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International Journal of Zoology Studies
ISSN: 2455-7269
Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 3; Issue 5; September 2018; Page No. 05-11

Habitat preference of endangered frog *Fejervarya greenii* (Amphibia: Dicroglossidae) in tropical montane cloud forests of Sri Lanka

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Abstract
The present study was conducted in tropical montane cloud forests of Horton Plains National Park in Sri Lanka, from January 2016 to December 2016, focusing on *Fejervarya greenii* which is an endemic, endangered and a rare species, in order to study its habitat preference in and around lentic and lotic water bodies with the purpose of providing information to support its conservation and management. The sampling process was taken place in randomly placed plots in and around five lentic water bodies and five lotic water bodies for three consecutive days per month throughout the year. Carrying out *F. greenii* census and obtaining information for habitat variables in both macro and micro level were based on these pots. For the determination of preferable microhabitats of *F. greenii* twenty 1m^2 quadrats were placed in occupied plots. The correlation between microhabitat variables and presence of *F. greenii* was analysed. Presence of *F. greenii* was recorded only in the area of 1m to the water and 3m to the terrestrial habitats, which confirms the semiaquatic status of *F. greenii* and suggests *F. greenii* as a habitat specialist preferring in and the immediate surroundings of lentic water bodies. Microhabitat variables such as water depth, water temperature, submerged plant cover, decaying plant matter and leaf litter, sand, substrate temperature, relative humidity and substrate relative moisture were identified as the factors determining microhabitat preference of *F. greenii*.

Keywords: *Fejervarya greenii*, macrohabitat, microhabitat, amphibia

1. Introduction

The accurate understanding of wildlife - habitat relations forms the foundation of sound management and conservation efforts (Babbitt *et al*., 2010) [1]. Obtaining precise information on amphibian habitat associations is a challenging process, due to the requirement of different habitat types for different maturity stages and species being cryptic throughout the year (Babbitt *et al*., 2010) [1]. An animal selects a habitat based on numerous behavioural and ecological aspects, then utilize it to perform their behaviours.

Simply, the habitat of an organism is considered as the place where that organism lives. In a broader way habitats are considered as the resources and conditions present in an area where an organism survive and reproduce (Hall *et al*., 1997; Krausman, 1999) [2, 3]. "Habitat selection is defined as the process whereby an individual chooses a habitat among available alternatives" (Babbitt *et al*., 2010) [1]. This habitat selection of an animal follows a hierarchical spatial scaling order (Johnson, 1980; Babbitt *et al*., 2010) [4, 1]. The first order selection (the broadest scale) consist the selection which determines the geographic range of an organism. The level of common features in the landscape is considered as the second order and it determines the home range. The third order selection is concerned with the particular sites resides within a home range (Johnson, 1980; Babbitt *et al*., 2010) [4, 1]. The second order is considered as the macro habitat and the third order is the microhabitat. The first order of habitat selection of *F. greenii* has been already determined which is the geographic range of *F. greenii* is central hills of Sri Lanka with an altitudinal range of 1700 – 2135 m a.s.l (Manamendra-Arachchi and De Silva, 2016) [5]. There is no information available regarding the preferred macrohabitats and microhabitats of *F. greenii* in montane cloud forests of Sri Lanka.

Fejervarya greenii (Boulenger, 1905) is an endemic, endangered and a rare species, which is restricted to the central hills of Sri Lanka, between the altitudinal range of 1700 – 2135m a.s.l. (De Silva, 2009; Manamendra-Arachchi and De Silva, 2016) [6, 5] (Figure 1). *F. greenii* can be seen in wetland habitats within montane forests and it is not associated with modified habitats (Manamendra-Arachchi and De Silva, 2016) [5]. Furthermore, it is a semi-aquatic species (Dutta and Manamendra-Arachchi, 1996) [7]. Though the breeding habits, morphometric data and maturity stages of *F. greenii* were investigated and were published (Weerawardhena, 2001) [8], The habitat preference of *F. greenii* in montane cloud forests of Sri Lanka was not subjected to research previously. The present study was conducted focusing on its preferred habitat conditions associated with montane cloud forests in Sri Lanka.

Fig 1: *Fejervarya greenii* (Amphibia: Dicroglossidae)

2. Materials and methods

2.1 Study site

The study was conducted in Horton Plains National Park (HPNP) which is located at the central hills of the upper montane forest zone of Sri Lanka from January to December 2016. HPNP has an altitudinal range of 1500 – 2524 m a.s.l (Whitmore, 1984) [9] and occupies an area of 3160 ha. It resides between 6^0 47'- 6^0 50' northern latitudes and 80^0 46' – 80^0 50' eastern longitudes (Green, 1990) [10]. HPNP occupies lentic habitats which are clustery distributed or isolated small pools while lotic habitats in HPNP include several tributaries and seven major streams with varying degrees of flow rates (De Silva, 2001) [11] (Figure 2).

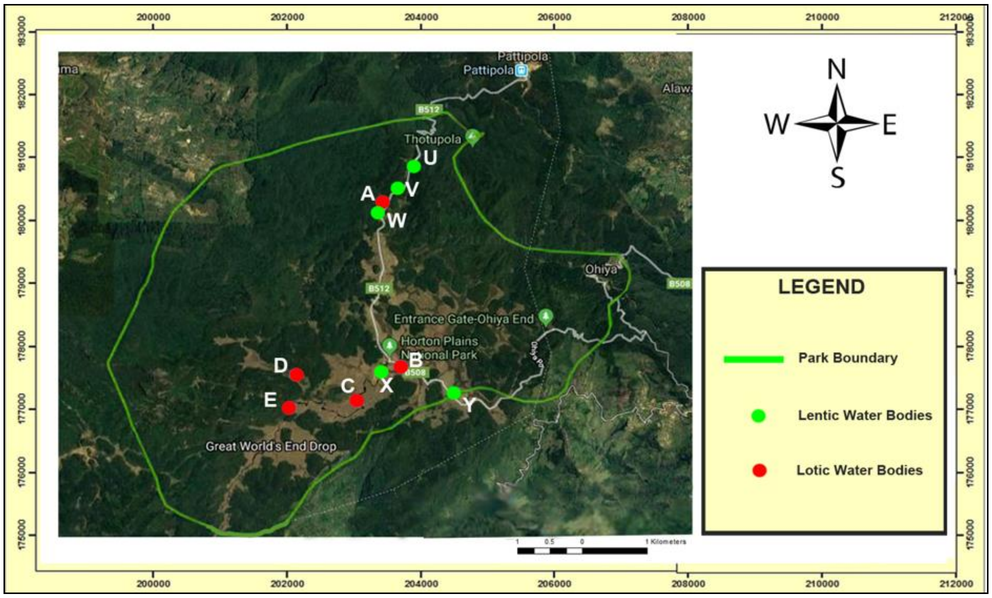

Fig 2: Locations of sampling sites inside the Horton Plains National Park

2.2 Sampling sites

Based on the semi aquatic status of *F. greenii* sampling was done in and around lentic and lotic water bodies. The *F. greenii* census was carried out in five lentic water bodies (U - 6°50'20.12"N, 80°48'42.82"E, V-6°50'1.05"N, 80°48'32.44"E, W- 6°49'52.15"N, 80°48'25. 29"E, X - 6°47'57. 13"N, 80°48'20. 58"E, Y - 6°47'46.27"N, 80°49'5.23"E) and five lotic water bodies (A-6°49'54. 31"N, 80°48'24. 61"E,B-6°48'3. 59"N, 80°48'35. 98"E, C- 6°47'40.84"N, 80°48'4.78"E, D-6°47'51. 01"N,80°47'31. 97"E,E-6°47'31. 18"N, °47'21. 53"E) for three consecutive days per month. GPS (Global Positioning System) points were recorded (Garmin etrex Euro hand held GPS receiver).

2.3 Field surveys and sampling

2.3.1 Macrohabitat preference

Three plots were placed around each sampling site. Each plot was 10 m in length. Plot A was 2 m in width where 1 m was in to the water body and 1 m to the land from the bank of the water body. Plot B and C were placed based on the established plot A. Plot B was placed 1 m away from the bank of the water body (From the boundary of the plot A) consisting a width of 2 m. Plot C was placed 3 m away from the bank of the water body (From the boundary of Plot B) and with a width of 2 m (Modified from, Faruk *et al*., 2013) [12]. All plots were surveyed in the morning (06:00hrs - 09:00hrs), noon (11:00hrs - 01.00hrs) and night (18:00hrs - 20:00hrs) by walking at a constant speed while scanning the vegetation below the height of 2 m, turning over small rocks, and causing slight movements to vegetation cover and searching for *F. greenii*.

Along each plot date, time, plot name and number, temperature (^{0} C) – ambient temperature 2 meters above the water surface / ground (Kestrel 4000 weather meter, USA), relative humidity- 2m above the ground (Kestrel 4000 pocket weather meter, USA), habitat relative moisture by collecting a substrate sample from the center of the plot. Relative moisture = {(weight of moist substrate sample – weight of dry substrate sample) / Weight of dry substrate sample}×100, availability of substrates (estimated as the proportion of the plot covered by each possible substrate such as submerged plant cover, bare water cover, short plants and shrubs cover, Grass cover, decaying plant matter and leaf litter, sand mud, gravel, rocks) were recorded.

Where possible *F. greenii* was captured with a net or by hand, they were held in a container to prevent repetition of the same individual (Wheeler and whelsh, 2008; Urbina and Galeano, 2009) [13, 14]. The container contained a small amount of water to prevent desiccation and overheat (Wheeler and Whelsh, 2008) [13] until the end of surveying the plot and they were released back into their captured habitat after the survey. In order to standardize measurements of frogs' hind limbs were grasped with one hand and the body of the frog was pressed lightly against a flat surface to elongate the body as much as possible (Haggarty, 2006) [15].

The Snout – vent length (SVL) (± 0.1 cm) (vernier caliper), maturity stage (male, female, juvenile, sub-adult: based on the presence /absence of nuptial pad and SVL), substrate type (submerged plant cover, bare water cover, short plants and shrubs cover, grass cover, decaying plant matter and leaf litter, sand mud, gravel, rocks), body surface temperature (non-contact UV thermometer, Switzerland), distance for the bank of the waterbody (measuring tape) were recorded when an individual frog was captured.

F. greenii was identified using keys of Manamendra-Arachchi and Pethiyagoda, 2006 [16]. Adult males and females were identified based on external morphology, which is the presence of nuptial pad on male forelimb first digit. After measuring snout to vent length (SVL), frogs were then assigned to an estimated age class category. SVL of mature males 3.03-4.18 cm, gravid females 3.25 - 4.98 cm and juveniles 1.30 – 2.26 cm (Manamendra-Arachchi and Pethiyagoda, 2006) [16]. If a frog's SVL is in between 2.27 – 3.24 cm, it was designated as a sub-adult.

2.3.2. Microhabitat preference

In plot A and B in and around lentic water bodies, ten 1m×1m quadrats were randomly placed (Urbina and Galeano, 2009) [14]. The quadrats were considered as "occupied", if at least one *F. greenii* was found occupying the quadrat (Sanchez *et al*., 2010) [17]. Sampling was carried out once a month from January 2016 to December 2016 during Morning (06:00hrs - 09:00hrs), noon (11:00hrs - 01:00hrs), night (18:00hrs – 20:00hrs). In order to record data, a standardized data sheet was used. In each quadrat, Dissolved Oxygen (DO) and temperature (YSI 550A Dissolved Oxygen Instrument), conductivity (YSI EcoSense EC300A conductivity meter), pH (YSI EcoSense pH 100A meter), water depth (meter ruler), substrate type (submerged plant cover, bare water cover, short plants and shrubs cover, grass cover, decaying plant matter and leaf litter, sand mud, gravel, rocks), availability of substrates (measured as % cover the quadrat), soil pH (Kelwey soil acidity and moisture tester), relative substrate moisture (by collecting substrate samples in the center of the quadrat), relative humidity in the center of the quadrat 2 meters above the pond/ ground (Kestrel 4000 pocket weather meter, USA), temperature (^{0}C) – ambient temperature 2meters above the water surface / ground (Kestrel 4000 weather meter, USA), substrate temperature at the center of the quadrat (non-contact UV thermometer, Switzerland) were recorded accordingly (Wyman, 1988; Iidos and Ancona, 1994; Hamer *et al*., 2002; Sanchez *et al*., 2009; Urbina and Galeano, 2009; Babbitt *et al*., 2010; Laura *et al*., 2010; Sparling, 2010)[18, 19, 20, 17, 14, 1, 21, 22].

2.4 Data analysis

The Minitab version 14.0 statistical software was used for statistical analysis. Graphical representations were created by using Microsoft excel 2010 software and also Minitab version14.0. Relative moisture was calculated using the below equation RM= {(weight of moist substrate sample – weight of dry substrate sample)/weight of dry substrate sample} x 100].

Variations in substrate relative moisture in months among plots were analyzed by subjecting data to one-way analysis of variance (ANOVA). The same statistical test was used to analyze monthly variations in average relative humidity and average ambient temperature in plots.

Non-Parametric Mann-Whitney U test at the significance level of $p<0.05$ was used to compare individual microhabitat variable between occupied and non-occupied quadrates.

3. Results and discussion

Amphibians require both terrestrial and aquatic habitats through their life cycle (Trenham and Shaffer, 2005) [23]. Lentic (ponds, pools) or lotic (streams) environments together form the aquatic amphibian habitats. Amphibians are known to utilize a broad range of habitats adjacent to these lentic and lotic water habitats (Semlitsch, 2003) [24].

3.1 Microhabitat Preference

A total of 340 individuals were recorded during the study period. The presence of *F. greenii* individuals was recorded only in and around lentic water bodies. Though it was mentioned that *F. greenii* maintained a dominance not only in lentic water bodies but also in lotic water bodies (De Silva, 2001) [11] during the current research there were no individuals of *F. greenii* recorded in lotic water habitats. The absence of *F. greenii* in lotic water bodies may be due to the presence of Rainbow trout (*Oncorhynchus mykiss*) inhabiting these lotic waterbodies. This fish species is the only single exotic species in HPNP, which was introduced in 1882 in order to support sport fishing (DWC, 2007) [25]. Hecnar (1997) [26] stated that the presence of introduced predatory fishes by humans has led to dramatic decline in amphibian species assemblages and community diversity. The reason is they can search out and easily capture amphibian eggs and larvae which will ultimately leads to declining in amphibian populations (Lips, 1998) [27]. Moreover, another reason may be the absence of the most preferred substrate type of *F. greenii* which is submerged plants in these lotic water bodies. Therefore, they found these habitats unavailable for them.

F. greenii was recorded in higher numbers in each month throughout the year in the area demarcated as Plot A. Relatively low number of *F. greenii* was recorded in Plot B throughout the year. No *F. greenii* was recorded in Plot C (Figure 3). Higher number of individuals were observed in plots with water in the study period indicates that this species is more inclined towards an aquatic environment.

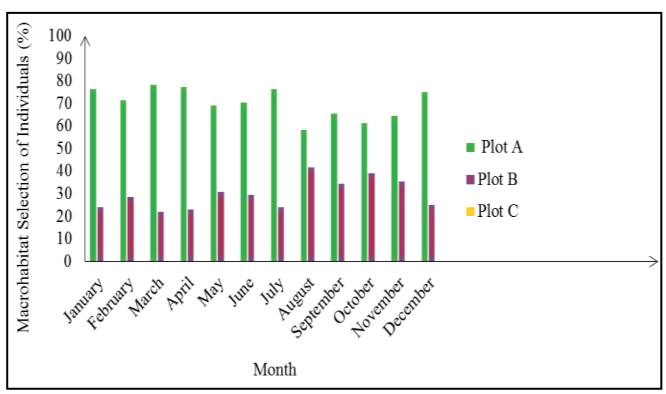

Fig 3: Monthly macrohabitat selection of *F. greenii* individuals

In HPNP lentic water bodies and the immediate surrounding is the preferred habitat of *F. greenii*. The immediate surrounding area of a lentic water body can be considered as an aquatic terrestrial ecotone (Pittman *et al*., 2008) [28]. This ecotone connects the water body and the surrounding area. Therefore, macrohabitats of *F. greenii* can be categorized as the aquatic lentic water bodies and the surrounding ecotone of lentic water bodies. These factors suggest that *F. greenii* is a habitat specialist preferring the lentic water bodies and the immediate surroundings. Plot A was selected by 74.24% of mature males, 93.87 % of mature females, 66.67% of sub-adults and 33.75% of juveniles. Plot B was mainly selected by juveniles (66.25%). Moreover, 25.76% of mature males, 6.13% of mature females and 33.33% of sub-adults were recorded in the plot B. *F. greenii* was not recorded in Plot C. Results indicated that both mature males and females tend to select plot A when compared to other plots (Figure 4).

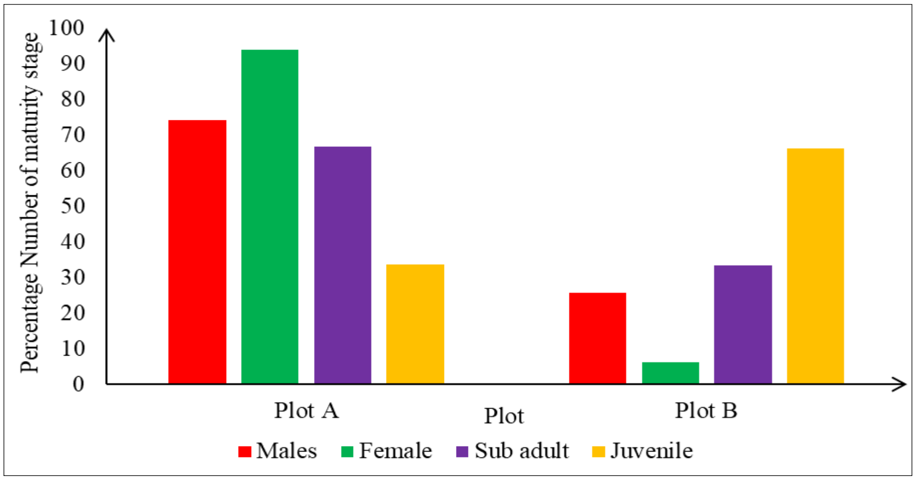

Fig 4: Variations in maturity stages in occupied plots.

The study revealed that although mature individuals preferred habitats where water was abundant, terrestrial band of 1-3 m was the preferred habitat of immature individuals mainly juveniles. This can be attributed to their behaviours observed in these habitat types. Calling, courtship and swimming behaviours were recorded only in the aquatic environment where as resting behavior was recorded only in the surrounding ecotone. In which calling and courtship beaviours were observed only in mature individuals and immature individualspreferredrestingmostofthetime(Prabhath,2016) [29].

Individuals of *F. greenii* were recorded in different distances from the bank of the water body. A total number of 182 individuals were recorded within a distance of 1.00 m from the bank to the water body. Furthermore, 55 individuals were recorded at a distance of 1.00 m from the bank of the water body to the land. Moreover, 78 individuals were recorded at the distance of 1 to 2 m band from the water body and 25 individuals were recorded at the distance of 2 to 3 m from the water body (Figure 5).

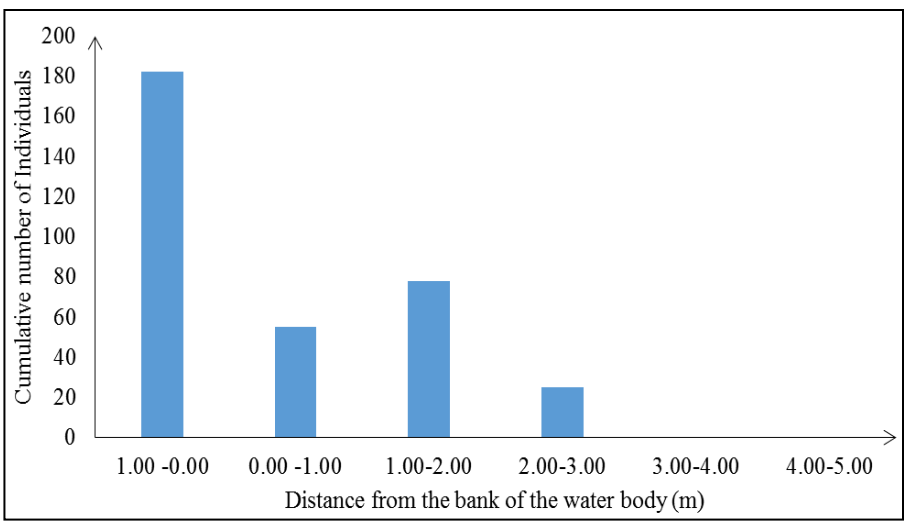

Fig 5: Variation between the distance from the bank of the water body and the number of individuals.
*The starting point of 0.0 m was placed on the bank of the water body.

Presence of *F. greenii* only in the areas 1m to the water and 3m to the terrestrial habitat in lentic water bodies confirmed that this is a semi aquatic species. This finding tallies with those of Manamendra-Arachchi and Pethiyagoda (2006) [16].

The aquatic water body and the ecotone provides every possible substrate type for *F. greenii.* Submerged plant cover was a unique substrate type found only in the aquatic habitat. Submerged plant cover, bare water cover, short plants and shrubs cover, grass cover, decaying plant matter and leaf litter cover, sand, mud, gravel and rocks were considered as possible substrate types for *F. greenii*. Submerged plant cover in both macro and micro level can be considered as the most highly prefferred substrate.

The presence of submerged plant cover and bare water cover was recorded only in plot A (55% and 10% respectively). In plots B and C, prominent substrates were short plants and shrubs, grasses, decaying plant matter and leaf litter (each about 20%). Though rocks were absent in plot A, rocks occupied 5% and 10% in plot B and plot C respectively (Figure 6)

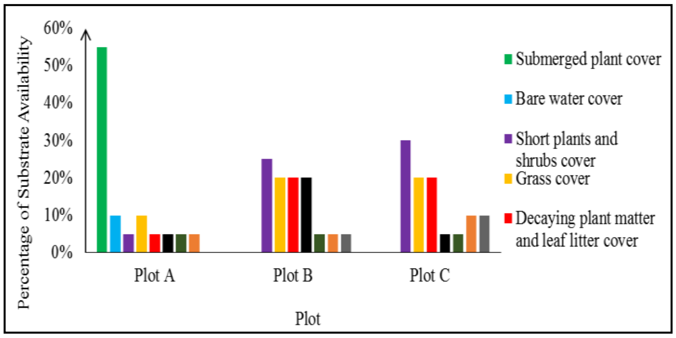

Fig 6: Substrate availability in plots.

Substrate relative moisture is known to effect amphibians in many ways including desiccation. They tend to utilize habitats with high moisture content in substrates in order to avoid desiccation (Seebacher and Franklin, 2011) [30]. Since the substrate relative moisture level decreased significantly with the increase in the distance from the water body, it may have directly affected the *F. greenii* and may have prompted them to avoid the drier areas. There was a significant difference ($p<0.05$) between substrate relative moisture levels in each plot [ANOVA, F=116.50, P = 0.000, ($P<0.05$)]. Substrate relative moisture decreased gradually from plot A to C. The highest value for substrate relative moisture was recorded from plot A with a value of 72.37%. An average value of 56.01% was recorded from plot B. Plot C had the lowest substrate relative moisture level with an average value of 32.40% (Figure 7).

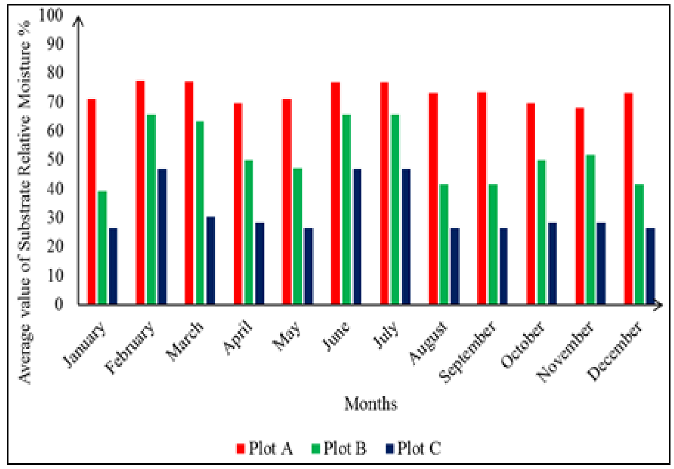

Fig 7: Variations in substrate relative moisture

Amphibians are known to utilize macrohabitats with significant ranges of ambient temperature and relative humidity (Gunasekara, 2015) [31]. Relative humidity along with temperature and rainfall together create an impact on global distribution of amphibians (Dey, 2010) [32]. Relative humidity alone determines the habitat suitability and activity of amphibians by acting on rates of water loss. The ambient temperature and relative humidity among the selected sites and plots at the HPNP did not differ significantly and hence did not affect *F. greenii* in any significant manner during the study period.

When a single sampling site was considered values of relative humidity in each plot were similar. But the values among sampling sites differed. Monthly average relative humidity values were not significantly different in each plot [ANOVA, F= 0.00, P=1.00 ($P>0.05$)]. Monthly average relative humidity values are given in Table 1.

Though the values among sampling sites were different, when a single sampling site was considered, values of ambient temperature in each plot were similar. The monthly average ambient temperature values did not differ significantly in each plot [ANOVA, F= 0.00, P=1.00 ($P>0.05$)]. The monthly average ambient temperature values are given in Table 1.

Table 1: Variations in monthly relative humidity and substrate relative moisture

Months	Average relative humidity (%) ± SD	Average ambient temperature (0C) ± SD
January	69.65±08.39	16.64±0.56
February	80.66±04.88	13.15 ± 0.29
March	80.02±06.72	16.17 ± 1.32
April	66.55±14.61	16.12 ± 1.44
May	68.15±10.40	14.16 ± 1.23
June	85.14±10.93	14.50 ± 1.29
July	86.60±10.39	13.94 ± 1.08
August	78.99±06.98	15.38 ± 1.20
September	72.18±09.82	16.79 ± 0.42
October	70.79±11.89	16.68 ± 0.64
November	72.30±01.87	16.50 ± 0.69
December	72.18±09.82	16.79 ± 0.42

3.4 Microhabitat preference

There is a significant correlation ($p < 0.05$) between environmental variables including water depth, water temperature, submerged plant cover, decaying plant matter and leaf litter, sand, substrate relative moisture, relative humidity, substrate temperature and the microhabitat selection of *F. greenii*. Presence of water is an important environmental parameter and most of the amphibians need water to complete their life cycle (Sparling, 2010) [22]. Due to the semi aquatic status of *F. greenii*, presence of ample volume of water is specifically essential, since fertilized eggs of *F. greenii* are deposited inside the water body on the pond bottom in close proximity to the banks of the water body (K. Ukuwela pers.comm). Water not only provide breeding habitats, but also provides a habitat to perform various behaviour such as calling, resting and feeding. *F. greenii* selects a range of 3.00 – 20.50 cm water depth closer to the bank of the water body.

F. greenii preferred a water depth ranges between 3.00 to 20.5 cm, a water temperature ranges between 13.60 – 20.60 ^{0}C and submerged plant cover with a range of 45.00 – 100.00 %. They were not found in habitats with not more than 20.00 % of decaying plant matter and leaf litter and 40.00 % of sand amount. A range of 14.02 – 22.80 ^{0}C substrate temperature, relative humidity with a range of 45.90 – 100.00%, substrate relative moisture ranges between 30.54 – 79.85% were preferred by *F. greenii* in their microhabitats (Table 2).

Access to a moist substrate or to a water body is a fundamental requirement of animals in order to prevent evaporative water losses from the body (Nagai *et al*., 1999) [33]. Amphibians are especially in need of this moist substrate or watery environment because of the tendency of high loss of water through their permeable skin. In order to prevent the excessive water loss *F. greenii* selects a substrate with relative moisture of 30.54 – 79.85%.

Table 2: Microhabitat variables in occupied quadrats by *F. greenii*

Variable	Mean ± SD	Minimum	Maximum
Water Depth (cm)	11.97 ± 2.09	3	20.5
Water Temperature (0C)	17.44±2.32	13.6	20.6
Submerged Plant Cover (%)	79.69±12.79	45	100
Decaying plant matter and Leaf Litter (%)	7.12± 5.15	10	20
Sand (%)	12.26±11.05	15	40
Substrate Temperature (0C)	17.53±2.48	14.02	22.8
Relative Humidity (%)	73.74±10.64	45.9	100
Substrate Relative Moisture (%)	65.09±12.73	30.54	79.85

Submerged plants, leaf litter and sand cover are the environmental variables which were used by *F. greenii* in their microhabitat selection. Substrates selected by amphibians can be inorganic (Sand) or organic (Submerged plants and leaf litter) (Babbitt *et al*., 2010) [1]. Sand cover with a range of 15.00 – 40.00 % provides resting sites for *F. greenii*. Presence of submerged plants (mainly *Isolepis fluitans*) is an indication of habitat suitability of *F. greenii*. The yellow mid vertebral band of *F. greenii* and the mid rib of *Isolepis fluitans* are similar in appearance which makes *F. greenii* hard to detect by predators. *F. greenii* selected habitats with a submerged plant cover range of 45.00 - 100.00 % with a relatively high average value of 79.69%. Leaf litter provides a good refuge for *F. greenii.* Leaf litter not only prevent excessive water loss of the frogs but also provide protection from predators (Urbina and Galeano, 2009) [14].

4. Conclusion

Being a semi aquatic species *F. greenii* selects an area of 1 m to the water and 3 m to the terrestrial habitat surrounding the water body as its macrohabitat which includes the water body and the aquatic terrestrial ecotone. This suggests the habitat specialist status of *F. greenii*. Moreover, this species is more inclined towards the aquatic environment while higher number of males and females occupy the water body, the terrestrial band of 1 – 3 m was selected by juveniles as their preferred habitats. The present study revealed that habitat variables such as water depth, water temperature, submerged plant cover, decaying plant matter and leaf litter, presence of sand, substrate relative moisture, relative humidity and substrate temperature had a correlation to *F.greenii* occupying a particular microhabitat. A water depth range between 7.00 to 20.5 cm, a water temperature range between 13.60 – 20.60 ^{0}C and submerged plant cover with a range of 45.00 – 100.00 % were selected by *F. greenii* in their microhabitats. Moreover, they were not found in habitats with not more than 20.00 % of decaying plant matter and leaf litter and 40.00 % of sand amount. Furthermore, a range of 14.02 – 22.80 ^{0}C substrate temperature, relative humidity with a range of 45.90 – 100.00%, substrate relative moisture ranges between 30.54 – 79.85% were preferred by *F. greenii* in their microhabitats.

5. Acknowledgment

We thank the Department of Wildlife Conservation Sri Lanka for providing us the permission (Permit Number: WL/3/2/11/15) to conduct this research and the staff of Horton Plains National Park for their great support. Our sincere gratitude goes to the Department of Zoology, University of Sri Jayewardenepura for the support we received in numerous ways. We are indebted to Dr. (Mr.) Anslem de Silva, Dr. (Mr.) Kanishka Ukuwela, Dr. (Mr.) Tharaka Mahaulpatha and Prof. (Mr.) Akira Mori for their valuable suggestions and helpful comments.

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