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WATER HYACINTH (*EICHHORNIA CRASSIPES*) AS AN OVIPOSITION SITE FOR THE DISEASE TRANSMITTING SNAIL *INDOPLANORBIS EXUSTUS* (DESHAYES, 1833) (GASTROPODA: PLANORBIDAE): IMPLICATIONS IN SNAIL MANAGEMENT

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Abstract. The water hyacinth (*Eichhornia crassipes*) is an invasive species in freshwater ecosystems with potential to alter habitat features and macroinvertebrate community composition. Many freshwater snails are associated with the water hyacinth for food and refuge. In the present study, an assessment of the water hyacinth as an oviposition site of the freshwater snail *Indoplanorbis exustus* was conducted through evaluation of the field collected plants. Apparently, the bulbous part of the water hyacinth was preferred over the leaves and the stems in terms of the egg capsules and eggs per egg capsule oviposited by *I. exustus*. A logistic regression indicated significant differences in the preferred oviposition sites by the snail. Owing to the invasion of the water hyacinth, the oviposition and further colonization by the snail *I. exustus* may be facilitated.

INTRODUCTION

Several species of freshwater snails serve as intermediate hosts for a number of helminthic parasites (Madsen and Hung 2015; WHO 2022). For instance, *Indoplanorbis exustus* (Deshayes, 1833) (Gastropoda: Planorbidae), common in different parts of India, is responsible for the transmission of the helminths causing schistosomiasis of a wide range of domestic animals (Parashar et al. 1986; Agrawal 2012; Bauri et al. 2015; Bulbul et al. 2021; Sonowal et al. 2021). A number of zoonotic diseases including metacercarial dermatitis are transmitted by *I. exustus*. In recent studies (Sangwan et al. 2016; WHO 2022), the distribution of *I. exustus* in various habitats has been studied along with the potential for the transmission of the helminth parasites (Tigga et al. 2014). The life history of *I. exustus* has been highlighted through the studies from Assam India (Islam 1977; Liu et al. 2010). Apparently, the transmission of the helminth parasites by freshwater snails is dependent on several abiotic and biotic factors that ensure sustenance of the population of the snail (Bulbul et al. 2022). The oviposition habitat selection by *I. exustus* in the freshwater habitat is certainly a key factor that determines the successful colonization and establishment of the population (Saijuntha et al. 2021). Among the various substrates, macrophytes are preferred for oviposition by *I. exustus* and other freshwater snails like *Racesina* spp. (Latchumikanthan et al. 2019). Generally, freshwater snails remained attached to different vegetative parts of the macrophytes and deposited eggs varying from 30 to 50 eggs per egg mass (Latchumikanthan et al. 2019). Thus, the macrophytes appear to be significant in the sustenance of snail population in the different freshwater habitats.

A typical mutualistic relationship between the snails and the macrophytes is observed in freshwater habitats (Lamberti et al. 1987). Snails living in association with macrophyte *Ceratophyllum* sp. exert potential beneficial effects by reducing bacterial and algal (*Cocconeis placentula)* load on macrophytes (Underwood et al. 1992). The removal of the epiphyton and the algae favours the growth of the macrophytes positively (Mulholland et al. 1985). Due to such mutualistic interactions (Brönmark 1989), different growth parameters of *Ceratophyllum* sp., measured in terms of the number of leaves, leaf nodes, growing tips and survival of leaves were found to be enhanced in presence of snails (Steinman et al. 1987) which act as a rich source of ammonia and phosphates. In return, the snails utilize the macrophytes for several different aspects including reproduction (Zealand and

Jeffries 2009; Hoverman et al. 2011). Macrophytes have been a common site for oviposition of freshwater snails in a number of instances including *I. exustus* (Islam 1977). By selecting macrophytes as oviposition sites, the snail *I. exustus* ensures the survival of the offspring in several ways including provision of resources and early food availability from the leaves of the macrophytes. Considering this macrophyte-snail relationship, it is thus evident that selection of suitable sites for oviposition is important for the sustenance of the snail population.

The invasive macrophyte *Eichhornia crassipes* (Martius) Solms-Laubach, 1883 (Pontederiaceae), commonly known as the water hyacinth, is abundant in many freshwater habitats in Kolkata, India. Although recognized for the detrimental effects in the freshwater community, the presence of water hyacinths is also linked with the increased abundance of macroinvertebrates (Marco et al. 2001; Masifwa et al. 2001). In almost all instances, an increase in the species richness and the augmented release of the nutrients in freshwater has been noticed with the presence of the water hyacinth (Rocha-Ramírez et al. 2007; Silva and Henry 2013). In the course of the survey of the water hyacinth in different water bodies of Kolkata, a consistent observation was made on the presence of the freshwater snail egg capsules on the stems and leaves. The egg capsules generally belonged to the snail *I. exustus* and thus an attempt was made to evaluate the role of the water hyacinth as an oviposition site for this snail species. Since the water hyacinth is an invasive species, the role in promoting the freshwater snails can prove harmful and degrade the invaded ecosystems further. An assessment of the role of the water hyacinth as a facilitator for the reproduction of *I. exustus* will enable focusing on regulating the populations of both macrophytes and snails. Besides, the role of macrophytes in the sustenance and spread of the freshwater snails in different habitats can be indicated through this appraisal.

MATERIALS AND METHODS

The study was conducted using the various ponds and lakes as the model freshwater habitats of Kolkata, India and adjoining areas during the monsoon period (July through October) of 2017 and 2018. Continuous and consistent sampling of the water hyacinth infested in the ponds was carried out through random picking of the whole plant from at least 24 water bodies (perennial small to large ponds with 30 to 90 cm depth on average and circular to rectangular in shape). The surface of these water bodies was dominated by the water hyacinth along with sporadic presence of *Marsilia quadrifolia*, *Pistia stratiotes*, *Lemna minor* and *Wolffia globosa.* The plants were selected from near the bank as well as from at least two meters away from the bank in a random manner. Both the tall non-bulbous and short bulbous types of water hyacinths were collected. Irrespective of the heterogeneity in the vegetative structures, the plants were collected and brought to the laboratory for morphological characterization and the assessment of snail eggs (Islam 1977; Sangwan et al. 2016).

In the laboratory, the plants were segregated and placed in a tray. Morphological variables were measured to the nearest 0.1 cm and the weight was taken in a pan balance (Afcoset, India ER-182A). Following the record of the morphological data, the various plant parts were checked for the presence of egg capsules. Subsequently the number of egg capsules and eggs laid were noted (Bulbul et al. 2020). The number of egg capsules was recorded separately for stems, leaves and bulbs. The egg capsules were scraped off using a fine sheet of fibre or blade and placed onto a glass slide to facilitate egg counting. The identification of the *I. exustus* egg capsule could be made based on the distinct lemon-yellow to golden-yellow colour with irregular but ovoid-to-round shape and columnar shape of the egg (Raut and Ghosh 1985; Pande et al. 2020). Different dimensions of leaves, stems, and bulbs of the invasive species were recorded to analyze if there is any selective preference or biasness of *I. exustus* for a particular microhabitat within this ramified weed mass during oviposition. The egg masses laid by other aquatic snails like *Racesina luteola*, *Gyraulus convexiusculus* and *Segmentina calatha* were also taken into consideration to assess the significance of a common invasive weed as oviposition substrate used by different aquatic snails from a comparative perspective.

A binomial generalized linear model (McCullagh and Nelder 1989) was applied to the data obtained on the number of egg capsules present in each stem or leaf or bulb of the plants. The differences in the pattern of egg-laying in the plant parts could be deduced through this analysis. In the logistic regression of the form y = 1 / (1 + exp (-(a + bx), where x represents the explanatory variable (i to n numbers) and y represents the eggs or the egg capsules deposited on the *E. crassipes* plant. A Wald's chi-square was used to justify the significance of the parameters of the logistic regression. In the present instance, the logistic regression was applied to the observed data on the eggs and the egg capsules (response variables) deposited on different plant parts (explanatory variable) to justify the differences, if any. The eggs oviposited by *I. exustus* were assumed to follow binomial (n, p) distribution with n replicates (number of eggs or egg capsules) for each combination of the explanatory variables (plant parts, viz. leaf, bulb and stem, in the plants). Here, the probability parameter p represents the linear combination of explanatory variables. A logit link was used and the parameters of the model were estimated through maximum likelihood using the software XLSTAT (Addinsoft 2010), and against each of the parameters a Wald's χ^2 value was used to judge the level of significance (McCullagh and Nelder 1989; Fox 2010). The parameters of the model were tested through Wald's chi-square for the significant contribution to the variations.

RESULTS

The sampling of the ponds and lakes revealed the presence of the macrophyte *E. crassipes* in almost all the ponds (n = 76) irrespective of the size of the ponds. Among the samples of *E. crassipes,* a total of 200 independent oviposition events were observed irrespective of the size of the egg capsules and the sites of oviposition (Figure 1 a and b). The freshwater planorbid snail laid eggs in a triple layered gelatinous ribbon-shaped matrix, and the number of egg capsules in an individual plant stem, leaf and bulbs revealed the presence of egg capsules of *I. exustus* in varying numbers. Irrespective of the sites of collection, the egg capsules of the snail *I. exustus* varied in the number of the eggs/capsule and oviposition sites (Figure 1). On an average, the number of eggs/capsule oviposited on the bulbs were greater than those observed in the stems and leaves (Figure 1 c, d and e). The number of capsules deposited was also higher in case of bulbs followed by leaves and stems. The logistic regression equation was significant for the explanatory variables (plant parts) and the eggs/capsule of *I. exustus*. The regression equations connecting the plant parts and the eggs deposited by *I. exustus* was: eggs = 1 / (1 + exp (-(-2.82-0.25*position))), where the parameters of the equation were significant at $p < 0.001$ (Intercept = -2.82 ± 0.06; Wald's χ^2 = 2341.03; position in the plant = -0.25 ± 0.03; Wald's χ^2 = 62.05). Since the plant parts were different in terms of the length and the width dimensions, the differences in the number of egg capsules deposited and the number of eggs/capsule are expected. This was reflected in the differences in the eggs/capsule in the three plant parts expressed through the logistic regression as: egg capsules = 1 / (1 + exp (-(-4.83 + 0.3*position))), where the parameters of the equation were significant at $p < 0.001$ (Intercept = -4.83 ± 0.12; Wald's χ^2 = 1648.46; position in the plant = -0.3 ± 0.07; Wald's χ^2 = 21.34). During the observations on the stems and leaves, the presence of the egg capsules of other freshwater snails was observed, particularly of *R. luteola* and *G. convexiusculus*, justifying that the freshwater snails use plants as oviposition sites.

DISCUSSION

The observation of egg capsules on the vegetative parts of the invasive macrophyte *E. crassipes* indicate the possible role of this plant in the dispersal of the snails

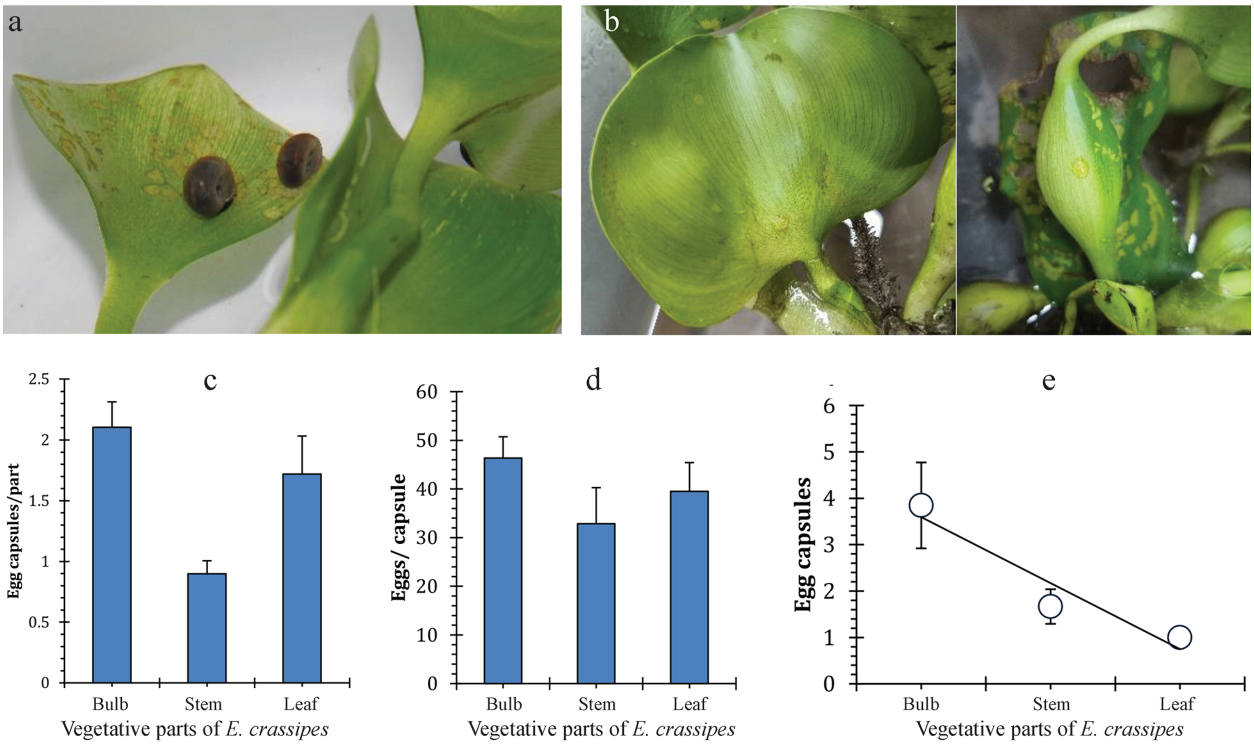

Figure 1. The number of egg capsules and the number of eggs per capsules of *I. exustus* observed in the different vegetative parts of the invasive weed *E. crassipes* (N = 96 number of water hyacinths observed over 76 lakes and ponds). (a) *I. exustus* on the leaves of water hyacinths, (b) Egg capsules of *I. exustus* on the leaves and stems of water hyacinths, (c) Eggs per capsule per plant parts, (d) Eggs per capsule and (e) Egg capsules laid per plant parts.

in different climatic conditions. In the present instance, the egg capsules were observed in different vegetative parts of the macrophyte with significant variations among the bulbs, leaves and stems. While the highest number of egg capsules was observed in the bulbous stems, the egg capsules were present in comparatively low numbers in the leaves of the plant. When viewed in terms of the contact with the surface and the water body, the stems and the roots of the water hyacinth are in contact with the water, but the leaves are rarely in contact with the water. Perhaps due to a low contact with the water, the leaves were less preferred as oviposition sites by the snail *I. exustus*. The leaves were also free from the eggs of other freshwater snails that tend to oviposit in the associated macrophytes. The number of eggs in each capsule remained higher for those that were oviposited on the stems than on the bulbs, owing to a larger surface area of the stems remaining submerged in the water. The oviposition strategies of the freshwater snails follow the mechanisms to avoid desiccation and water loss by the egg capsules and continuous availability of the water for the developing embryos. In many instances, the oviposition of the snails associated with the macrophytes substantiate the proposition that prevention of water loss and continuous availability of the water to the egg capsules are two vital factors required for the development of the embryos and the successful hatching of the juveniles (Bulbul et al. 2020, 2022). In a comparative study, *I. exustus* was found to lay a greater number of egg capsules when compared with *Radix rufescens* (*Lymnaea acuminata*). However, the number of egg capsules with the mean number of eggs/capsule was higher in case of *R. rufescens* than that of *I. exustus* (Pande et al. 2020).

The macrophyte *E. crassipes* can act as an organizer of the macroinvertebrate communities, which is evident from several studies (Rocha-Ramírez et al. 2007; Ohtaka et al. 2011; Silva and Henry 2013). The invasive nature of the water hyacinth and the ability to organize the freshwater community t can be considered as a probable reason for the spread of the associated organisms (Barker et al. 2014). As observed in the present study, the availability of the eggs of the snails *I. exustus* suggests the possible involvement of the water hyacinth in the dispersal and the spread in different water bodies within the concerned regions. Freshwater snails are considered as a concern to public health due to their involvement in the transmission of helminth diseases (Mattison et al. 1995; Madsen and Hung 2015; Campbell et al. 2017). In continuation with earlier studies including the recent survey of the presence of *I. exustus* in the water bodies indicated a population abundance in different time periods. The presence of macrophytes in the water bodies is a redundant feature and appears to assist the colonization and the establishment of the snails at a local as well as regional scale (Lodge 1985; Underwood et al. 1992). However, the preference of macrophytes for oviposition remains to be explored further to highlight the propagation of the population of the freshwater snail *I. exustus*. This may further be viewed in the context of the reproduction (Bulbul 2020) and the global distribution of the snail *I. exustus* (Liu et al. 2010). Besides, given that many freshwater snail species are capable of using the macrophytes as oviposition sites, it remains to be seen how the co-occurring snail species (Pande et al. 2020) are able to evade competition.

In freshwater ponds and lakes of India and many other tropical and sub-tropical countries, the abundance of freshwater snails is directly related to the availability of macrophytes (Zealand and Jeffries 2009; Hovermann et al. 2011). Macrophytes being a preferable oviposition site for several snails facilitate the sustenance of the snail populations and thereby enable transmission of helminthic diseases. This is particularly relevant for the snail *I. exustus* recognized for the transmission of schistosome parasites to various domesticated animals (Bauri et al. 2015; Bulbul et al. 2021). The snail is abundant in several different places in India (Raut et al. 1992; Bulbul et al. 2020) and elsewhere with dense vegetation being the main factor in the concerned habitats. The egg capsules of *I. exustus* were found in the different ramified parts of the water hyacinth (*E. crassipes*). Apparently, the rough under surface of leaves and bulbs of water hyacinths act as a preferable oviposition site for a number of snail species including *I. exustus.* Short bulbous plants were found to harbour most of the egg capsules sampled throughout the study period rather than the tall non-bulbous variant. In addition to the water hyacinth, several other macrophytes can also act as suitable oviposition sites for *I. exustus*. The risk of the spread of *I. exustus,* however, remains higher with the water hyacinth. Since the water hyacinth is an invasive species, it has a superior colonization ability and establishment in newer areas which will further facilitate the spread of the snail *I. exustus*. Therefore, the regulation of the snail *I. exustus* would require simultaneous regulation of the macrophytes like water hyacinth. Assessment of the role of other macrophytes in the sustenance of the population of *I. exustus* is therefore required to prevent the spread and regulation of helminth-borne diseases.

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All the authors provide consent for publication of this article in Zoology and Ecology.

Contribution of the authors

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Competing Interests

As authors of this article we declare no competing interest.

Availability of data and materials

The data concerning experiments of the present study can be shared upon authentic and reasonable request.

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