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PLATINUM OPEN ACCESS

Amphibian abnormalities and threats in pristine ecosystems in Sri Lanka

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Abstract: Amphibian abnormalities are caused by numerous etiologies prevailing in the environment. Since amphibians are good bio indicators of the environment, amphibian abnormalities are popularly known as a veritable ecological screening tool to assess ecosystem health. The present study was carried out encompassing within and outside the Horton Plains National Park areas, from January to November 2017. Distribution of amphibian morphological abnormalities were assessed in and around the five lentic water bodies through gross visual encounter. Six quadrates of 1m×2m were randomly placed in each sampling site. Frequency and composition of amphibian abnormalities were assessed in a total of 694 amphibians, belonging to four families and 11 species. Thereby, 4.5% and 80.87% abnormality indexes were accounted for respectively within and outside the park, comprehended surficial abnormalities, ectromelia and femoral projection abnormality types. Surficial abnormalities were the most predominant in both localities, generally occurring at the hind limb region of pre-mature stages of *Taruga eques*. Two lentic water bodies were identified as "abnormality hotspots" within and outside the Horton Plains National park; however, a multiplicity of possible combinations of potential causes of abnormalities were present in the environment. Hence, finding the exact causes of amphibian abnormalities are an extremely difficult exercise in the field.

Keywords: Abnormality, Horton Plains National Park, morphological, *Taruga eques.*

Abbreviations: DWC—Department of Wildlife Conservation | GPS—Global Positioning System | HPNP—Horton Plains National Park | IUCN—International Union for Conservation of Nature | OHPNP—Outside the Horton Plains National Park | RH—Relative Humidity | T amb—Ambient temperature | Tw—Water temperature.

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Author contribution: All authors contributed equally. GKVPTS was the main researcher on this research project, field sampling, data collection, data analysis and preparation of the paper. WADM was the supervisor of this piece of research and provided immense guidance and support from the initiation of the project until the completion, with her valuable experience and knowledge. AdS provided some important relevant literature and valuable suggestions with his expertise in field studies, which contributed a lot to improve parts in the methodology section and to prepare the manuscript.

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Wildlife Circle
University of Sri Jayewardenepura

INTRODUCTION

"Abnormality" refers to "any deviation from normal morphology, independent of whether its origin was developmental or acquired after proper development" (Lunde & Johnson 2012). Both amphibian malformations and deformities are included in amphibian abnormalities (Reeves et al. 2008). Amphibian abnormalities can be classified mainly as surficial abnormalities (infectious diseases/cysts and wounds), skeletal abnormalities and eye abnormalities (Linder 2003; Reeves et al. 2008). Amphibian abnormalities have interconnected with many factors including, chemical contaminants (Bridges et al. 2004; Lunde & Johnson 2012), trematode, cestode, and nematode parasites (Ankley et al. 2004; Imasuen & Ozemoka 2012), predators (Johnson et al. 2006; Johnson & Bowerman 2010) and UV (Ultraviolet) radiation (Blaustein & Johnson 2003; Lannoo 2008).

Anthropogenic activities have been recognized as a main element for the modification of aquatic habitats (Johnson & Chase 2004). Especially nutrient loading in freshwater ecosystems (particularly ponds) directs to the acceleration of eutrophication that result in shifting the community composition. This results in various amphibian abnormalities.

Helminth infections are considered as a major governing factor for existing amphibian abnormalities and linked with numerous factors. Most of the trematode infections in amphibians are recorded from the habitats associated with agriculture and cattle farms. Trematodes in the genus *Ribeiroria* occur in lentic aquatic water bodies (Johnson et al. 2004). Aquatic snails in lentic aquatic water bodies serve as intermediate host for *Ribeiroria* parasite. When high nutrient levels are present the snail densities increase exponentially. Free swimming cercariae emerge from infected snails then penetrate and encyst as metacercariae in the second intermediate host (Jayawardena et al. 2010b) often around the developing limb buds of amphibian larvae, leading to improper limb development (Blaustein & Johnson 2003). In addition to that frequent exposure of amphibians to chemical contaminants accumulated in lentic water bodies leads to a reduction of immunity strength. As a result of that amphibians become highly susceptible to parasitic infections (Kiesecker 2002; Budischak & Belden 2009; Lunde & Johnson 2012). Cestodes are one of the major parasite groups which infect the amphibians. Cestodes commonly occur within the musculature of the body and in the hind limbs of adult and juvenile frogs (Gillilland & Muzzall 2002) and a number of metacercariae of helminthes has been recorded in the leg musculature of deformed amphibians (Gillilland & Muzzall 1999).

There is a considerable effect on anuran abnormalities from predators existing in natural environments (Johnson & Bowerman 2010). Most of these amphibian abnormalities are caused by aquatic predators such as dragonfly larvae (Bowerman & Johnson 2010; Johnson & Bowerman 2010), small fishes, crabs, crayfishes (Johnson et al. 2001a, 2006), diving beetles, predatory odonate nymphs and water scorpions (Ballengee & Sessions 2009). Smaller aquatic predators (insect larvae, small fishes) attack the exposed portions of larval and metamorphosis stages of anurans such as the tail or limbs (Bowerman & Johnson 2010). A traumatic loss of an entire limb (Lannoo 2008), however, and wounds of amphibians can be seen after the metamorphosis which are produced by the attack of large vertebrate predators (Bowerman & Johnson 2010).

Leech attack causes some of the abnormalities of amphibians and many abnormality studies have proved that leech attacks cause a high prevalence of missing limbs (ectromelia) or parts of the limbs (Johnson et al. 2001a, 2002, 2006; Ballengee & Sessions 2009; Bowerman & Johnson 2010).

Considering worldwide abnormality studies it can be seen that most of them have been carried out as laboratory studies, related with chemical inductions (Burkhart et al. 1998; Lajmanovich et al. 2003) and parasitic inductions (Johnson et al. 2001a; Stopper et al. 2002) to frog embryos and tadpoles in various limb bud stages. Only a few studies, however, have been conducted to investigate abnormalities of amphibians in the field (Johnson et al. 2001b; Peltzer et al. 2011). As regards the fact that Sri Lanka is a biodiversity hotspot, only four studies have been mentioned (Rajakaruna & Samarawickrama 2007; de Silva 2009, 2011; Meegaskumbura et al. 2011). Though, abnormality surveys were based on selected regions of the country, it's more valuable to extract data from pristine ecosystems with the purpose of identifying the actual threats of amphibian's survival. Therefore, the present research was undertaken to assess the amphibian abnormalities and their possible predators which cause amphibian abnormalities encircling the area in and around the lentic water bodies within and outside the World Heritage Horton Plains National Park as a comparison of threats and abnormality types possessed by amphibians against two different localities to ultimately fulfill the knowledge gap of field studies pertinent to amphibian abnormalities.

MATERIALS AND METHODS

a) Study site

The study was conducted within and outside the Horton Plains National Park (HPNP) from January to November 2017. HPNP is located between 6.802 northern latitudes and 80.807 eastern longitudes (Green 1990) which occupies an area of 3,160ha and is contiguous with the Peak Wilderness Sanctuary to the west. It is in the eastern extremity of the Nuwara Eliya District in Sri Lanka within the range of 2,100-2,300 m elevation (DWC 2007). Tropical montane cloud forests and wet pathana grasslands are the two distinct habitats in the park (Gunatilleke & Gunatilleke 1990) with a narrow ecotone belt of shrubs and herbs between the two.

Most of the lentic water bodies are surrounded by three main grass types (*Chrysopogon nodulibarbis, Andropogon polyptychos*, and *Garnotia exaristata*) and one bamboo species (*Arundinaria densifolia*) in the grassland habitat (DWC 2007). The sampling was conducted in selected five lentic water bodies on four days per month, providing equal effort to each sampling site. Three lentic water bodies (A, B and C) were selected within the HPNP and two (D and E) were selected outside the HPNP based on the availability of amphibians (Figure 1A). Sampling sites, outside the HPNP were located within a range of 1,170.9–1,864.7 m elevation and mainly associated with forested area. GPS (Global positioning system) points of these sampling sites were recorded using Garmin etrex Euro hand held GPS receiver.

Six quadrates each 1 x 2 m were placed within each sampling site for the sampling of amphibians. Three quadrates were randomly placed in the area 1m from the pond bank outside the pond and three quadrates were randomly placed inside the pond in the area of 1m from the pond bank (Faruk et al. 2013). Anuran species (larvae, metamorphs, juveniles, and adults) and leeches in these quadrates were surveyed from morning to after noon (08.00–16.00 h) and during the night (18.00–20.00 h). Moreover, vegetation within each plot was searched even when slight movement was detected. Head lamps and torches were used for nocturnal searches. Larval amphibians were captured using active sweeps (Dodd 2010), metamorphic anurans were captured with a dip net or by hand (Ouellet et al. 1997) and adult amphibians were captured with a dip net or by hand (Wheeler & Whelsh 2008; Urbina & Jenny 2009). A small amount of water was added to the container to prevent overheating and desiccation (Wheeler & Whelsh 2008). Overcrowding based on the container size and Tamb was avoided (Lunde & Johnson 2012). At the end

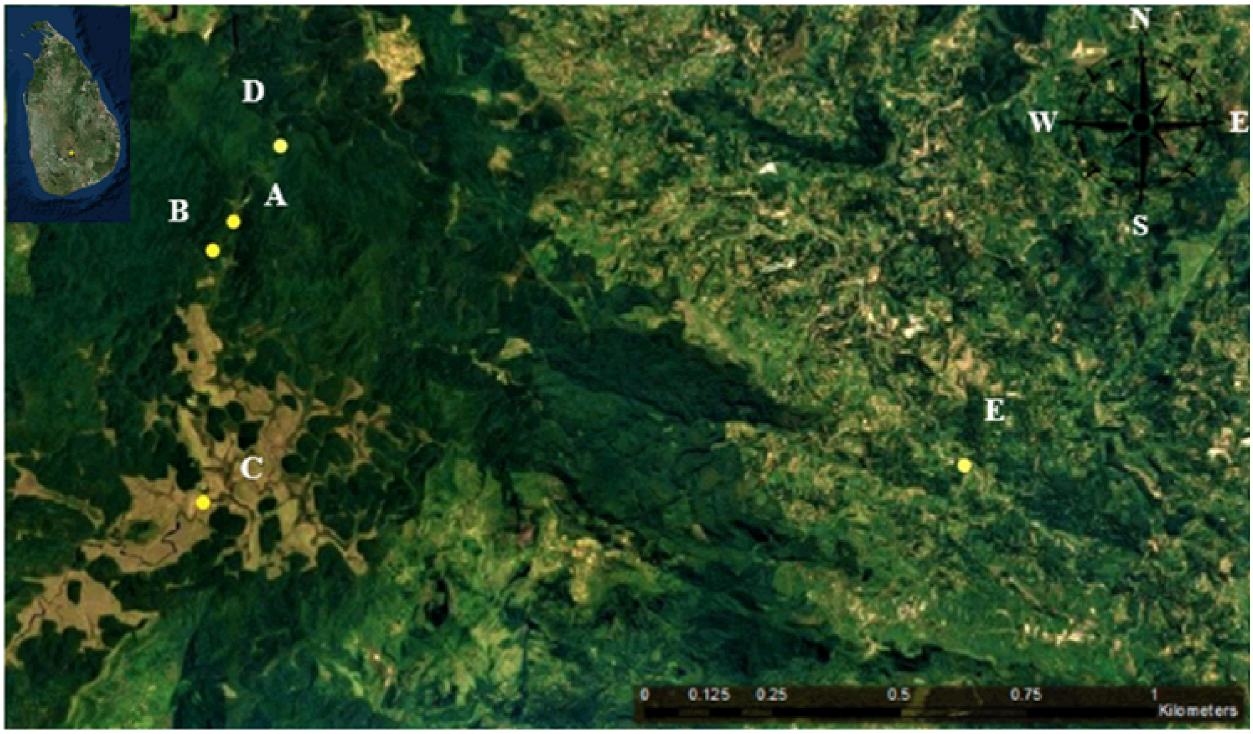

Figure 1. (A). Locations of sampling sites within and outside the Horton Plains National Park. [Lentic water bodies within the HPNP]. A (6.839^{0}N and 80.812^{0}E), B (6.834^{0}N and 80.809^{0}E), C (6.801^{0}N and 80.807^{0}E), D (6.850^{0}N and 80.818^{0}E), E (6.805^{0}N and 80.908^{0}E).

of the survey they were released back to their habitat after removing the ectoparasites from the attached skin surface of the amphibians. Amphibian species and their stages were identified using the amphibian guides of Dutta & Manamendra-Arachchi (1996), Manamendra-Arachchi & Pethiyagoda (2006), and de Silva (2007). When an amphibian was captured, (a) it was identified to species level, (b) life stage noted, (c) presence or absence of the abnormalities assessed (in larvae, metamorphs, juvenile and adult stages) (If abnormalities were present, they were recorded with the count, according to the abnormality classifications of guides) and recorded.

b) Identification of amphibian abnormalities

Amphibian physical abnormalities (assessed the abnormalities which appeared externally) were associated with the loss of body parts or organs. External abnormalities were identified through gross visual inspections (both dorsal and ventral sides of the amphibian body) and classified based on Meteyer (2000), Johnson et al. (2001b), Johnson et al. (2002), Lannoo (2008), Rajakaruna et al. (2008), Reeves et al. (2008), Johnson & Hartson (2009), and Peltzer et al. (2011) guides. Surficial abnormalities (cysts) were identified by careful examination of the external body surface (dorsal side, ventral side, around the fore limbs and hind limbs) and using the external morphological features of the infection. Cysts of infected amphibians are found as clear or brownish colour (Johnson et al. 2004), round, swelling nodules of the musculature (Ostler 2004). Moreover, Johnson et al. (2004), Ostler (2004), de Silva (2007, 2009), and Jayawardena et al. (2010a, b) were referred to for the identification of cysts. Based on the number of cysts (surficial), severity of amphibian infections were classified as mild and moderate. Mild infectious amphibians possess 1–3 nodules and moderate infectious amphibians possess 4–6 nodules (de Silva 2009).

c) Identification and sampling of possible predators of amphibians

Leeches were identified by careful examination of the external body surface of the anuran larvae, metamorphs, juveniles, adults and also the grasses and shrubs which were adjacent to the bank of the water body. Possible aquatic invertebrate predators of the amphibian life stages (larvae, metamorphs, juveniles and adult) were sampled by using the 0.36 × 0.22 m dip net, performing 2m sweeps at 11 evenly spaced points around each pond's perimeter (Bowerman & Johnson 2010). Captured aquatic predatory varieties were identified, counted, recorded and released to the same lentic water body. Amphibian invertebrate predators in the ponds were classified as either abundant (<100 individuals trap per day) or rare (<10 individuals trap per day) based on density capture per day according to Bowerman & Johnson (2010). Possible large vertebrate predators (aquatic birds) of amphibians were assessed based on observations in and around the lentic water bodies.

Aquatic invertebrate predators of amphibians were identified based on the morphological characteristics (nature of the abdomen, presence of exoskeleton and arrangement of gills) with the help of Curtis (2011) and Quek et al. (2014). Possible large vertebrate predators (aquatic birds) of amphibians were identified (colouration of feathers and beak and foot type) using Harrison (2011), a bird guide. All types of abnormalities, aquatic invertebrates and large vertebrate predators as possible threats of amphibians (which cause amphibian abnormalities) were photographed using a digital camera.

d) Determination of the environmental variables

Environmental variables of lentic water bodies were measured to find out the habitat suitability for amphibians (Hamer & Lane 2002; Urbina & Jenny 2009; Dodd 2010; Sparling 2010). The atmospheric data temperature (ºC) and relative humidity (RH) were assessed. Ambient temperature (Tamb) was recorded 2m above the water surface / ground (using Kestrel 4000 weather meter, USA) and relative humidity (RH) data were recorded 2m above the ground. In addition to that soil pH values were obtained using Kelwey soil tester, under the soil chemical data. Furthermore, dissolved oxygen (DO) (YSI 550A Dissolved Oxygen Instrument), water temperature (Tw), pH (YSI Eco Sense pH 100A meter), and conductivity (YSI Eco Sense EC300A Conductivity meter) were measured using analysis of water physiochemical data. Soil chemical data and water physiochemical data were recorded once a month in each plot (as mentioned above) using a standardized data sheet.

e) Data analysis

The Minitab version 17.0 statistical software was used to calculate the mean and standard deviation (SD) values of environmental variables and possible predators of amphibians in each sampling site. Graphical representations were created using Microsoft Excel 2013 software. Abnormality index (AI) was calculated using the equation,

AI = (Total number of abnormal amphibians / Total number of amphibians inspected) × 100%

RESULTS

a) Abundance of amphibian species

A total of 694 amphibians belonging to four families and 11 species were recorded during the study in the five lentic water bodies studied. Five-hundred-and-eleven individuals were examined inside the HPNP and 183 individuals were examined outside the HPNP (Table 1).

b) Amphibian abnormalities

Out of the 511 amphibians examined inside the HPNP, only 23 (4.50%) had abnormalities; however, 80.87% of amphibians outside the HPNP, possessed abnormalities. *Taruga eques* had the most number of abnormalities in both localities—22 inside the HPNP and 147 outside the HPNP. This was 99.30% of AI outside the HPNP and 95.70% of AI inside the HPNP with respect to *T. eques*. Surficial abnormalities (Image 1) were more dominant than ectromelia and femoral projection abnormality types in both localities, thereby mild infections (60.00%) (Image 1A and 2B) and moderate infections (77.55%) (Image 1C) were predominant respectively within and outside the HPNP (Figure 2A). Cysts were recorded only in *T. eques*, accounted for 5.76% within the HPNP and 100.00% outside the HPNP (Figure 2B). Ectromelia was recorded in both *T. eques* (Image 2A) (0.58%) and *M. greenii* (Image 2B) (0.74%) species. Femoral projection was found only in *F.* cf. *limnocharis* (Image 2C) which accounted for 5.56% frequency of the abnormalities. Comparing the abnormality index with amphibian life stages (Figure 2C), no abnormal larvae were found in both localities. Abnormal metamorphs were found only inside the HPNP which accounted for 4.35% of the abnormalities. A high AI, however, was observed in the juvenile stage than in adults in both localities—56.52% and 77.70% within and outside the HPNP. Moreover, most of the abnormalities were found at the hind limb region of amphibians (Figure 2D). When comparing the AI of amphibians in each sampling site, no abnormal amphibians were recorded in pond C. Amphibians with ectromelia were only recorded within the HPNP in sampling site B; however, 1.16%, 14.39%, 100%, and 2.78% abnormality percentages were recorded in A, B, D, and E ponds, respectively (Table 2). With reference to national conservation states (determined by IUCN), 94.71% of the amphibians found in the HPNP were endangered, thereby 4.55% had abnormalities, whereas in contrast 100% of the amphibians were abnormal outside the HPNP.

Table 1. Amphibian abundance in and around the lentic water bodies within and outside the Horton Plains National Park.

Locality type	Family & Species	Abundance	Abnormality Index (%)
HPNP	**Dicroglossidae**		
	Minervarya greenii	135	0.74
	Microhylidae		
	Microhyla zeylanica	11	00
	Microhylidae		
	Uperodon palmatus	03	00
	Rhacophoridae		
	Pseudophilautus alto	02	00
	Pseudophilautus frankenbergi	01	00
	Pseudophilautus microtypanum	12	00
	Taruga eques	347	6.34
OHPNP	**Bufonidae**		
	Duttaphrynus melanostictus	06	00
	Dicroglossidae		
	Euphlyctis cyanophlyctis	04	00
	Minervarya kirtisinghei	08	00
	Fejervarya cf. *limnocharis*	18	5.55
	Rhacophoridae		
	Taruga eques	147	100

c) Comparison of amphibian predatory density in each sampling site

Damselfly larvae *Elattoneura leucostigma* were the most abundant possible predatory type of the amphibians. Damselfly larvae (Image 3A) were recorded in A, B and C sampling sites within the HPNP. Water scorpions (Image 3C), dragonfly larvae *Orthetrum glaucum* (Image 3B), Pond Herons *Ardeola grayii* (Image 3E) were recorded only in pond A. Leeches (Image 3D) were recorded in A and B sampling sites. Crabs *Perbrinckia glabra* (Image 3D) were only found in sampling site C. No possible predators of amphibians were recorded in pond E. Aquatic beetles were only recorded in pond D (Table 3).

d) Environmental variables of sampling sites

Highest conductivity was recorded in sampling site E. It may due to the fact that the water was contaminated with agro-chemicals. Moreover, D lentic water body recorded the least water pH value which indicates acidic conditions and that more anthropogenic stressors are present in the water body (Table 4).

DISCUSSION

Both amphibian malformations and deformities are included in amphibian abnormalities (Reeves et al. 2008). Some of the abnormalities are external physical abnormalities and others are internal (Blaustein & Johnson 2003; Spolyarich et al. 2011). The present study did not find any abnormalities in the family Bufonidae coinciding with the studies of Lannoo (2008) who

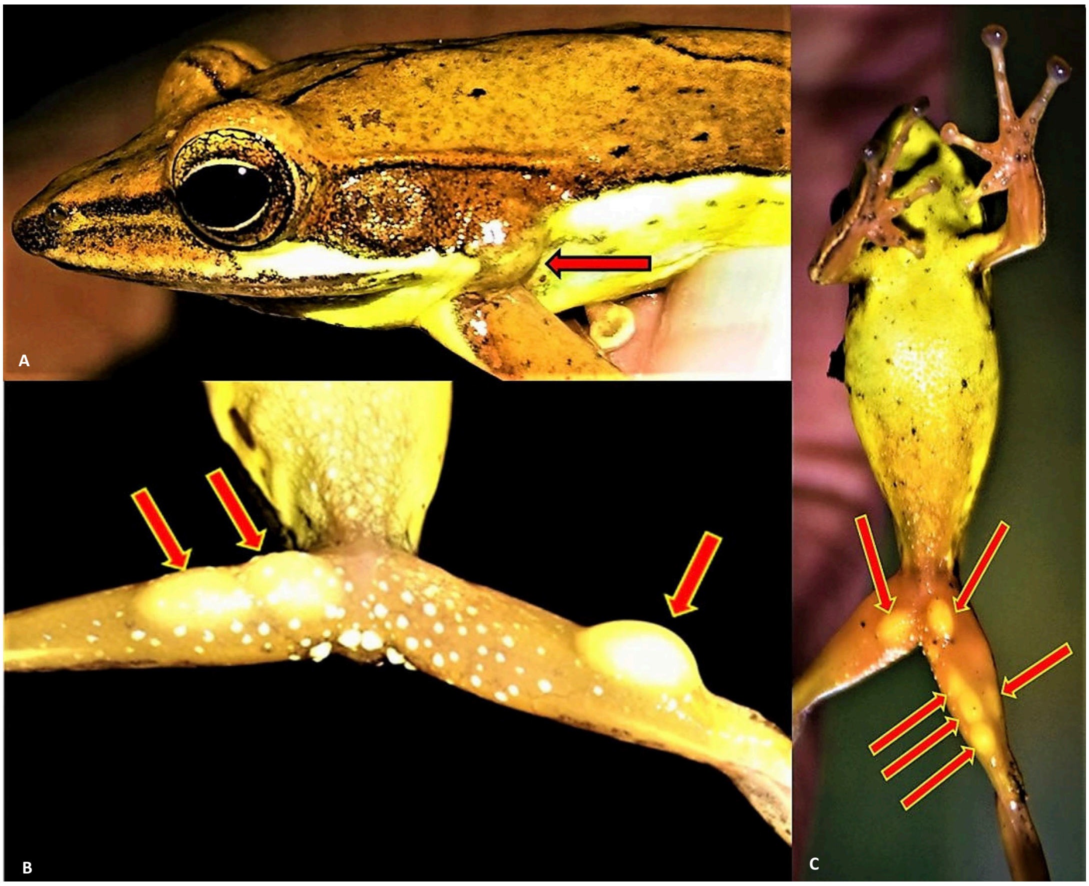

Image 1. A and B—mild infection: infected *Taruga eques* amphibians with clear colored 1–3 cysts (surficial abnormalities) as round swelling nodules of the musculature and subcutaneous tissue of the skin | C—moderate infection- Infected *Taruga eques* amphibians with 4–6 cysts. © G.K.V.P.T. Silva.

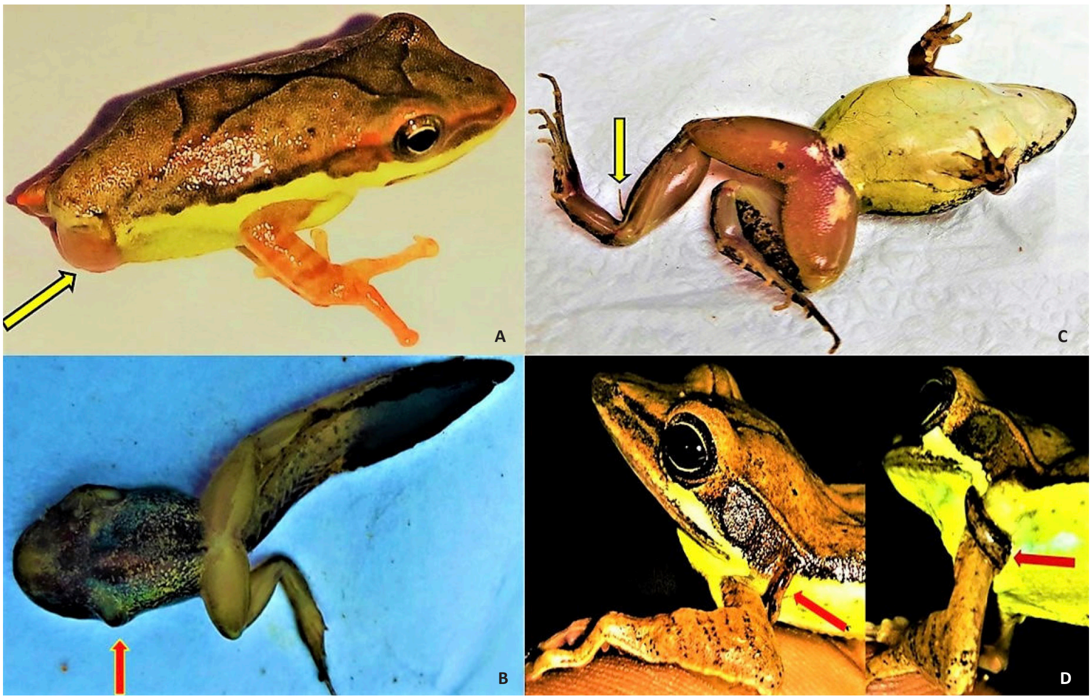

Image 2. A—Ectromelia in *Taruga eques* | B—Ectromelia in *Minervarya greenii* metamorph | C—femoral projection abnormality in *Fejervarya* cf. *limnocharis* | D—leeches attached to the external body surface of *Taruga eques.* © G.K.V.P.T. Silva.

Table 2. Different abnormality types and abnormality indices recorded in each sampling site.

Locality type	Sampling site	Ectromelia	Infections	Femoral projections	Abnormality index (%)
HPNP	A	-	4	-	01.16
	B	3	16	-	14.39
	C	-	-	-	00.00
OHPNP	D	-	147	-	100.00
	E	-	-	1	02.78

Table 3. Comparison of density of amphibian possible predators in each sampling site number of predators trapped/observed per day.

Locality type					
	HPNP		OHPNP		
Predator	A	B	C	D	E
Aquatic beetles	1.89 ±0.93	-	-	-	-
Crustaceans	-	1.44±1.01	-	-	-
Damselfly larvae	1 .78±1.92	104.33±31.89	1.22±1.09	-	-
Dragonfly larvae	11.56±6.73	-	-	-	-
Leeches	01.56±2.01	5.44±2.74			
Pond herons	02±0.87	-	-	-	-
Water scorpions	1 .56±1.01	-	-	-	-
Aquatic beetles	-	-	-	01±1.12	-

Table 4. Environmental variables in and around the lentic water bodies in each sampling site.

Site	Environmental variables (Mean ± SD)						
	T_{amb} (˚C)	RH	Soil pH	DO (mg/L)	T_w (˚C)	Water pH	Conductivity (μS/cm)
A	16.29 ± 0.30	94.11±2.57	5.90±0.38	4.67±0.19	15.73±0.53	8.14±0.68	18.56±5.68
B	16.15±0.58	95.05±2.06	5.91±0.50	4.55±0.26	15.75±0.45	7.93±0.62	17.07±5.79
C	15.88±0.32	94.50±2.77	5.88±0.45	4.65±0.23	16.01±0.66	8.30±0.66	20.95±7.13
D	16.80±0.67	95.03±2.66	4.49±0.18	4.52±0.16	16.21±0.77	6.89±0.90	18.94±4.03
E	15.41±0.89	91.95±8.14	5.15±0.45	4.66±0.19	16.99±0.38	6.95±0.93	25.65±4.50

observed that the Bufonidae family is less susceptible to abnormalities.

Previous studies have indicated that less than 5% abnormality prevalence of the population in a particular area or site is normal (Johnson et al. 2002; Kiesecker et al. 2004; Piha & Pekkonen 2006; Lunde & Johnson 2012). The abnormality prevalence within the HPNP was 4.50%, which is within the accepted range; however, abnormality prevalence outside the HPNP was 86.55% and should be considered as abnormally high (Piha & Pekkonen 2006). Lentic body wise, pond B inside the HPNP had 14.5% abnormality prevalence and pond D had abnormality prevalence of 100%. These high abnormality prevalent sites were classified as ''hotspots'' with respect to abnormalities (Johnson & Bowerman 2010). B lentic water body was located within the pristine ecosystem of HPNP. Moreover, all the water quality parameters were also within the standard levels for amphibian survival. Therefore, further experiments must be carried out to find out the exact cause for abnormalities in B water body. In contrast, D lentic water body was located on the way to HPNP and most visitors use it as a garbage dumping site (which may be the reason for reporting less DO and soil pH values). As a result of that, this high nutrient content of the water body provides a better environment to increase the parasitic density. It might be the major reason for recording 100% of surficial abnormalities in D lentic water body. Since, observed

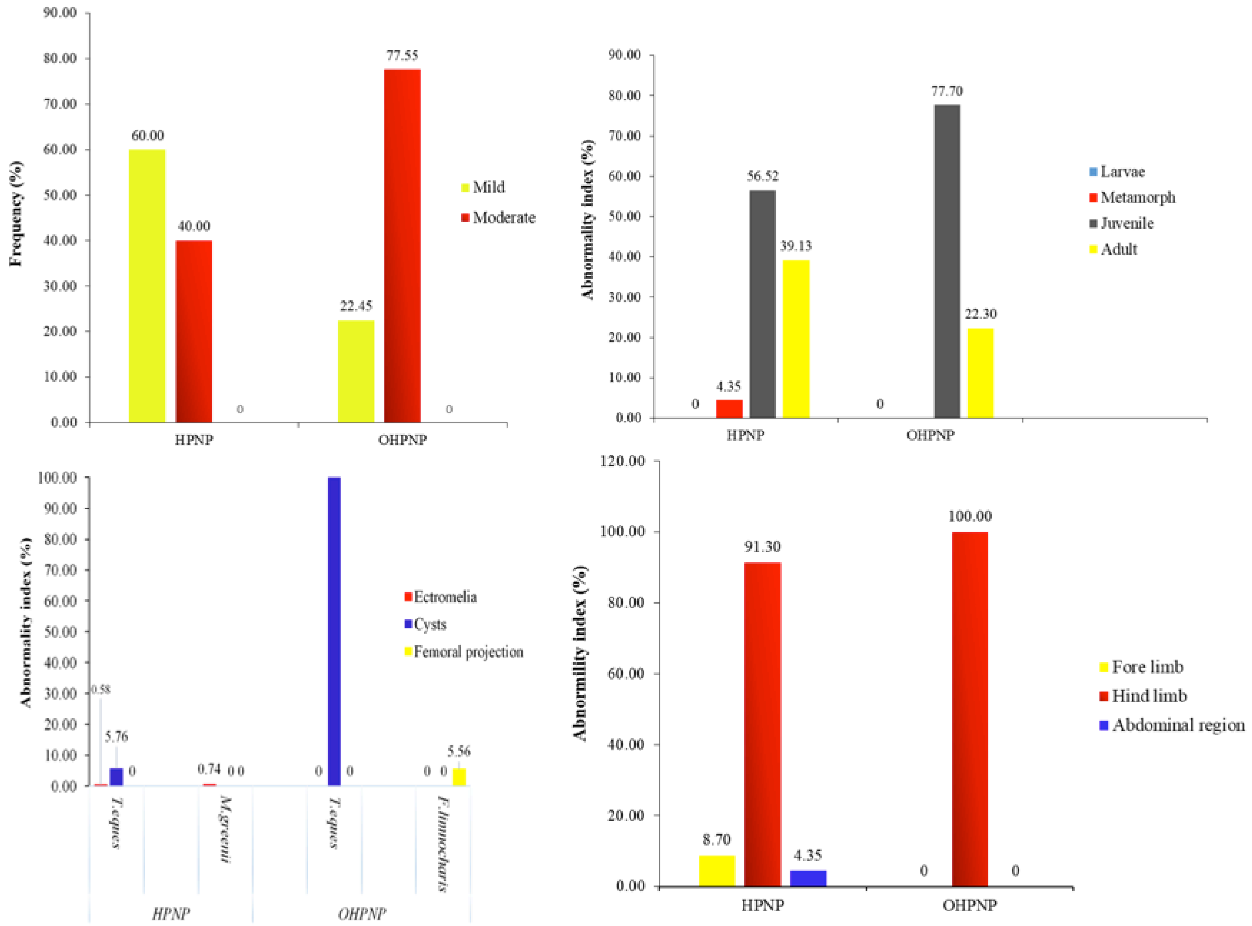

Figure 5. A—comparison of the infection severity within and outside the Horton Plains National Park (HPNP) | B—comparison of abnormality types recorded in different amphibian species found within and outside the HPNP | C—comparison of Percentages of abnormality indices recorded from different amphibian life stages within and outside the HPNP | D—comparison of abnormalities based on the body region where the abnormalities were recorded.

abnormality prevalence of B and D lentic water bodies were greater than the expected baseline range, further investigations are warranted to find out the locality specific causes (Lunde & Johnson 2012).

Highest number of amphibian abnormalities were reported in pre-matured stages of amphibians which may for the reason that either adult populations of amphibians are less susceptible for abnormalities against different environmental stressors or they have reduced survivorship. The survival of abnormal amphibians declines due to a high predation pressure and inability to capture its prey (Lunde & Johnson 2012). Since abnormal adult amphibians are unable to survive long periods of time in the environment, frequently a smaller number of adult amphibian populations are discovered with abnormalities (Goodman & Johnson 2011; Lunde & Johnson, 2012). Previous abnormality studies have also observed that most of the amphibian abnormalities are associated with hind limbs (Ouellet et al. 1997; Johnson et al. 2002; Piha & Pekkonen 2006), which may be resulted by the attack of natural pradators when they try to avoid the escape of amphibians. Results of the present study also tallies with these findings as most of the abnormalities found as cysts arise within the musculature found in the hind limbs.

Surficial abnormalities (cysts) were the predominant abnormality type recorded in this study. Even the HPNP comprises optimal conditions for growth of *Batrachochytrium dendrobatidis*, in the most observed cysts may be caused by parasitic helminths infected after limb bud stages of amphibians since they were located within musculature, mostly around limb structures and were easily visible to the naked eye as swelling round nodules; in contrast pathogenic cysts are microscopic and confined only to the epidermis (Lunde & Johnson 2012). Lunde & Johnson (2012) also recorded that the high number of amphibians inhabiting lentic water bodies are commonly infected with many trematode species that

Image 3. Possible predators of amphibians which cause amphibian abnormalities. A—Damselfly larva | B—Dragonfly larva | C—Water scorpion | D—Crustacean (crab) | E—Pond Heron. © G.K.V.P.T. Silva.

form cysts under the skin within musculature.

Amphibian abnormalities due to trematode infections depend on the stage of limb development at which infections occurred (Schotthoefer et al. 2003). Therefore, timing of the infection is a critical determinant in forming abnormalities (Jayawardena et al. 2010b). Infection coincides at pre-limb bud developmental stage of the tadpoles which results in high mortality rate with axial abnormalities. Trematode infections acquired at limb bud stage also produces high abnormality rate including ectromelia (Schotthoefer et al. 2003; Jayawardena et al. 2010b). Further more, there is no any effect to limb development and survival of tadpoles when they are infected at the paddle stage (Schothoefer et al. 2003; Jayawardena et al. 2010b). However after the infection at the paddle stage of amphibians, encysted parasites are able to remain viable even at the adult stage of amphibians (Imasuen & Ozemoka 2012). This was also observed in the amphibians in the present study. Existence of amphibian predators in ponds generally increase the trematode infection of amphibians (Thiemann & Wassersug 2000; Lunde & Johnson, 2012). Trematodes commonly occur in the lentic water bodies with dragonfly larvae (Bowerman & Johnson 2010; Lunde & Johnson, 2012). These two factors may have contributed to amphibian abnormalities in the lentic water bodies that were studied.

All the amphibian abnormalities cannot be explained away as parasitic infections. Lannoo (2008) and Lunde & Johnson (2012) observed that high level of ectromelia are present in frogs even when parasites are absent. In addition to parasites, amphibian predators (vertebrates and invertebrates) play a major role in limb abnormalities in amphibians (Bowerman & Johnson 2010). Larval amphibians are attacked by small predators including aquatic invertebrates; however, traumatic loss and injuries in limbs of amphibians (after metamorphosis) are mostly caused as a result of failed predatory attempts by large vertebrate predators (Bowerman & Johnson 2010). Bowerman & Johnson (2010) observed a direct relationship between the leech density and the abnormality level in field studies and laboratory induction of leeches. Leeches act as predators at amphibian larval stages and act as parasites of both larval and adult stages (Schalk & Forbes 2002; McCallum et al. 2011). Damselfly larvae was the abundant possible predators recorded in site B and all the ectromelia were observed in the same pond. Therefore, there is a high possibility of these predators causing the ectromelia observed in site B.

Water quality values of all sampling sites were included to the estimated optimum ranges (Sparling 2010) for amphibian growth and development; however, with respect to soil pH values, even moderate acidic condition is identical to HPNP (Chandrajith et al. 2009), strongly acidic conditions can't be expected within the sampling sites outside the HPNP, which indicate that both sampling sites outside the HPNP may have exposed to some anthropogenic activities.

Multiplicity of possible combinations of potential causes of abnormalities are present in the environment. Hence, finding the exact causes of amphibian abnormalities are an extremely difficult exercise in the field. The fact that abnormalities are linked with ecology, epidemiology, and developmental biology further increases the complexity of this problem.

CONCLUSIONS

Taruga eques was the most susceptible amphibian species to abnormalities irrespective of the locality type, indicated that amphibians may have been exposed to some stress conditions linked with multiple factors. Present research findings revealed that toads are less susceptible to amphibian abnormalities. Since, B and

D lentic water bodies were discovered as abnormality hotspots, further experiments are crucial at those particular lentic water bodies, for determining the site-specific cause for amphibian abnormalities. The present study clearly discloses further that Sri Lankan tree frogs are in a critical state. Since *Taruga eques* is confined only to the central hill regions of the country with declining population and all the amphibians which belong to endangered conservation state had abnormalities outside the HPNP, it is clear that mandating urgent measures to carryout extensive research-based conservation work before including it to critically endangered state is needed.

The present research data reveals that potential predators of amphibians are provided with excellent environmental conditions for their existence within the HPNP as a protected area in contrast to areas outside the HPNP. The present research, however, suggests that exigency of extensive research-based studies for the identification of complex causes for abnormalities in amphibians integrated with many disciplines, including ecology, toxicology, parasitology, and developmental biology. These studies will be important not only as ecological or evolutionary influences, but as significant implications for conservation and contemporary concerns over widespread amphibian population losses.

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