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Distributional Pattern and Environmental Response of Snail in South-central Bhutan

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Abstract

Terrestrial and freshwater snails are adapted to specialised ecological niches with a low mobility. They are highly susceptible to environmental changes, rapid urbanisation and the associated development activities. Snail diversity in different habitats and response of snails to environmental variables were studied in south-central Bhutan. Terrestrial habitat was subdivided into undisturbed and disturbed category (Dekidling and Gelephu gewogs respectively) under Sarpang district. Snails were collected from 88 (44 plots each) random sampling quadrats (20 x 20 m) with subplots of 5 (1 x 1 m) each in the main plots. Sampling in freshwater (nine plots) was carried out in 100 m interval with 20 m stretch. A total of 4,477 individuals comprising of 35 snail species belonging to 11 families were collected. There was significant difference in species richness in all the habitat types ($p < .05$). Terrestrial and freshwater snail species richness, diversity, evenness and abundance had no significant correlation with biotic factors except altitude (r = .65)and vegetation cover (r = .65) had significant positive correlation to the indices. The undisturbed terrestrial habitat supports more of small-body snails with higher species richness. However, disturbed terrestrial habitat supports large-body snails with less species richness but with higher species abundances. *Melanoides tuberculata* and *Tarebia lineata* were rarely recorded in freshwater habitat indicating the needs for further studies in monsoon season.

Keywords: Disturbed habitat, diversity, environmental variables, freshwater snail, species richness, terrestrial snail, undisturbed habitat

Introduction

Bhutan is a landlocked country located on the southern slope of the Eastern Himalaya with an area of 38,394 km^2. Considering its size, Bhutan has the most diverse ecosystem in Asia (Myers et *al.*, 2000). However, current information of country's biodiversity is typically subjected towards higher groups of flora and fauna, particularly vascular plants, vertebrate taxa and some selective hexapoda groups (Wangdi and Sherub*,* 2012; Nidup *et al.*, 2014). For other taxa such as molluscs, though they are the key regulating components of ecosystems, their information is incomplete (Chiba, 2007). This could be the probable reason for limited evaluation of many macro and micro invertebrates in global conservation "Red List" (Filipe *et al.*, 2013).

In the past, data on molluscs of Bhutan were rarely published although Nordsieck (1974) described a molluscan species: *Phaedusa bhutanensis* Nordsieck in 1974. However, in recent years, endemic land snail species (*Truncatellina bhutanensis* Gittenberger, Leda and Sherub,

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2013) was documented and more than 200 different land snail species are reported (Gittenberger *et al.*, 2013).

Terrestrial snails as pest of vegetables and crops are challenging (Ramzy, 2009) and also some snails (land and freshwater) serve as intermediate hosts which are harmful for animal health (Mas-Coma, 2004; Furst *et al.*, 2012). However, they are vital in every natural ecosystem as scavengers, calcium cyclers, environmental toxin cleaners such as cadmium, and as food for birds, fish and human beings (Imevbore and Ajayi, 1993; Lange and Mwinzi, 2003; Madsen and Hung, 2014). Aquatic snails are filter feeders which process detritus and decaying organic matter (Mitra, Dey and Ramakrishna, 2005).

Terrestrial and freshwater snails have low mobility and are adapted to very specialised ecological niches (Budha, 2016). They are highly susceptible to environmental changes, rapid urbanisation, expansion of settlement, and associated development activities. Thus, with rapid socio-economic development in Bhutan, unavoidable disturbances to environment and biodiversity are generated (Wangdi and Sherub, 2012).

With limited literature available on snails and environment dynamics in Bhutan (MOAF, 2016), it is timely that scientific studies on snail diversity and distribution are carried out to minimise the adverse environmental impacts on terrestrial and freshwater snails. Therefore, this study focused on the study of terrestrial and freshwater snails with the main objectives of comparing species diversity, richness and abundance among different habitats and to assess the response of snails with different environmental variables.

Materials and Methods

Study area

Sarpang (26°52’28.04” N and 90°29’17.84” E) is located in south-central Bhutan and has an area of 1,655 km^2. Sarpang encompasses one *Drungkhag* and 12 *Gewogs*. It is characterised by low lying plains in the south and rugged and steep terrains in the north region with elevation ranging from 200 m above sea level (asl) to 3600 m asl (Dzongkhag Administration Sarpang, 2016).

The study was carried out at Dekidling gewog (26°54'45.97" N and 90°21'55.57" E), and Gelephu gewog (26°52'14.76" N and 90° 29'11.41" E) maintaining Samtenling gewog (26°54'32.78" N and 90°25'48.23" E) as buffer (Figure 1). In 2014, the annual rainfall of Sarpang district was 1,278.5 mm and mean annual temperature was 22.4 °C. Due to higher number of households, human population (Table 1) and also as commercial hub for central Bhutan, Gelephu may have higher anthropogenic disturbances to natural resources than Dekidling gewog (Gelephu Developmental Control Regulation, 2013).

Movement of cargo vehicles and other transport means through international route (India) might affect the dispersal of exotic flora and fauna. For example: spread of invasive

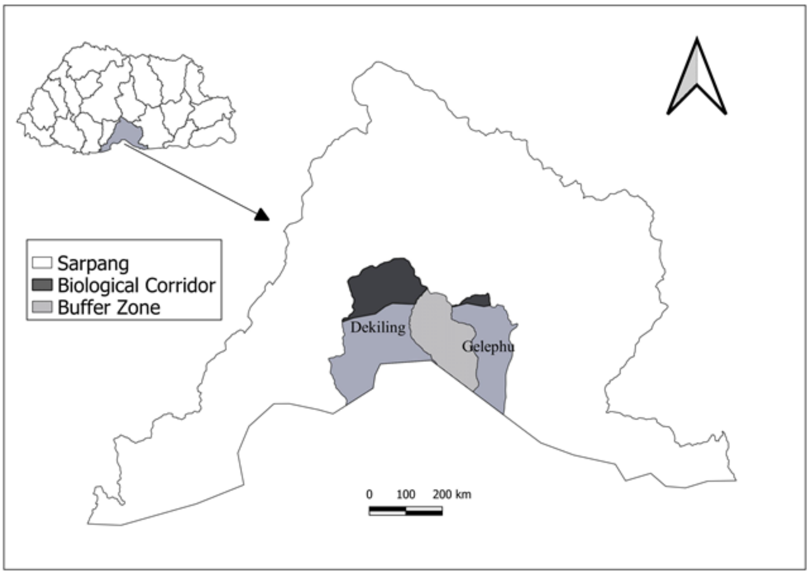

Figure 1: Map of the study area

Table 1: General information of the study area

Gewog	Area (km^2)	Altitude range (m asl)	Climate	Forest cover (%)	Household	Population
Gelephu	54	201-1602	Warm sub-tropical	79.8	2,635	13,174
Dekidling	113	293 - 1590	warm sub- tropical	88.6	732	3,835

African Giant Snail (*Lissachatina fulica)* at Gyelpozhing (Mongar District) was suspected to be introduced through material transport for hydropower dam construction from India (Penjor, 2013). Therefore, in this study, Gelephu gewog was considered as a disturbed habitat and the Dekidling gewog as undisturbed habitat (urban and rural respectively).

Terrestrial snail survey

A combination of direct search in random sampling (Nekola, 2003; Gittenberger *et al.,* 2013) and leaf litter-sieving method in non-random subplots were used. The minimum distance between the sample plots were maintained at 100 m (Hamburger *et al*, 1992). The quadrats (20 x 20 m) were established at every 100 m distance and 44 sample plots were selected using GIS fishnet plot generation method (Nekola, 2003).

At each randomly selected plot, one person/ hour (i.e., one searcher active for one hour) intensive search for snails was carried out (Cameron, 2013). In addition, topsoil from 5 non-randomly (based on personal observation) selected subplots of 1 m^2 in each sampling plots were sieved (E. Gittenberger, Pers. Comm., 8 September 2016).

Freshwater snail

Systematic sampling was carried out in 20 m stretch along the selected streams with 100 m distance between plots. Snails were searched using steel sieves (1.5 mm and 5 mm) attached to wooden handle (E. Gittenberger, Pers. Comm., 8 September 2016). Submerged/ emergent vegetation, floating plants, leaves and other litter were screened (Sallam and El-Wakeil, 2012). Rocks were hand-washed to dislodge the snails in a bucket (Bousset *et al.,* 2004).

Materials

Sieves (two sieves with mesh-widths of 1.5 mm and 5 mm), metal ring with 1.5 mm wire mesh fixed on a long stick, forceps, GPS, magnifying lens, plastic containers, rack and pH tester were used for data collection. Specimens were identified using 'A field guide to freshwater molluscs of Kailali, far western Nepal by Budha (2016)', 'Pictorial Handbook: Indian Land Snails (selected species) by Mitra, Dey and Ramakrishna (2005)' and 'A systematic revision of the land snails of the Western Ghats of India by Raheem *et al*. (2014)'. The specimens are preserved in the College of Natural Resources, Lobesa, Bhutan.

Determining the snail diversity, species richness and abundance

Shannon-Wiener diversity indices were used to determine species diversity among different habitats, which is expressed as:

$(H') = \Sigma PiLnPi$

Where: Σ is the sum of the calculations, Ln = the natural log, $Pi = ni/N$, ni = the number of individuals in species i and the abundance of species i.

Species Richness (S_R) = (S-1)/ Log N

Where: S = the sum of species

N = the total number of all species

Species Evenness (HE) = H'/ LogS

Where: H' = Shannon's diversity

S = the sum of species

Microsoft Excel, SPSS and PC-ORD software were used for data cleaning, storing and analyses.

Results and Discussion

Species composition of snails

A total of 4,477 snails belonging to 11 families, 22 genera and 35 species were collected from 88 terrestrial plots and nine freshwater plots (Kalikhola River, under Gelephu Gewog) during the field survey (Table 2). Among 11 snail families, 7 families were recorded from terrestrial habitat and 4 families from freshwater habitat (Figure 2a) and a total of 11 subfamilies, 16 genera and 29 species were recorded from terrestrial habitat. Among these, 2 families, 3 subfamilies, 5 genera, and 10 species were recorded only for the undisturbed habitat (Figure 2b). However, a distinct snail species was recorded in disturbed habitat (Table 2). The altitude of freshwater plots ranged from 238 to 244 m asl. In freshwater habitat, 4 families with 6 genera and 6 species were recorded (Table 2).

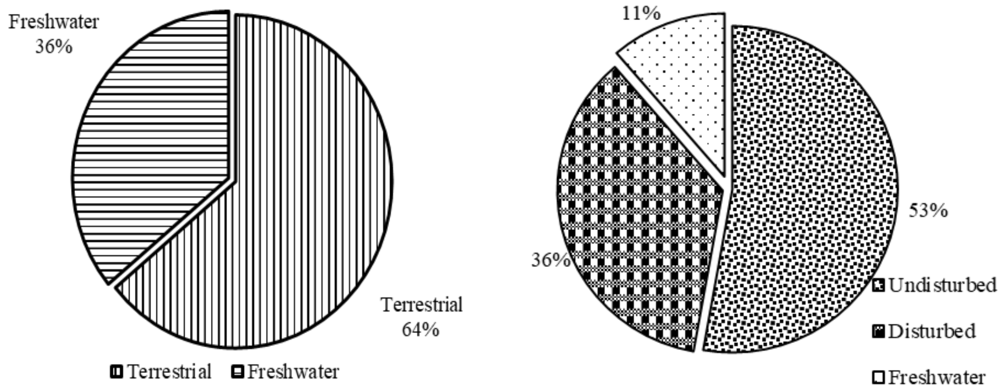

Figure 2: (a) Percentage of terrestrial and freshwater snail families (b) Percentage of species in undisturbed, disturbed and freshwater habitats

Species richness

The undisturbed habitat showed higher number of species (S_R = 9.67) than disturbed habitat (S_R = 6.06) and freshwater habitat (S_R = 1.40). The highest number of snail species recorded from terrestrial habitat belonged to Ariophantidae (*N* = 14; 48.24%) and Subulinidae (*N* = 8; 27.58%) families. Species richness under different families in two types of habitat is shown in Figure 3a. Ariophantidae family was represented by two species (*Khasila climacterica* and *Thebaldius* sp.(2), higher in undisturbed than in disturbed habitat (*N* = 12). Achatinidae, Streptaxidae, Bradybanidae and Camaenidae families were the lowest number of snails recorded from terrestrial habitat (*n* = 1; 3.44%).

Mann-Whitney test showed significant differ-

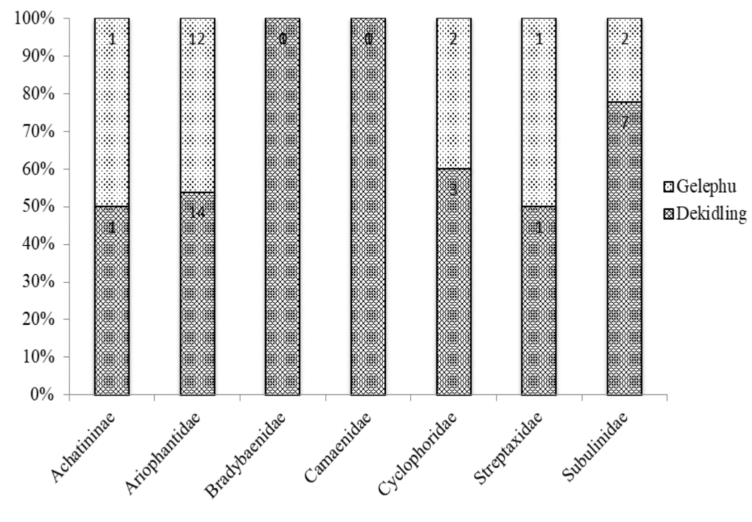

Figure 2: (a) Percentage of terrestrial and freshwater snail families (b) Percentage of species in undisturbed, disturbed and freshwater habitats

Table 2: Classification of collected snails under Dekidling and Gelephu gewog

Family	Subfamily	Species	Counts	
			Undisturbed	Disturbed
Terrestrial snails				
Ariophantidae	Achatininae	*Lissachatina fulica*	20	56
	Ariophantinae	*Ariophanta* sp. (dextral)	74	294
		Ariophanta sp. (sinistral)	5	6
	Durgellinae	*Sitala infula*	5	5
	Girasiinae	*Cryptaustenia ovata*	67	7
		Cryptaustenia bensoni	48	35
	Macrochlamydinae	*Khasiella climacterica*(u)	3	0
		Macrochlamys indica	39	44
		Macrochlamys sp. (1)	127	41
		Macrochlamys sp. (2)	14	40
		Macrochlamys sp. (3)	33	351
		Macrochlamys sp. (4)	12	9
		Oxytesta sp.	78	8
		Theobaldius deplanatus	15	3
		Theobaldius sp.	1	0
Bradybaenidae(u)	Bradybaeninae(u)	*Bradybaena radicicola*(u)	2	0
Camaenidae(u)	Camaeninae(u)	*Landouria huttoni*(u)	5	0
Cyclophoridae	Cyclophorinae	*Cyclophorus* sp. (1)	3	5
		Cyclophorus sp. (2)	15	8
	Pterocyclinae(u)	*Pterocyclus* sp.(u)	16	0
Streptaxidae	Enneinae	*Gulella bicolor*	1	1
Subulinidae	Subulininae	*Allopeas gracile*	5	3
		Allopeas clavulinum(u)	1	0
		Bacillum erosum(u)	1	0
		Glessula gemma	20	9
		Glessula sp. (2)(u)	1	0
		Glessula sp. (3)(u)	6	0
		Glessula sp. (4)(u)	1	0
		Glessula sp. (5)(d)	0	1
Freshwater snails				
Lymnaeidae		*Lymnaea acuminata*(d)		24
Pachychilidae		*Brotia costula*(d)		1,959
Paludomidae		*Paludomus conica*(d)		908
Thiaridae		*Melanoides tuberculata*(d)		20
		Tarebia lineata(d)		1
		Thiara scabra(d)		21

Note: (u): taxa only recorded in undisturbed habitat, (d): taxa only recorded in disturbed habitat ence in species richness between undisturbed and disturbed habitats (U = 701.00, Z = -2.23, p = .026, r = -0.237). Bonham *et al.* (2002) also reported similar result in species richness (higher) in natural forest and in plantation sites at Tasmania. However, this is not always true for all snail species as there are several native species that are higher in modified habitat of Mount Kenya (Tattersfield *et al.*, 2001).

Diversity

The Shannon-Weiner diversity index (H') of snail in the undisturbed, disturbed and freshwater habitats were H' = 2.61, H' = 2.15 and H' = 1.30 respectively. Overall, the terrestrial snail diversity of the study area was (H' = 3.03).

There was no significant difference in snail diversity between the undisturbed and disturbed habitats; (U = 757.00, Z = -1.76, p = .078, r = -0.18). Similarly, there was no significant difference in snail diversity among the undisturbed, disturbed and freshwater diversity ($H_{(2)}$ = 7.40, p = .25).

Table 3: Spearman's rho correlation coefficient between terrestrial snails and environmental variables

	H'	**E$_H$**	**S$_R$**	**Abundance**
Canopy	0.199	0.205	0.225	-0.053
S. cover	0.042	0.073	0.023	-0.012
Biopre	-0.028	-0.033	-0.086	-0.004
Aspect	-0.075	0	-0.042	0.176
Slope	-0.047	0.167	0.105	-0.123
Altitude	0.169	0.12	0.178	-0.057

Note: S. cover: Soil cover and Biopre: Biotic pressure

Table 4: Kendall's tau_b correlation between freshwater snails and environmental variables

	H'	**E$_H$**	**S$_R$**	**Abundance**
W. Temp	0.279	0.372	-0.093	0.316
Biopre	-0.4	-0.215	-0.277	-0.329
Veg. cover	0.704	0.503	0.235	0.651
pH	0.121	0.182	0.364	-0.059
Altitude	0.588	0.471	0.647	0.457

Note: W. temp: Water temperature, Biopre: Biotic pressure, Veg. cover: Vegetation cover

Species abundance and composition

A total of 1,544 terrestrial snail individuals (undisturbed = 618, disturbed = 926) and 2,933 freshwater snails from freshwater habitat were recorded. The most abundant freshwater snail species recorded was *Brotia costula* (n = 1959; 66.79%) followed by *Paludomus conica* (n = 908; 30.95%). Whereas, *Tarebia lineata* (n = 1; 0.03%) was the least recorded snail species.

The species richness recorded in the disturbed habitat was less than that of the undisturbed habitat. In contrast, the abundance of snail species was higher (19.95%) in disturbed habitat (disturbed = 59.97%; undisturbed = 40.03%). More recognisable indicator of disturbed habitat, the widespread *Lissachatina fulica* (Nurinsiyah *et al.*, 2016) was found abundant in disturbed habitat indicating the replacement of native species. Thus, the higher snail abundance with low species richness (small body-sized snails in undisturbed and large body-sized snails in disturbed) indicates disturbed area and the area could be in the verge of native species being replaced by exotic species.

The species relative abundance, however for undisturbed and disturbed habitat was not significant; (U = 904.00, Z = -.53, p = .593, r = -

0.05). Similarly, Kruskal-Wallis test ($H_{(2)}$ = .293, p =.834) showed no significant difference in relative abundance of snails among the undisturbed, disturbed and freshwater. Similarly, no significant difference among undisturbed, disturbed and freshwater snail relative abundance was indicated by Kruskal-Wallis test ($H_{(2)}$ = .293, p =.834) (figure 4.3). However, Giri and Singh (2013) and Dorji *et al.* (2014) reported that abundance of macroinvertebrate depends on degree of human disturbance.

Relationship between species richness and environmental variables

The species richness (S_R) was positively correlated with slope (r_s = .105, p = .335), altitude (r_s = .178, p = .097) and soil cover (r_s = .023, p = .832). However, S_R was negatively correlated with aspect (r_s = -.042, p = .702) and disturbances (r_s = -.086, p = .424) (Table 3).

As forest cover maintains the moisture and direct solar radiation to forest floor, it helps in gastropoda community composition (Ranckaet *al.*, 2015). Thus, there was significant positive correlation between the species richness and forest canopy (r_s = .225, p = .023). However, to some extent, species rishness relationship with forest cover depends on types of species (Chiba, 2007). The finding of this study indicates that the *Cryptaustenia bensoni*, *Glessula* spp. and *Theobaldius* sp. are depended on canopy cover whereas *Macrochlamys indica*, *Macrochlamys* sp. (1) and *Bradybaena radicicola* are not.

Kendall's tau_b correlation showed no significant correlation between the freshwater snail species richness and vegetation cover (τ = .235, p = .424) and pH (τ = .364, p = .647). As aquatic vegetation, particularly the deep rooted plants, help in shading the water (Hubendick, 1958); water temperature (τ = -.093, p = .766) and biotic pressure (τ = -.277, p = .328) were negatively correlated with the freshwater snail species richness.

Altitude is significantly correlated with species richness (τ = .647, p = .019) (Table 4). However, it is difficult to conclude with this study as all the freshwater plots were located in the plain area with less altitudinal range; 238 to 244 m asl.

Relationship between diversity and environmental variables

Among the environmental variables considered in the terrestrial snail study, altitude (r = .169, p = .116), forest canopy (r = .199, p = .062) and soil cover (r = .042, p = .697) showed positive correlation with diversity indices (*H'*) (Table 3). Similar results were reported by De Chavez *et al.* (2010) at Mt. Makiling in Laguna, Luzon.

Species diversity (*H'*) of the freshwater snails showed no significant correlation with water temperature (τ = .279, p = .372), vegetation cover (τ = .704, p = .372), pH (τ = .121, p = .662) and altitude (τ = .588, p = .032) using Kendall's tau_b correlation test (Table 4). Again, aspect (r_s = -.075, p = .492), slope (r_s = -.047, p = .666) and disturbances (r_s = -.028, p = .116) showed no significant correlation with terrestrial snail diversity. Biotic pressure (τ = -.400, p = .158) also showed no significant correlation for freshwater snail diversity. However, these factors can affect the diversity as well as abundances of both freshwater and terrestrial snails. Due to the shorter period of study and time for data collection, the association could not have been established well.

Relationship between species abundance and environmental variables

In the terrestrial habitat, only aspect showed no significant correlation with species abundance (r = .176, p = .102). Slope (r = -.123, p = .256), canopy (r = -.053, p = .625), soil cover (r = -.012, p = .911), disturbances (r = -.004, p = .968) and altitude (r = -.057, p = .596) also showed no significant correlation with species abundances. However, Labaune and Magnin (2002) found that the species density decreased with elevation even in short elevation gradients.

Species abundance of freshwater snails also showed no significant correlation with water temperature (τ = .316, p = .303), pH (τ = -.059, p = .831), altitude (τ = -.457, p = .092) and biotic pressure (τ = -.329, p = .239). While vegetation cover showed significant correlation (τ = .651, p = .024). As forest cover maintains the moisture and direct solar radiation to forest floor, it helps in gastropoda community composition (Rancka *et al*., 2015).

Distributional pattern and influence of environment variables

A Canonical Correspondence Analysis (CCA) showed that the distribution patterns of terrestrial snail species richness, evenness and diversity differed between undisturbed and disturbed habitat. The number of snail species decreased from undisturbed to disturbed habitat. There-

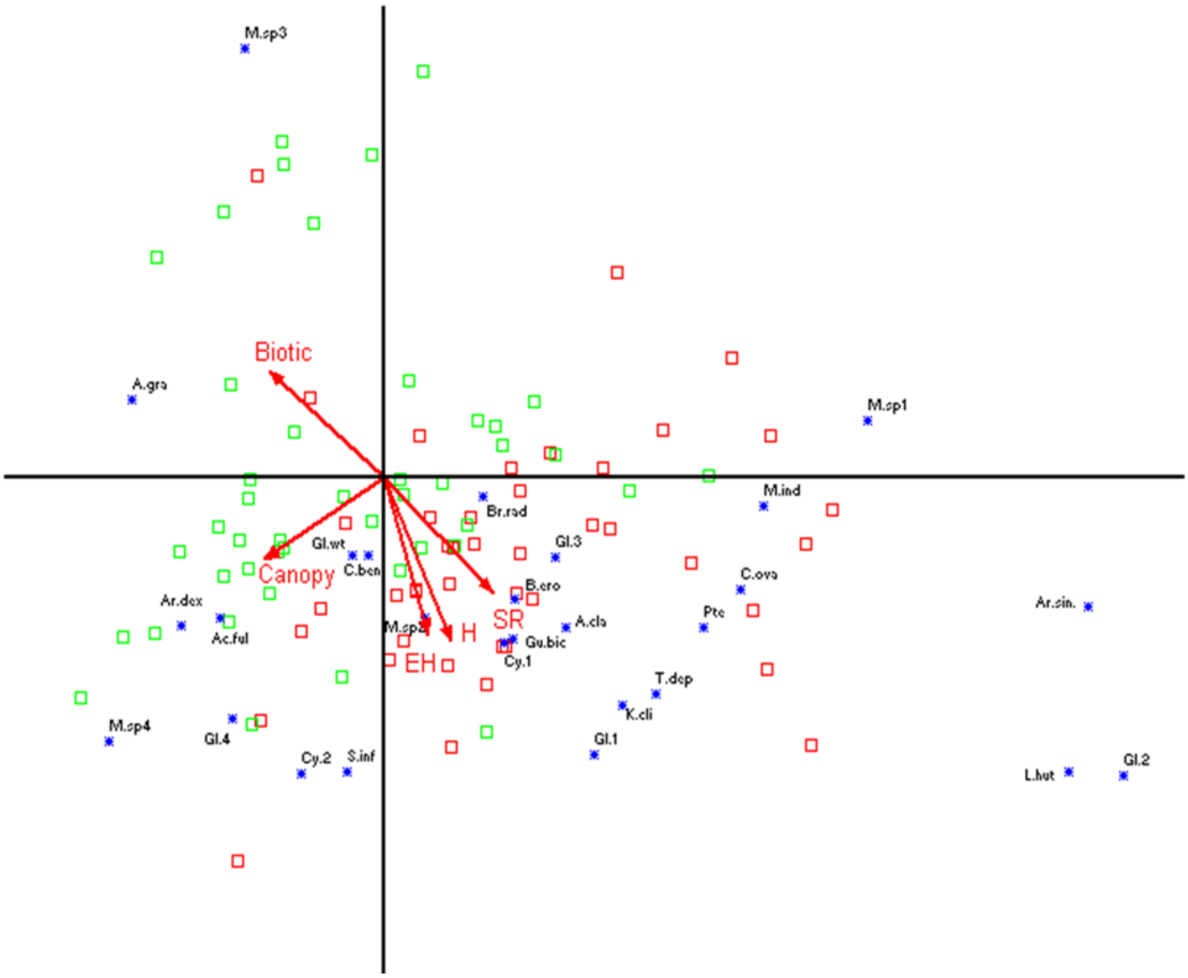

Figure 4: Ordination for distributional pattern and environmental response of terrestrial snail in undisturbed and disturbed habitat

Note: Canopy: canopy cover, Biotic: Biotic pressure/anthropogenic disturbances: Species code; A.gra: *Allopeas gracile*, A.cla: *Allopeas clavulinum*, Ar.dex: *Ariophanta* sp. (dex), Ar.sin: *Ariophanta* sp. (sinis), B.ero: *Bacillum erosum*, Br.rad: *Bradybaena radicicola*, C.ova: *Cryptaustenia ovata*, C.ben: *Cryptaustenia bensoni*, Cy.1: *Cyclophorus* sp. (1), Cy.2: *Cyclophorus* sp. (2), Ac.ful: *Lissachatina fulica*, Gl.wt: *Glessula gemma*, Gl.2: *Glessula* sp. (2) Gl.3: *Glessula* sp. (3), Gl.4: *Glessula* sp. (4), Gu.bic: *Gulella bicolor*, M.ind: *Macrochlamys indica*, K.cli: *Khasiella climacterica*, L.hut: *Landouria huttoni*, M.sp1: *Macrochlamys* sp. (1), M.sp2: *Macrochlamys* sp. (2), M.sp3: *Macrochlamys* sp. (3), M.sp4: *Macrochlamys* sp. (4), Oxy: *Oxytesta* sp. T.dep: *Theobaldius deplanatus*, Pte: *Pterocyclus* sp., S.inf: *Sitala infula* and T.sp: *Theobaldius* sp. (2)

fore, higher snail assemblages were observed in undisturbed habitats. Species diversity, richness and evenness were negatively correlated to the anthropogenic disturbances (r = -.541). However, canopy cover had no correlation with diversity, richness and evenness (Figure 4).

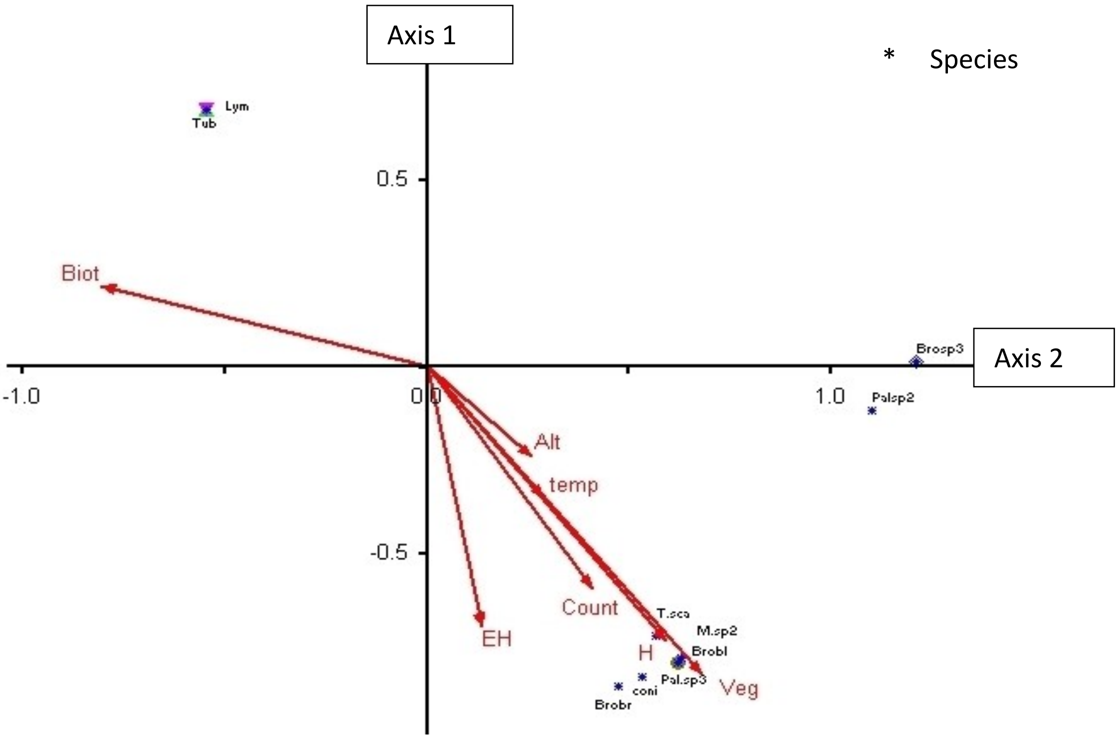

Figure 5: Non-metric multidimensional scaling ordination diagrams showing distributional pattern and environmental response of freshwater snail

Note: Veg: Vegetation cover, Biot: Biotic pressure/anthropogenic disturbances, Alt: Altitude, temp: Temperature: Species code: Lym: *Lymnaea acuminata*, Brobr: *Brotia costula* (brown), Brobl: *Brotia costula* (blcak), Brosp3: *Brotia costula* (ring), Coni: *Paludomus conica*, Palsp2: *Paludomus conica* (b), Pal.sp3: *Paludomus conica* (c), Tub: *Tarebia lineata*, M.sp2: *Melanoides tuberculata* and T.sca: *Thiara scabra*

Lymnaea acuminata and *Tarebia lineata* were positively associated with disturbances in the freshwater habitat. Vegetation cover (r = .806) was the most influential variable for the snail diversity and abundance (Figure 4). *Paludomus conica* (b) and *Brotia costula* (ring) do not have any correlation with environmental variables considered in this study.

Conclusions

The data collection on the distribution pattern and environmental response of snails were collected and analysed for 88 plots and 9 plots from terrestrial and freshwater habitat respectively in between December 2016 to January 2017 in Gelephu. A total of 35 snail species belonging to 11 families were recorded. There was significant difference in species richness in all the habitats (U = 701.00, Z = -2.23, p = .026, r = -0.237). However, no significant differences were recorded between species diversity (U = 757.00, Z = -1.76, p = .078, r = -0.18) and relative abundance (U = 904.00, Z = -.53, p = .593, r = -0.05) among the habitats. Species diversity, richness and evenness were negatively correlated to the anthropogenic disturbances (r = -.541). Vegetation cover (r = .806) was the most influential variable for the snail diversity and abundance.

This study was conducted in a short period of time and the relationship between snail composition and environmental variables were not completely studied. Therefore, there is a need for longer duration study in terrestrial as well as freshwater habitats. Invasive snail species and intermediate hosts were not included in this study. So, identification and classification of invasive and endemic snails with reference to intermediate hosts and their ecological roles can have greater impact on the conservation of species and management of habitats.

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