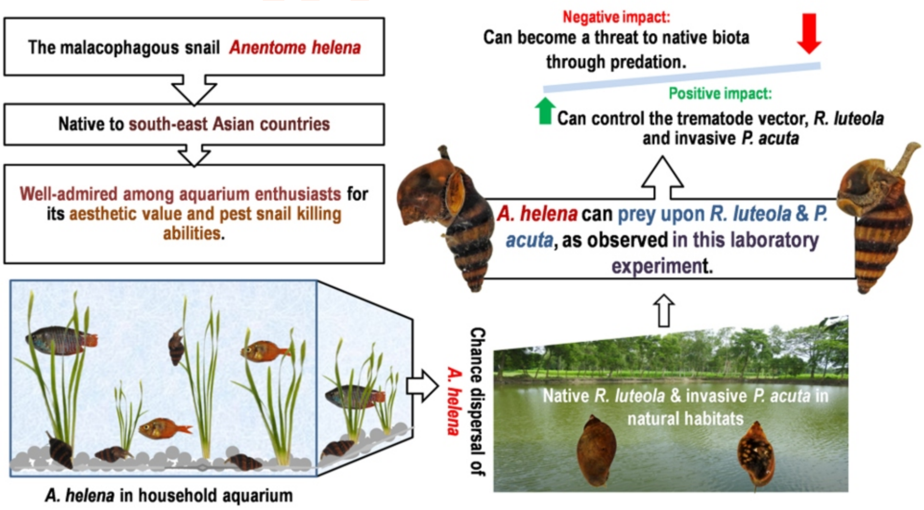
Abstract

Aim: The aim of the present study was to estimate the probable impact of predation of the exotic snail *Anentome helena* on two co-occurring freshwater snails, *Racesina luteola* and *Physella acuta*, in the laboratory set-up.

Methodology: In the initial experiment, the predatory efficiency of *A. helena* was observed using *R. luteola* and *P. acuta* as prey, separately, under varying prey and predator densities. In the following experiment, the prey snails were provided in both conspecific and heterospecific conditions with different predator densities to deduce the prey preference of *A. helena*, if any.



Results: The experiments show that *A. helena* can consume a considerable number of *R. luteola* and *P. acuta*. The predation rate of *A. helena* varied significantly ($p < 0.0001$) with different sizes of prey, prey and predator densities. Although the predation rate differed among conspecific and heterospecific prey conditions, *A. helena* did not demonstrate any significant prey preference towards any of the prey.

Interpretation: The study revealed that the chance dispersal of predator *A. helena* from household aquaria to natural habitats may directly impact the population of the freshwater snail *R. luteola* and *P. acuta*.

Key words: Assassin snail, Aquatic snail, Invasive snail, *Physella acuta*, Prey preference

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Introduction

The dispersal of the freshwater invertebrates across the land is often facilitated by vectors such as water, wind or terrestrial vertebrate species (Bilton *et al.,* 2001). With the growing popularity of aquarium ornamental pets, the global aquarium industry serves as an alternative process for the translocation of non-native organisms beyond their native range. For instance, the spread of several non-native molluscs is linked with their increasing demand in aquarium trades as observed worldwide (Ng *et al.,* 2016a), including in India (Raut and Aditya, 1999). Introduction or unintentional release of the exotic molluscs from the aquaria increase the chance of their establishment as observed for *Melanoides tuberculata* (Müller, 1774) in New Zealand (Duggan, 2002) and *Pomacea canaliculata* (Lamarck, 1822) in South-East Asia (de Brito and Joshi, 2016). The unintentional or deliberate introduction of non-native snails from aquaculture or aquariums cause considerable negative impacts on the native fauna. For instance, the invasive golden apple snail *P. canaliculata* consume the early life stages of native snails and amphibian eggs (Kwong *et al.,* 2009; Karraker and Dudgeon, 2014).

Among different freshwater snails in the aquarium trade, *Anentome helena* (von dem Busch, 1847) (Gastropoda: Nassariidae) bears considerable demand for its predatory efficiency upon the aquarium pest snails. Commonly known as the "assassin" snail, *A. helena* was first described from Java, Indonesia (Philippi, 1847) and distributed in few South-East Asian countries (Fischer 1891; Chan, 1997). In recent years, the widespread sale of *A. helena* has been observed from different aquatic pet shops and markets of Kolkata, India. This particular factor raises concern for the chance dispersal and possible colonisation of *A. helena* in the natural water bodies. For instance, the widespread use of *A. helena* as an aquarium pet resulted in its first non-native establishment in Singapore (Ng *et al.*, 2016b) and is thought to be a possible threat as possible colonisation of *A. helena* in the freshwater habitats can render the freshwater snails vulnerable to predation (Bogan and Hanneman, 2013; Paul *et al.,* 2021).

In order to justify the prospective impact of *A. helena* on the freshwater snails of Kolkata, India, experimental observations were made on its predation on two co-occurring pulmonate snails, *Racesina luteola* (Lamarck, 1822) (earlier known as *Lymnaea luteola*) (Gastropods: Lymnaeidae) and *Physella acuta* (Draparnaud, 1805) (earlier known as *Physa acuta*) (Gastropods: Physidae). The prey snails, *R. luteola* and *P. acuta* exist together in different freshwater bodies, including the sewage drains, temporary pools and ponds (Aditya and Raut, 2002a; Paul and Aditya, 2021). The native snail *R. luteola* is responsible for spreading trematode infections in animals and men (Raut, 1986). On the other hand, *P. acuta* is native to North America and is now considered as a globally invasive species (Vinarski, 2017). It is evident from the earlier studies that the predation of native predators on *R. luteola* and *P. acuta* varied with prey size, prey and predator densities (Raut and Nandi, 1984; Aditya and Raut, 2002a). The present study was undertaken to estimate the probable impact of predation of the exotic predator snail *A. helena* on two co-occurring freshwater snails, *R. luteola* and *P. acuta*, in the laboratory set-up. The purpose was to understand the consumption pattern and prey choice of the exotic predator snail.

Materials and Methods

Collection and maintenance of snails: The exotic carnivorous snail *A. helena* were brought from the pet market of Galiff Street, Kolkata, India. In the laboratory, the stock of *A. helena* was maintained in plastic trays (30 x 25 x 10 cm; Tarsons, India) filled with aged tap water. As food, we provided ample tubificid worms and non-operculate snails with regular cleaning of the rearing trays and replenishing the food items. Although the shell length of a fully grown *A. helena* can range up to 30 mm, in the present instance, the snails of the size class 18 to 20 mm (in shell length) were selected (being the dominant size class) for the experiments. In all instances, *A. helena* was fed to satiation and then kept in starvation for 24 hrs to coequal the hunger level before their use in the experiments. This step was carried out to minimise disparity among the predators regarding their hunger level and ability to search the prey.

Using an aquatic net fitted with a long handle (200 μm mesh size), *R. luteola* and *P. acuta* were collected from the sewage drains and ponds in and around Kolkata. In addition, the snails that remain attached with the submerged and emergent vegetations (*Alternanthera philoxeroides, Vallisneria spirallis, Jussiaea repens, Nymphoides cristatum, Lemna minor*) in the ponds were also picked by hand as and when found to assure adequate supply. The prey snails were maintained in aged tap water-filled plastic trays (30x25x10 cm; Tarsons, India) with lettuce leaves in the laboratory.

Experimental design: A heterogeneous population of the snails were present in the collection, dominated by two particular size classes (based on shell length) that were assigned as small (5-7 mm) and large (8–10 mm) sizes. Following measurement using a vernier calliper (Insize, Brazil), the snails were segregated according to the size classes mentioned. In the first experiment, *R. luteola* and *P. acuta* were placed separately (*i.e.,* conspecific condition) in the experimental containers with different densities (n= 5, 10, 20). In addition, *C. helena* was placed in the same containers in different densities (n= 5, 10, 20) to observe the effect of predator density on the consumption rate. The second experiment was carried out to appraise the effects of - (a) prey heterogeneity on the predation on *R. luteola* and *P. acuta* and (b) prey preference of *A. helena*. To fulfil the second experiment, the prey snails were placed in three different ratios (*R. luteola*: *P. acuta*- 10:0 and 0:10 for conspecific and 5:5 for heterospecific prey condition) to various densities (n= 1, 2 and 4) of *A. helena* in the experimental containers.

For each experiment, at least nine replicates were considered for each predator and prey combination and size class. The selection of prey and the predator were made such that a particular prey or predator snail individual was never used more than once, complying with the norms of replication (Hurlbert, 1984). In selecting the predator and prey snails in the experiments, strict norms of randomisation were applied, and the experiments were carried out at two different time phases, abiding by the norms of interspersion (Hurlbert, 1984). The experiments were carried out in plastic containers filled with at least 2 l of tap water at laboratory temperature 25±1 ˚C and a photoperiod of 14:10 hrs. The number of prey snails consumed by *A. helena* was noted every 48 hrs interval for both experiments.

Data analyses: Apart from evaluating the mean prey consumption by *A. helena*, the effects of the prey density, combinations of the prey and the predator density were assessed through ANOVA. The prey density, predator density and the prey combinations were considered explanatory variables against the prey consumed as the response variable. To determine if *A. helena* prefers or avoids any prey snail over the other, the selectivity index (S_i) (Manly's alpha) for each prey species was calculated by the equation- $S_i = \alpha_i / \sum \alpha_i$ (Chesson, 1978; Rehage *et al.*, 2005) and the values of α_i were determined by-

$$\alpha_i = \frac{c_i}{n_i} \Big/ \sum_{1}^{n} \frac{c_j}{n_j}$$

(Manly, 1972; Chesson 1978), where α_i= selectivity index for *R. luteola* or *P. acuta*, c_i= number of *R. luteola* or *P. acuta* consumed, n_i= abundance of *R. luteola* or *P. acuta*, c_j= number of prey consumed and n_j= abundance of total prey in the experimental setup. Value of S_i greater than 0.5 indicates prey preference, and lower than 0.5 shows avoidance towards any prey species. The values of S_i for all replicates were further analysed using a one-tailed t-test to determine if the avoidance or preference was statistically significant. ANOVA and t-test were analysed (Zar 1999) using a licensed copy of XLSTAT version 2010.04.02 (Addinsoft 2010).

Results and Discussion

On the worldwide scale, various exotic non-mollusc and mollusc (Kwong *et al.,* 2009; Kreps *et al.,* 2012) predators exhibited negative impacts on the native snails. In the present study, the exotic predatory snail *A. helena* consumed a significant number of *R. luteola* and *P. acuta*, with variations related to the density and size classes of the prey. The consumption of *R. luteola* by *A. helena* ranged from 0.94±0.22 (mean±SE) to 2.72±0.15 for smaller size class and from 1.27±0.15 to 4.11±0.17 for larger size class per 48 hrs owing to different prey and predator densities (Fig. 1). Similarly, in the case of *P. acuta*, the consumption rate varied from 1.72±0.31 to 5.55±0.35 for smaller size class and from 0.83±0.14 to 3.16±0.21 for larger size class in 48 hrs (Fig. 1).

The result of ANOVA indicated that prey size had the most significant effect (for *R. luteola*- $F_{1,306}$= 125.7, p< 0.0001 and for *P. acuta*- $F_{1,306}$= 283.7, p< 0.0001), followed by predator density (for *R. luteola*- $F_{2,306}$= 27.1, p< 0.0001 and for *P. acuta*- $F_{2,306}$= 75.6, p< 0.0001) on the predation rate of *A. helena* on both prey species. Although the changes in prey density of *R. luteola* did not have any impact, the predation rate varied significantly with varying density of *P. acuta* ($F_{2,306}$= 23.9, p< 0.0001). *A. helena* consumed the larger size of *R. luteola* more than the smaller snails whereas consumed a high number of smaller *P. acuta* compared to the larger size class when present with the conspecifics. Selectivity of prey sizes in predation has several consequences in the predator-prey interactions. For

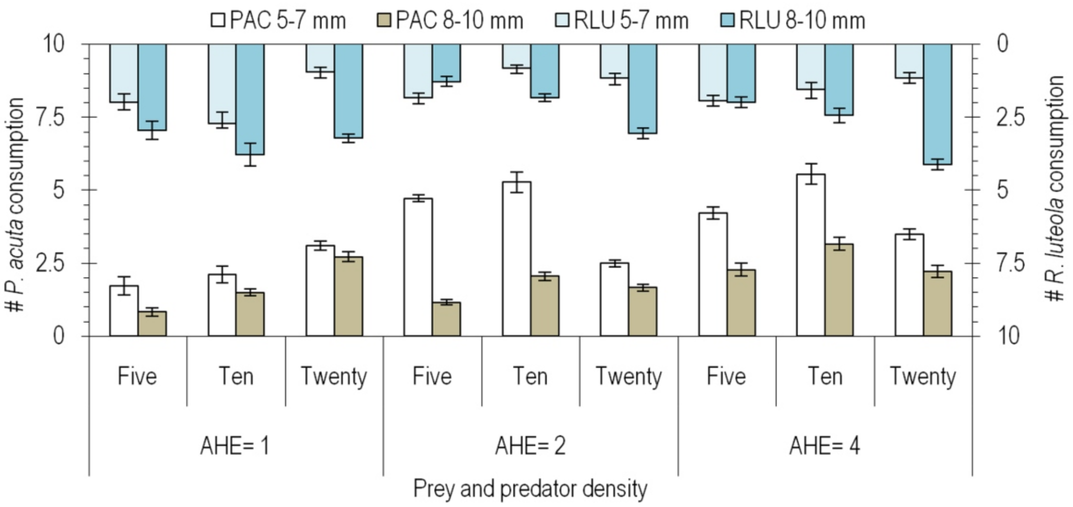

Fig. 1: The predation rate (mean±SE) of *A. helena* (AHE) on *R. luteola* (RLU) and *P. acuta* (PAC) (when provided separately) at the end of 48 hrs under varying prey size, prey and predator densities.

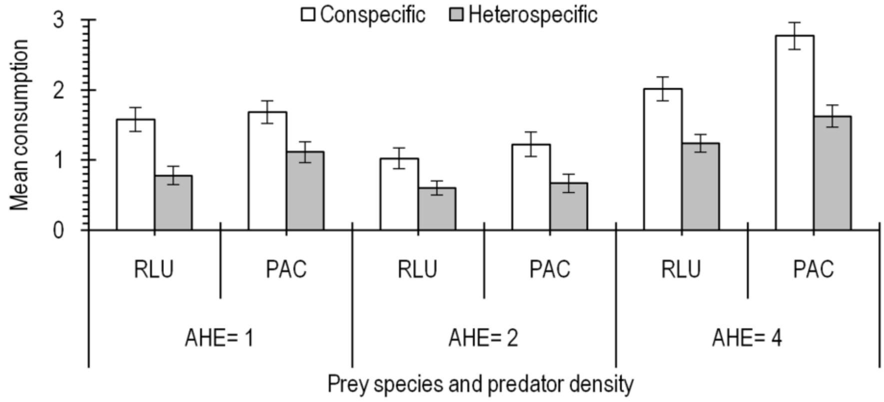

Fig. 2: Consumption of *A. helena* (AHE) per 48 hours (mean±SE) on *R. luteola* (RLU) and *P. acuta* (PAC) on the conspecific and heterospecific condition under different densities.

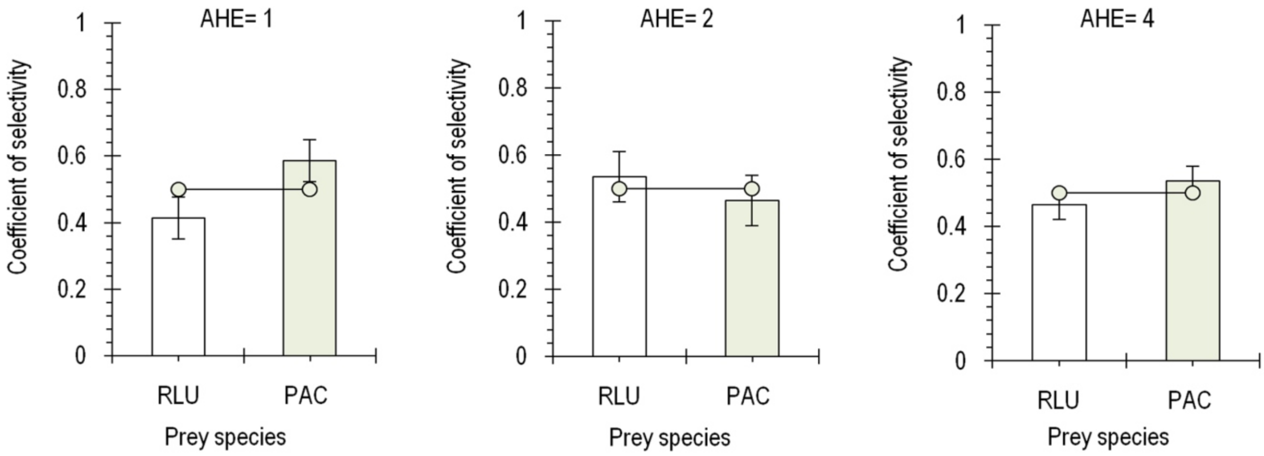

Fig. 3: Selectivity index (±SE) of *A. helena* (AHE) for *R. luteola* (RLU) and *P. acuta* (PAC) in different predator densities. The horizontal lines correspond to null hypothesis of no or equal preference towards any of the prey in each plot.

instance, size-selective predation can reduce the prey abundance and alter the size distribution structure of prey population through specific size selection (Kerfoot and Peterson, 1980).

The consumption rate on *R. luteola* ranged from 0.6±0.1 to 2.01 and from 0.66 to 2.73 on *P. acuta* as a result of different predator densities and prey heterogeneity (Fig. 2). The results of ANOVA represented that both predator density (for *R. luteola*- $F_{2,294}$= 16.1, p< 0.0001 and for *P. acuta*- $F_{2,324}$= 29.8, p<0.0001) and prey heterogeneity (for *R. luteola*- $F_{1,294}$= 22.2, p< 0.0001 and for *P. acuta*- $F_{1,324}$= 26.6, p< 0.0001) resulted in significant changes in the predation rate of *A. helena* for both prey species. In all instances, *A. helena* consumed comparatively fewer prey snails on the heterospecific prey conditions. Although the selectivity index values differed slightly from 0.5 for all predator densities, we observed no statistically significant prey selectivity of *A. helena* towards any of the prey snails (Fig. 3). The negative effect of heterogeneity of prey species on predation rate was due to alternative prey, which was equally palatable to the exotic predator.

A similar observation was made on the same species pair of snails when exposed to an indigenous predacious water bug, *Spherodema rusticum* (Hemiptera: Belostomatidae), which did not demonstrate any prey selectivity (Aditya and Raut, 2002a). Therefore, *R. luteola* and *P. acuta* remained equally vulnerable to predators like water bugs and exotic predator even though *P. acuta* has a considerably stronger defense strategy than other species, as observed against a predatory leech, *Hebbdella conifer* (Clitellata: Glossiphoniidae) (Wilken and Appleton, 1991). Although no firm conclusion about the correlation between predation rate and predator density can be drawn from the experiments, the predation rate per individual decreased in case of higher densities than single predator. This result implied the presence of mutual interference between the individuals of *A. helena* when confined to a smaller space. As mutual interference is a well-observed phenomenon during predation (Kratina *et al.,* 2009) and is thought to be a stabilising mechanism of prey-predator dynamics, detailed observations can be carried out in this regard.

The invasive snail *P. acuta* has been reported to compete with several cohabiting native freshwater gastropods. *P. acuta* was reported to replace several native freshwater snails in Mozambique (Dobson, 2004) and reduced the growth of a native snail by competition in Australia (Zukowski and Walker, 2009). The competitive success of an invader depends on high fecundity, flexible diets or utilisation of resources with more efficiency (Boland *et al.,* 2008; Morrison and Hay, 2011). As *P. acuta* cohabits with native *R. luteola* and utilises a similar niche in sewage canals and ponds, *P. acuta* may compete with *R. luteola* in the previously described way. Therefore, *A. helena* may influence the competitive interaction between these two snails through predation, if any. Previously, native predators such as *Glossiphonia weberi* (Blanchard), *S. rusticum* and *S. annulatum* were proposed as potential biological control agents against the trematode vector *R. luteola* and invasive snail *P. acuta* in India (Raut and Nandi, 1984; Roy and Raut, 1994; Aditya and Raut, 2002a, b). Apparently, the results of the experiments indicated that the predatory snail *A. helena* can consume considerable number of *R. luteola* and *P. acuta* under single and double prey combinations. However, variations in the prey snail consumption by *A. helena* varied with the size and relative density of the prey.

A possibility of the regulatory impact of *A. helena* on *R. luteola* and *P. acuta* can be anticipated in freshwater conditions. However, the proposal as a potential biocontrol agent is a matter of immense concern, based on the theme of the invasional meltdown (Simberloff and Von Holle, 1999). As the exotic predators are often assumed to be risky than the native predators (Salo *et al.,* 2007), an ill-conceived biological control program can cause considerable damage to the native non-target organisms. Similarly, besides predation on *R. luteola* and *P. acuta, A. helena* can predate upon several freshwater snails of West Bengal, India (Paul *et al.,* 2021). Considering the potential impact of exotic predator snail *A. helena* in the Indian context, the aquarium owners should be more aware during the aquarium water disposal to minimise the chance of unintentional introduction of this species to natural habitats.

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Add-on Information

Authors' contribution: R. Karmakar: Executed the experiment, analyzed; **P. Paul:** Executed, analyzed, drafted; **G. Aditya:** Conceptualized, statistics, data analysis and compilation of MS.

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