Punjab University Journal of Zoology

OPEN ACCESS

39(1): 69-77 (2024)
https://dx.doi.org/10.17582/journal.pujz/2024/39.1.69.77

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Research Article

Diversity and Habitat Preferences of Amphibians in Abbottabad, Pakistan

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Article History
Received: November 30, 2022
Revised: March 05, 2024
Accepted: March 26, 2024
Published: May 24, 2024

Authors' Contributions
AS collected the specimens and data, and wrote the manuscript. SA designed the study and helped in the collection of specimens. SAM analysed data and constructed the phylogenetic trees. WK arranged figures and tables. WAP proofread the manuscript. FSA helped in the measurements of the amphibians.

Keywords
Amphibians, Diversity, Frogs, Habitat, Systematics, Toads

Abstract | Pakistan has reported twenty-four amphibian species, nine of which are endemic to the country. Amphibians in Pakistan are diverse, falling into four families: Dicroglossidae, Microhylidae, Bufonidae, and Megophryidae. The current study was carried out to investigate the amphibian fauna of several localities in District Abbottabad. From June 2019 to August 2020, a field survey was undertaken in District Abbottabad. Total dissolve solvent (TDS), Dissolve solvent (DO), Electrical Conductivity (EC), PH, Humidity, and Temperature (C) of several water bodies were also measured. A total of 100 specimens from the District were collected and stored in a 10% formalin solution. This study discovered that five species of anurans, i.e. *Duttaphrynus stomaticus*, *Duttaphrynus melanostictus*, *Euphlyctis cyanophlyctis*, *Hoplobatrachus tigerinus*, and *Nanorana liebigii* are members of the Bufonidae and Dicroglossidae families, respectively. Morphometric measurements were made using a non-digital vernier caliper with a resolution of 0.05 mm. HW, HL, SVL, TFL, THL, TBL, HnL, TD, END, IND, IOD, ED, LF, LH, FAL, LHU, UEW, PL, and PW were all measured. This research discovered that *D. melanostictus* and *E. cyanophlyctis* were also found but in lower numbers than *D. stomaticus*. The District had the lowest amount of *H tigerinus* as well as *N. liebigii* records. There is need to discovered more amphibians fauna in the District Abbottabad and all over Pakistan to update the identification keys of Amphibians.

Novelty Statement | The study will assist to know the distributional pattern of the amphibians fauna and hence its conservation in various terrestrial environments and water bodies of the Abbottabad district.

To cite this article: Sheraz, A., Ahmed, S., Mehmood, S.A., Khan, W., Panhwar, W.A. and Awan, F.S., 2024. Diversity and habitat preferences of amphibians in Abbottabad, Pakistan. *Punjab Univ. J. Zool.*, **39**(1): 69-77. https://dx.doi.org/10.17582/journal.pujz/2024/39.1.69.77

Introduction

There are 7,044 amphibian species worldwide, serving as an important evolutionary link between terrestrial and aquatic environments (Frost, 2013), and they are classified into three orders: Anura (frogs and toads), Gymnophiona (Caecillians), and Caudata (Salamanders). These cold-blooded vertebrates regulate body temperature based on air temperature (Shah and Tiwari, 2004). Amphibians have skin glands that produce mucus and poison granules, notably bufotoxin from parotid glands in toads and frogs (Stebbins and Cohen, 1995). They have low metabolic rates and low energy and food requirements.

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The term "Anura" refers to frogs and toads, the larvae of which are known as tapole.

Metamorphosis in amphibians includes rapid growth, the loss of gills and gill sacs, and the formation of webbed toes, huge eyes, and short forelimbs in frogs (Duellman *et al.*, 2012). Metamorphosis maintains physiological changes by being regulated by the thyroid gland's thyroxine hormone (Galton, 1992). Pakistan, located between the Oriental and Palearctic regions, is home to 24 amphibian species, nine of which are endemic, in different settings such as the Indus Valley and the Himalayan region (Khan, 2002, 2004, 2006). Pakistani amphibians are divided into four families: Dicroglossidae, Microhylidae, Bufonidae, and Megophryidae (Masroor, 2012).

Adult amphibians have similar diets, primarily devouring insects and tiny organisms, with different reproductive techniques based on feeding habits and habitat (Khan and Malik, 1987b; Khan, 1999b). Pakistan's environment is threatened by loss of breeding habitat (GOP, 1999), necessitating checklists by (Khan, 2006).

Herpetology is the scientific study of reptiles and amphibians such as newts, salamanders, frogs, toads, turtles, lizards, snakes, and crocodiles. All of these animals are referred to as herpetofauna (Shah and Tiwari, 2004). Herpetofauna is a major indication of climate change, therefore they can be found in a variety of habitats all across the world, save on isolated islands. However, amphibians and reptiles are under severe threat due to habitat loss, deforestation, pollution, climate change, fragmentation, exploitation, endocrine-disrupting pollutants, urbanization, introduced species, chemical use such as fertilizers and pesticides, and ozone layer destruction, all of which have a significant impact on amphibian and reptile populations, eyes, skin, and eggs.

Contaminated agricultural areas are largely to blame for the global loss of anuran species (Petrov, 2004). The removal of herpetofauna from the environment will disrupt prey-predator dynamics, algae dwellers, and invertebrate populations. Anthropogenic activities have a significant impact on the herpetofauna population (Baig *et al.*, 2006).

There are several contributing factors to the decline of amphibians including habitat degradation and alteration (Cushman, 2006). The alteration of the physical structure of habitats is one of the five factors affecting the structure and composition of resident biological communities (Karr, 1991). Thus, the disturbance of physical habitats in aquatic environments influences amphibian communities as much as any other source of pollution and, often, inadequate habitat conditions can obscure the effects of pollutants (Barbour *et al.*, 1999). Dissolved oxygen, temperature, pH, salinity and water conductivity, organic carbons, and pollutants are important factors in the habitat of amphibians, which can affect survival, growth, maturation, and physical development (Sparling, 2010).

Amphibians are commonly utilized for demonstration and testing in laboratories around the world for learning. *Rana tigrina* is one of several species used for school, college, and academic demonstrations (Khan, 2006). The current survey was carried out to investigate the fauna of amphibians and their dispersal in various terrestrial environments and bodies of water in the Abbottabad district.

Materials and Methods

Study area

Abbottabad, a district in Khyber Pakhtunkhwa that is part of the Hazara Division, was chosen as the study location because amphibian fauna of the district abbottabad was remained undiscovered. The Abbottabad district is located between 33° 49' and 34° 22' N and 72° 55' and 73° 31' E, and it encompasses around 715 square miles (1,850 Km^2). Major James Abbott, Hazara's first deputy commissioner (1849-1853), inspired the district's name. Nearby districts include Huripur, Mansehra, and Muzaffarabad. The district was established by the Khyber Pakhtunkhwa Wildlife (protection, management, preservation, and conservation) Act of 1975, which included Ayubia National Park and Qalandrabad Game Reserve. These two areas make up only 6% of the district's overall land area. Abbottabad is at an elevation of around 1232 meters above sea level (IUCN, 2004). It has mild weather in June and July, and cold weather in December and January. Heavy rainfall during the monsoon season (July-September) causes flooding in many regions of the city (Rahman and Tariq, 1996).

Samples collection

The survey was conducted out in Abbottabad district to gather and observe amphibian species. Water samples were collected from various water channels in the district, and ecological parameters such as temperature (°C), total dissolve solids (TDS), electric conductivity (EC), dissolve oxygen (DO), PH, temperature (air), and humidity of terrestrial habitat were also measured (Table 2). The samples were gathered between June 2019 and August 2020. Samples were gathered during the day from several water bodies in Abbottabad city, while the majority of the samples were obtained in the evening from District Abbottabad using a hand net or scoop net. The sample was also collected with bare hands (Baig *et al.*, 2008).

Preservation

The collected samples were stored in plastic bottles with a formaldehyde (10%) solution. Samples were labeled with hard chart paper that included information such as

the date of collection, the name of the species, codes, and collection locations. To preserve the frog for a long time, a 10% formaldehyde solution was injected into its body with a disposable syringe.

Identification

Identification keys of amphibians, centered on morphological characters were provided by Khan (2006) and Masroor (2012). Species and families which are not presented in the current study are denoted by "*".

Identification key of amphibian's families

1. Parotid glands found…….Bufonidae
Parotid glands not found……2
2. Horizontal pupil……Dicroglossidae
Vertical pupil……..3
3. Broad mouth and head; warty body; definite post orbital tuberculated ridge……Megophryidae*
Narrow mouth and head; smooth body with small smooth tubercle……Microhylidae*

Key to Family Bufonidae

1. Cranial crest on head…….2
No cranial crest on head……...3
2. Definite tympanum; canthal, Supraorbital, postorbital an orbito-tympanic crests found……. Duttaphrynus melanostictus
3. Tibial glands found……..4
No tibial glands…….5
4. Weak spinulated line present on tarsal folds…… Duttaphrynus stomaticus
Longitudinal lines on dorsal sides; tarsal fold found…. Bufotes latastii*
5. Vertebral lines present; toes with single subarticular tubercles.....Bufotes pseudoraddei*
Vertebral line absent; dorsal side have marbled pattern; first, second and third finger have two subarticular tubercles……..Bufotes baturae*

Key to Family Dicroglossidae

1. Indefinite tympanum……2
Definite tympanum……3
2. Smooth dorsal body with small tubercles present on edges….Nanorana vicina*
Deeply tuberculated dorsal body……Nanorana liebigii
3. Partially webbed toes; tubercle on inner metatarsal; toad like habitus……..Sphaeroteca breviceps*
Half webbed toes; inner metatarsal not like above…….4
4. Toes somewhat webbed otherwise not depressed; mid-dorsal line absent……Euphlyctis cyanophlyctis sensu lato
Clear webbed toes; mid-dorsal line present……5
5. Webbed toes to the tips; length of body often 80 mm or greater…..Hoplobatrachus tigerinus
Length of body not more than 40 mm; partially webbed toes……..Fejervarya limnocharis sensu lato*

Measurements of morphometric characters

Morphometric character measurements were obtained with a non-digital Vernier caliper, measurements were taken (nearest to 0.05 mm), and species were photographed in the field with a Canon Power Shot G9 X, 20.1 Megapixels camera. Morphometric measurements were taken at the Hazara University Lab in Mansehra. Watters *et al.* (2016) detailed the morphometric features that were used to record the measurements.

Measurement of limnological and environmental parameters

The water samples were collected from different water bodies of the district, limnological parameters like temperature (°C), total dissolve solids (TDS), electric conductivity (EC), dissolved oxygen (DO) and PH were measured. Environmental parameters were also measured which included temperature (air) and humidity of the terrestrial habitat.

Statistical analysis

The collected data of ecological parameters, identified fauna and distribution of amphibians were analyzed by using statistical software such as Past 3.1 and Microsoft Excel.

Results and Discussion

In the course of the present research work, field surveys were conducted in different areas of district Abbottabad; Galliyat, Thandiyani, Normang, Harno, Dhamtour, Dobathar, Khutialia, Sherwan, Shimla hills, Qalandrabad, Mangal, Boi, Havaliyan, Sajikot, Gulaga from June 2019 to August, 2020. A total of 100 specimens were collected from district Abbottabad and these specimens were identified up to the species level. A total of five species of amphibians were identified. These five identified species of amphibians belong to two families; Dicroglossidae and Bufonidae under four genera such as *Duttaphrynus, Hoplobatrachus, Euphlyctis* and *Nanorana.*

Duttaphrynus stomaticus (Lütken, 1864)

Indus valley toad

Wider head; broader interorbital distance than the width of upper eyelid; cranial crests absent; tympanum distinct; kidney shaped parotid glands; first finger is larger than second; tibial glands are present; adult's SVL is between 38.1-64.9 mm. Toes are blunt; small warts are present on skin. Color of body is usually normal grayish to black (in juvenile's stages these molting centers are pinkish color) but more visible in subadult stages; muddy white ventral surface; color of upper lip is cream (Figure 1A, F). They are mostly loner but they may rest together. They hide themselves in holes, sand or wet soil (Daniel, 1963a) (Tables 1, 2).

Table 1: Morphometric analysis of amphibians fauna of district Abbottabad.

Morpho-metric Parameters	*Duttaphrynus stomaticus* (n=53)		*Duttaphrynus melanostictus* (n=14)		*Euphlyctis cyanophlyctis* (n=23)		*Hoplobatrachus tigerinus* (n=5)		*Nanorana liebigii* (n=5)	
	Range	mean±SD	Range	mean± SD	Range	mean±SD	Range	mean±SD	Range	mean±SD
HL	10.1-16.8	13.7±1.6	13.9-25.1	19.86±4.27	11.1-19.9	16.1±2.8	13.1-21.1	17.4±3.6	15.9-23.9	19.6±3.3
HW	12.1-22.1	17.5±2.2	17.9-35.9	26.73±7.09	13.1-22.9	18.5±3.2	10.8-19.1	15.2±3.6	16.1-27.9	21.9±4.6
IOD	2.1-5.9	3.9±0.8	2.1-6.1	4.40±1.08	1.1-4.1	2.7±3.8	1.1-3.1	1.9±0.7	3.1-4.1	3.8±0.4
ED	2.1-7.9	6.1±1.3	6.1-10.9	8.09±1.36	4.1-7.9	6.2±1.2	5.1-7.7	6.5±1.2	6.7-7.8	7.1±0.4
IND	1.1-7.1	2.5±1.0	2.1-4.9	3.21±1.06	1.1-2.9	1.5±0.6	1.1-2.1	1.7±0.5	4.1-6.1	5.2±1.0
END	2.1-4.1	3.3±0.7	2.9-4.1	3.27±0.42	1.1-3.1	2.7±0.6	2.1-4.9	3.5±1.1	2.1-3.2	2.8±0.4
SVL	38.1-64.9	50.4±5.9	48.9-98.1	70.78±17.64	35.9-61.1	50.7±8.1	35.1-60.1	48.5±11.2	45.1-73.7	55.5±11.7
TBL	14.9-23.9	20.0±2.1	19.9-38.1	27.86±6.35	18.9-30.9	25.8±4.2	17.8-30.1	24.4±5.1	26.7-40.7	33.1±5.9
TFL	21.1-34.9	27.6±3.4	29.1-51.9	39.63±8.18	25.9-41.9	35.4±5.4	21.1-41.7	33.3±9.3	36.1-53.2	43.5±7.3
THL	9.8-22.9	17.6±3.2	9.1-34.1	25.71±7.66	14.1-29.9	23.1±5.1	14.8-30.8	21.9±6.6	25.8-37.9	30.4±5.5
TD	2.1-4.9	3.7±0.7	3.1-6.9	4.44±0.97	3.1-5.9	4.3±0.9	3.1-4.9	4.1±0.7	-	-
UEW	3.1-5.9	4.6±0.7	4.1-6.9	4.93±1.04	2.1-4.9	3.6±0.8	2.1-4.1	3.3±1.0	4.1-5.1	4.5±0.5
LHU	6.9-18.1	10.3±2.1	9.9-17.9	12.97±2.20	7.1-13.9	10.5±1.8	6.7-10.9	8.3±1.8	8.7-12.1	10.7±1.4
HnL	6.9-11.1	8.0±1.0	11.1-18.1	14.60±2.10	6.1-11.1	9.0±1.8	5.1-10.1	8.1±2.1	10.1-15.9	12.7±2.1
FAL	6.1-14.9	12.0±1.8	11.1-24.1	17.31±3.23	6.1-14.9	10.2±2.2	6.1-10.9	8.8±1.7	10.1-17.7	13.5±3.1
LF	23.1-39.7	32.8±4.0	34.1-60.1	44.88±7.15	20.3-39.9	29.7±5.2	17.9-31.1	25.2±5.3	28.9-45.3	36.9±6.3
LH	47.1-76.9	65.2±7.0	60.9-119.9	93.20±21.30	62.1-100.7	84.3±14.1	55.1-102	79.7±20.6	90.4-131.8	107.0±18.6
PW	4.1-11.9	6.9±1.6	4.1-9.1	6.69±1.73	-	-	-	-	-	-
PL	8.1-18.9	12.9±2.1	8.1-24.1	15.39±5.35	-	-	-	-	-	-

HL, head length; HW, head width; ED, eye diameter; IOD, interorbital distance; END, eye-nostril distance; IND, internarial distance; SVL, snout-vent length; THL, thigh length; TFL, tarsus foot length; TBL, tibia length; TD, tympanum diameter; LHU, length of the humerous; FAL, forearm length; UEW, upper eyelid width; PW, parotid gland width; PL, parotid gland length.

Table 2: Statistical analysis on the basis of morphometric characters and morphometric ratios of Amphibians fauna of district Abbottabad.

Morpho-metric parameters	*Duttaphrynus stomaticus* (n=53)		*Duttaphrynus melanostictus* (n=14)		*Euphlyctis cyanophlyctis* (n=23)		*Hoplobatrachus tigerinus* (n=5)		*Nanorana liebigii* (n=5)	
	Range	mean±SD	Range	mean± SD	Range	mean±SD	Range	mean±SD	Range	mean±SD
HL/HW	0.64-0.98	0.79±0.07	0.67-0.88	0.75±0.06	0.72-1.00	0.87±0.06	1.10-1.21	1.15±0.05	0.86-0.99	0.90±0.05
HL/SVL	0.22-0.33	0.27±0.02	0.25-0.33	0.28±0.02	0.29-0.34	0.32±0.02	0.34-0.37	0.36±0.02	0.32-0.40	0.36±0.03
SVL/LH	0.64-0.87	0.77±0.06	0.64-0.85	0.76±0.06	0.55-0.70	0.60±0.05	0.59-0.64	0.61±0.02	0.50-0.56	0.52±0.03
TBL/LH	0.28-0.36	0.31±0.02	0.27-0.33	0.30±0.02	0.27-0.34	0.31±0.01	0.29-0.35	0.31±0.02	0.30-0.32	0.31±0.01
THL/LH	0.16-0.33	0.27±0.03	0.15-0.30	0.27±0.04	0.22-0.30	0.27±0.02	0.24-0.30	0.27±0.02	0.27-0.29	0.28±0.01
LHU/LF	0.19-0.49	0.31±0.06	0.27-0.31	0.29±0.01	0.31-0.42	0.35±0.03	0.28-0.37	0.33±0.03	0.26-0.30	0.29±0.02
FAL/LF	0.23-0.44	0.37±0.04	0.37-1.52	1.27±0.27	0.29-0.39	0.34±0.03	0.32-0.38	0.35±0.02	0.34-0.39	0.36±0.02
IOD/UEW	0.51-1.58	0.87±0.23	0.41-1.24	0.91±0.21	0.27-4.85	0.73±0.93	0.51-0.76	0.57±0.11	0.76-1.00	0.85±0.10
SVL/LF	1.34-1.87	1.54±0.13	1.28-1.96	1.56±0.23	1.38-1.74	1.72±0.17	1.69-2.14	1.92±0.16	1.36-1.66	1.51±0.14
PL/PW	1.00-3.10	1.95±0.47	1.00-3.08	2.37±0.67	-	-	-	-	-	-

Duttaphrynus melanostictus (Schneider, 1799)

Asian common toad

Wider head; clear tympanum; palm of hands contains metatarsal tubercles; distance between interorbital is greater than the width of upper eyelid; oval parotid gland; warts are thorny black; constant belly with light color and no dark patches; adult SVL is 48.9-98.1 mm (Tables 1, 2). They are usually present in moist places, near water canals, but some are present in hilly areas of Gujarat (Sarkar, 1984). In water eggs of this species is rounded with grass, but when there is no plant in water than eggs are present in the bottom (Figure 1B, G).

Euphlyctis cyanophlyctis sensu lato (Schneider, 1799)

Skittering frog

Rana cyanophlyctis Schneider, 1799: 137.

Dicroglossus cyanophlyctis Deckert, 1938: 138.

Rana (Euphlyctis) cyanophlyctis Dubois, 1981: 240.

Figure 1: Color variation of amphibian species of Abbottabad in their natural habitat (A–E) dorsal and (F–J) lateral views: (A & F) *Duttaphrynus Stomaticus* (B & G) *Duttaphrynus melanostictus* (C & H) *Euphlyctis cyanophlyctis* (D & I) *Hoplobatrachus tigerinus* (E & J) *Nanorana liebigii*.

Diagnosis

Wider head but not long; interorbital distance is larger than width of upper eyelid; tympanum is definite; snout is blunt; it is brown, yellowish brown or olive in color; toes are fully webbed; smooth skin from ventral side; tubercles are present on dorsal side along with dark patches and distinct pores; same pattern observed on hind limbs and for limbs; adult's SVL is about 55.9-61.1 mm. Tadpoles of *Euphlyctis cyanophlyctis* is grown about 65-75 mm of length (Figure 1C, H). This species is always present below 1300 m elevation. Usually, *E. cyanophlyctis* is present in manmade surroundings (Dubois, 1975) (Tables 1, 2).

Hoplobatrachus tigerinus (Daudin, 1802)

Tiger frog

Rana tigerina Daudin, 1802: 64.
Rana tigerina Gravenhorst, 1829: 39.
Dicroglossus tigerinus Deckert, 1938: 138.
Hoplobatrachus tigerinus Dubois, 1992: 315.

Diagnosis

Head width is shorter than long; less distance between interorbital than the width of upper eyelid; snout is pointed; tympanum is clear; pointed fingers, second finger is shorter than first; thick toes with somewhat swollen tips; fully webbed toes; Nostrils are present nearer to mouth; Dorsal side is olive green, green and brown with irregular patches and dark spots on it (Figure 1D, I) Ventral side is white; hind limbs and fore limbs have unsightly marks, but thighs are fully marbled and mostly possessed yellow and black color; clear mid dorsal line; adult's SVL is about 58.7-60.1 mm (Tables 1, 2).

Nanorana liebigii (Gunther, 1860)

Liebig's frog

Rana liebigii Gunther, 1860: 157.
Rana (Rana) liebigii Boulenger, 1920: 8.
Nanorana liebigii Chen *et al.,* 2005: 239.
Nanorana (Paa) liebigii Chen *et al.,* 2010: 2.

Diagnosis

Head thickness is somehow wider, then long; tympanum is indefinite; nostrils are closer to snout and eye; distance between interorbital is shorter than the width of upper eyelid; tubercle formed on inner metatarsal; tip of snout contains tibiotarsal articulation; dorsal side is smooth; tips of toes are somewhat swollen, completely webbed; SVL is about 45.1-73.7 mm (Tables 1, 2 and Figure 1E, J).

Ordination on the bases of morphological characterization of amphibians' fauna

Cluster analysis

Phylogenetic tree was made according to morphometric data. This tree was made up of two clades, I and II. Two species of toads; *Duttaphrynus stomaticus* and *Duttaphrynus melanostictus* were clustered in clade I due to close similarity. Three species of frogs; *Euphlyctis cyanophlyctis, Hoplobatrachus tigerinus* and *Nanorana liebigii* were grouped in clade II on the bases of close relation with each other (Figure 2).

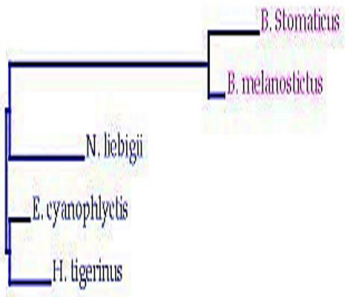

Figure 2: Phylogenetic tree based on the morphometric characters.

Principal coordinate analysis (PCoA)

PCoA plot was generated on the basis of morphometric data of amphibian's fauna showed the close resemblance among collected species of amphibians from different localities. According to this graph species of toads; *Duttaphrynus stomaticus* and *Duttaphrynus melanostictus* were plotted in the same region (0 to 0.8). The species of frogs; *Euphlyctis cyanophlyctis* and *Hoplobatrachus tigerinus* were plot in the region of (0 to -0.4) due to the close resemblance with each other. *Nanorana liebigii* showed a small difference from rest of two species so that it was plotted in the region (0 to -0.6) horizontally (Figure 3).

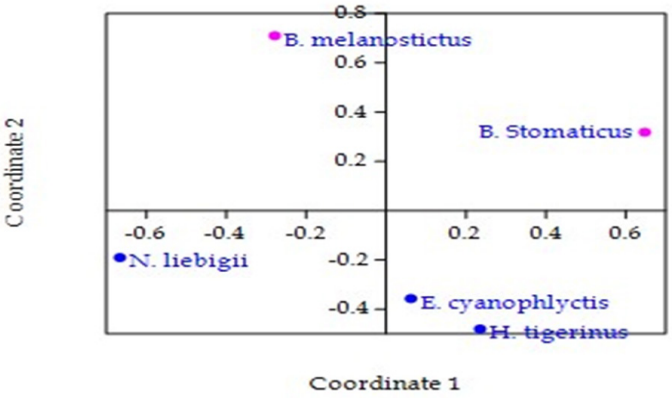

Figure 3: PCoA graph showed the variation among anurans on the basis of morphometric data.

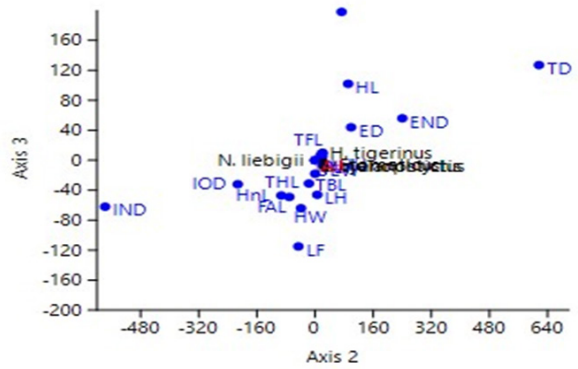

Figure 4: DCA graph on the basis of morphometric variations.

Detrended coordinates analysis (DCA)

For detrended coordinate analysis morphometric parameters like HL, HW, ED, END, IND, IOD, TD, SVL, THL, TBL, TFL, PW, PL, LF, LH, UEW, LH, FAL and HnL were used. In this graph TD, LF and IND are slightly positive correlated with each other. This graph showed the morphometric parameters of frogs; *Euphlyctis cyanophlyctis*, *Hoplobatrachus tigerinus* and *Nanorana liebigii* (Figure 4).

Ordination of amphibians' fauna on the basis of environmental variable

The samples of water were collected from different water channels of the district and ecological parameters like temperature (°C), total dissolve solids (TDS), electric conductivity (EC), dissolve oxygen (DO), PH, temperature (air) and humidity of terrestrial habitat were also measured (Table 3).

Detrended correspondence analysis (DCA) ordination of ponds

DCA ordination of all collected specimens of amphibians from various water bodies of Abbottabad District showed that some of ponds like Kalapul, Samandarkata and Nathiagali showed that some of the ecological parameters; AT, WT, PH, DO and HU. EC and TDS show negative correlation with rest of parameters (Figure 5).

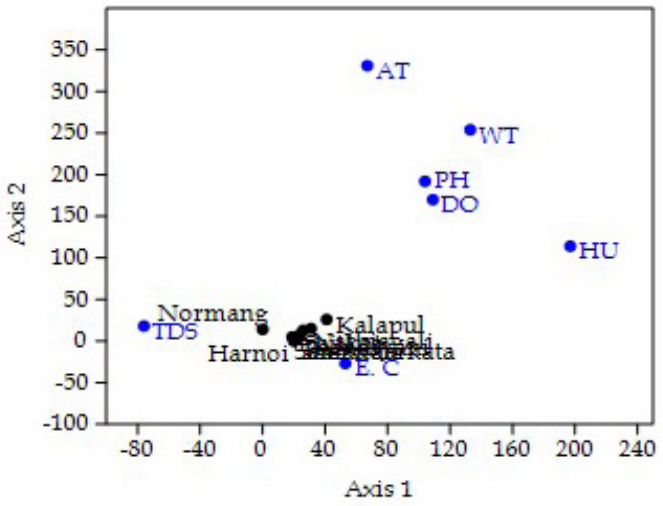

Figure 5: DCA graph represented the pond ordination of Abbottabad District.

Table 3: Ecological and limnological parameters of district Abbottabad from June, 2019 to August, 2020.

S. No	Parameters	Standard*	Localities							
			Sajikot	Sher-wan	Nathiagali	Thandi-yani	Harnoi	Nor-mang	Kala-pul	Saman-darkata
1.	Electrical Conductivity (µS/cm)	1000	617	961	619	793	1440	636	402	1069
2.	PH	7.0-8.5	5.9	7.0	7.2	7.6	7.8	7.6	7.1	7.7
3.	Total Dissolved Solids mg/L	500	290	452	291	373	677	662	189	567
4.	Dissolved Oxygen (mg/L)	3	2.92	2.98	1.69	1.86	2.91	2.79	2.88	1.69
5.	Humidity %	-	44	65	82	56	77	48	88	76
6.	Air Temperature (°C)	-	33	25	30	33.3	27.5	35.8	23.4	32.6
7.	Water Temperature (°C)	-	28.4	30.3	26.1	25	26.2	25.3	27.9	24.4

* There is no specific standard value for humidity, air and water temperatures.

Principle coordinate analysis (PCoA) of environmental variables

On the basis of morphometric data PCoA graph was plotted. This graph showed correlation among different water bodies. Water parameters of Samandarkata, Sherwan and Harnoi showed the close resemblance due to the same environmental conditions. Sajikot, Thandiyani, Kalapul and Nathiagali showed close correlation with each other on the basis of environmental similarities. While Normang showed different environmental condition as compared to other localities (Figure 6).

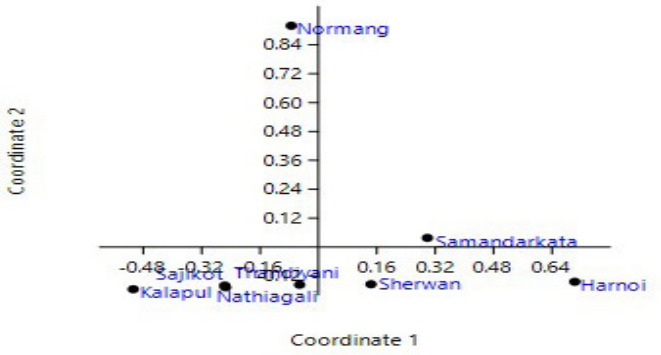

Figure 6: PCoA graph showed the Coordination among environmental variables based on morphometric data.

Neighbor joining clustering of localities and environmental variables

The Pond localities of Nathiagali, Kalapul and Sajikot showed the similarity in environmental conditions, while Harnoi, Samandarkata, Sherwan, Normang, Thandiyani share the similar environmental conditions (Figure 7).

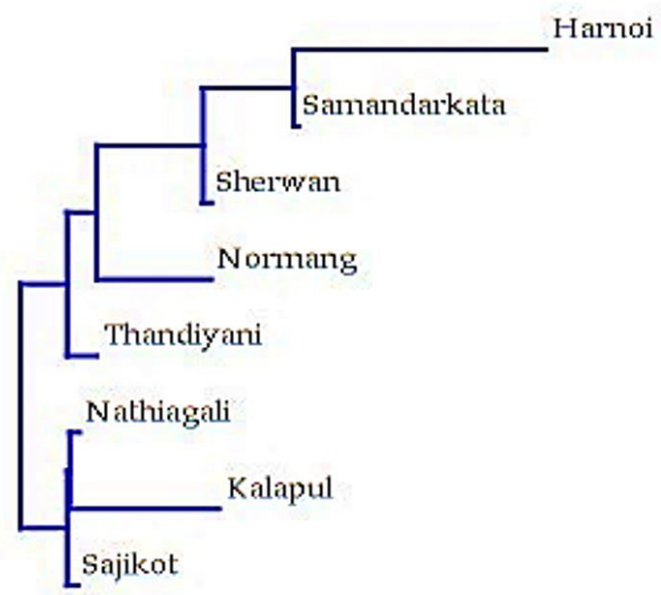

Figure 7: Neighbor joining cluster of localities and environmental variables of district Abbottabad.

As amphibians are an important part of number of species of reptiles, birds and mammals, as well as the main predators for number of groups of insects. So, their extinction will have profound impacts on a large part of the food chain. First specie of Amphibians isolated from District Abbottabad was *Duttaphrynus stomaticus*. According to the morphometric measurements of *Duttaphrynus stomaticus*, it's Head length (HL) was 13.7±1.6mm, Tarsus foot length (TFL) was (27.6±3.4mm), Hand length (HnL) (8.0±1.0mm), Snout vent length (SVL) (50.4±5.9mm), Tympanum diameter (TD) (3.7±0.7mm) and Eye diameter (ED) was (6.1±1.3mm). These results were similar to the study conducted by Awan, 2019 according to which HL (14.1±2.7mm), TFL (26.0±4.4mm), HnL (11.2±1.6mm), SVL (57.3±9.3mm), TD (3.9±0.7mm) and ED (5.7±0.8mm) were measured during examination. Similar study conducted by Ali *et al.* (2017), and found the similar results as mentioned in the above study that is their HL was measured (1.82±0.266cm), TFL (2.06±0.487cm), HnL (c1.21±0.451m), SVL (5.60±0.694cm), TD (0.35±0.051cm) and ED (0.40±0.079cm).

Second species of amphibians isolated from district Abbottabad was *Duttaphrynus melanostictus*. According to the morphometric measurements of *Duttaphrynus melanostictus,* its interorbital distance (IOD) was 2.1-6.1mm, Tympanum diameter (TD) was 3.1-6.9mm. Tabial length (TBL) 19.9-38.1mm, Snout vent length (SVL) 48.9-98.1mm, Upper eye width (UEW) 4.1-6.9mm, Parotid gland width (PW) 4.1-9.1mm, Parotid gland length (PL) 8.1-24.1mm, Head length (HL) 13.9-25.1mm and Head width (HW) was 17.9-35.9mm. These results were similar to the study conducted by Awan, 2019 according to which IOD (4.0-8.0mm), TD (4.0-6.0mm), TBL (18.3-39.0mm), SVL (41.3-98.5mm), UEW (3.5-6.0mm), PW (4.3-10.0mm), PL (.0-23.0mm), HL (15.4-29.0mm) and HW (23.0-42.3mm) were measured during examination. Similar study conducted by Khan, 2001 and similar results as mentioned in the above study that is their IOD was measured (10-11(♂) (♀), TD (4-5 (♂) (♀), TBL (30-34(♂) (♀), SVL (65-80(♂)80-95(♀), UEW (6-7(♂) (♀), PW (9-10(♂)8-10(♀), PL (13-22(♂)20-24(♀), HL (23-25(♂)23-27(♀) and HW (24-27(♂)23-36(♀).

Third species of amphibians isolated from District Abbottabad was *Euphlyctis cyanophlyctis* sensu lato. According to the morphometric measurements of *Euphlyctis cyanophlyctis* sensu lato, its Eye diameter (ED) was 6.2±1.2mm, Tarsus foot length (TFL) was 35.4±5.4mm, Tympanum diameter (TD) 4.3±0.9mm, Head length (HL) 16.1±2.8mm, Snout vent length (SVL) 50.7±8.1mm and Hand length (HnL) 9.0±1.8mm. These results were similar to the study conducted by Awan, 2019 according to which ED (6.2±0.7mm), TFL (30.4±4.9mm), TD (5.0±0.7mm), HL (14.5±1.9mm), SVL (56.3±8.1mm) and HnL (12.7±2.3mm) were measured during examination. Similar study conducted by Ali *et al.* (2017), and found the similar results as mentioned in the above study that

is their ED (3.47±0.458mm), TFL (22.77±0.909mm), TD (3.52±0.475mm), HL (14.75±0.761mm), SVL (44.60±2.197mm) and HnL (11.99±0.381mm).

Fourth species of amphibians isolated from District Abbottabad was *Hoplobatrachus tigerinus*. According to the morphometric measurements of *Hoplobatrachus tigerinus* its Eye diameter (ED) was 6.5±1.2mm, Head length (HL) 17.4±3.6mm, Hand length (HnL) 8.1±2.1mm, Snout vent length (SVL) 48.5±11.2mm, Tympanum diameter (TD) 4.1±0.7mm and Tarsus foot length (TFL) 33.3±9.3mm. These results were similar to the study conducted by Awan, 2019 according to which ED (9.6±2.2mm), HL (29.8±9.2mm), HnL (17.8±4.4 mm), SVL (90.0±19.4mm), TD (7.7±1.9mm) and TFL (52.5±15.0 mm) were measured during examination. Similar study conducted by Ali *et al*. (2017), and found the similar results as mentioned in the above study that is their ED (4.90±0.74mm), HL (28.10±5.92mm), HnL (15.13±1.62mm), SVL (81.90±22.13mm), TD (7.32±1.57mm) and TFL (36.60±6.54 mm).

Fifth species of Amphibians isolated from District Abbottabad was *Nanorana liebigii.* According to the morphometric measurements of *Nanorana liebigii*, its Snout vent length (SVL) was 55.5±11.7mm, Eye diameter (ED) 7.1±0.4mm, Tibial length (TBL) 33.1±5.9mm and Head width (HW) 21.9±4.6mm. These results were similar to the study conducted by Awan (2019) according to which SVL (62.4mm), ED (6.0mm), TBL (36.8mm) and HW (24.8mm) were measured during examination. *D.stomatics* is national toad of Pakistan so that is why it is commonly present throuhtout the country.

Conclusions and Recommendations

Based on our findings, we have concluded that five species of anurans were reported from the District Abbottabad, including two species of toads, *Duttaphrynus stomaticus* and *Duttaphrynus melanostictus*, and three species of frogs, *Euphlyctis cyanophlyctis*, *Hoplobatrachus tigerinus*, and *Nanorana liebigii*. This study discovered that *Duttaphrynus stomaticus* is common in District Abbottabad, while *Duttaphrynus melanostictus* and *Euphlyctis cyanophlyctis* are also present although in lesser numbers. *Hoplobatrachus tigerinus* and *Nanorana liebigii* were the two species with the fewest records from Abbottabad.

Acknowledgements

Dr. Inamullah's hands-on effort and input are gratefully acknowledged by the author. The success of this research would not have been feasible without him.

Conflict of interest

The authors have declared no conflict of interest.

References

Ali, W., Javid, A., Khan, W.A., Hussain, A., Rizwan, M., Ameer, M. and Sajid, A.Q. 2017. Diversity and habitat preferences of herpetofauna at Kalabagh game reserve, district Mianwali, Punjab, Pakistan. *Russian J. Herpetol.,* **24**(4): 267-274.

Awan, F.S. 2019. Biosystematics of amphibians of Haripur district with additional notes on amphibians of Northern Pakistan. M. Phil thesis. Department of Zoology, Hazara Unversity Mansehra-Pakistan.

Baig, K.J., Awan, M.R. and Ashraf, N., 2006. Ecological studies and zoogeographic affinities of the amphibians and reptiles found in Chagai desert, Balochistan, Pakistan. *Pakistan J. Zool.*, **38**: 145-151.

Baig, K.J., Masroor, R. and Arshad, M., 2008. Biodiversity and ecology of the herpetofauna of Cholistan Desert, Pakistan. *Russ. J. Herpetol.*, **15**: 193-205.

Barbour, M.T., Gerritsen, J. and Snyder, B.D., 1999. *Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish*. US Environmental Protection Agency, Office of Water Washington, DC.

Cushman, S.A., 2006. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biol. Conserv.*, **128**: 231–240. https://doi.org/10.1016/j.biocon.2005.09.031

Daniel, J.C., 1963a. Field guide to the amphibians of weastern India. Part I. *J. Bombay Nat. Hist. Soc.*, **60**: 415-438.

Dubois, A., 1975. Un nouveau sous-genre (*paa)* et trois nouvelles espèces du genre Rana. Remarques sur la phylogènie des Ranidès (Amphibiens, Anoures). *Bull. Mus. Nat. Hist. Nat.*, **3**: 1093-1115.

Duellman, W. and Zug, R.G., 2012. *Anura*. Encyclopedia Britannica Online.

Fahimi, H., Broomand, S., Mashayekhi, M. and Kazemi, M., 2015. *The herpetofauna of Iran: Checklist of taxonomy, distribution and conservation status*.

Frost, D.R., 2013. Amphibian species of the world: http://research.amnh.org/herpetology/amphibia/index.htm1. New York: American Museums of Natural History.

Frost, D.R., 2016. *Amphibian species of the world: An online reference*. Version 6.0. New York: American Museum of Natural History.

Galton, V.A., 1992. The role of thyroid hormone in amphibian metamorphosis. *Trends Endocrinol. Metab.*, **3**: 96-100. https://doi.org/10.1016/1043-2760(92)90020-2

GOP, 1999. *Biodiversity action plan of Pakistan*. Islamabad: Ministry of Environment/IUCN/WWF.

IUCN Pakistan, 2004. State of the Environment and Development. IUCN

Karr, J.R., 1991. Biological integrity: A long-neglected aspect of water resource management. *Ecol. Appl.*, **1**: 66–84. https://doi.org/10.2307/1941848

Khan, M. and Malik. S., 1987b. Reproduction strategies in a subtropical anuran population in arid Punjab, Pakistan. *Biologia*, **33**: 279-303.

Khan, M.S., 2002. A checklist and key to the Amphibia of Pakistan. *Bull. Chicago Herpetol. Soc.*, **37**: 158-163.

Khan, M.S., 2004. Annotated checklist of amphibians and reptiles of Pakistan. *Asiatic Herpetol. Res.,* **10**: 191-201.

Khan, M.S., 2006. *Amphibians and reptiles of Pakistan*. Malabar, Florida, USA: Krieger Publishing Company.

Khan. M.S., 1999b. Food particle retrieval in amphibian tadpoles. *Zoo Print J.*, **14**: 17-20. https://doi.org/10.11609/JoTT.ZPJ.14.5.17-20

Masroor, R., 2012. A contribution to the herpetology of northern Pakistan. *SSAR, Ithaca Rev. JoTT,* **4**: 2670. https://doi.org/10.11609/JoTT.o3218.2670-2

Minton, S.A., 1962. An annotated key to the amphibians and reptiles if Sind and Las Bela, West Pakistan. *Am. Mus. Novit.*, **2081**: 1-21.

Petrov, B., 2004. The herpetofauna (Amphibia and Reptilia) of the eastern Rhodopes (Bulgaria and Greece). *Biodiv. Bulgar.*, **2**: 863-879.

Rahman and Tariq, 1996. *Language and politics in Pakistan*. Oxford University Press, pp. 211-214.

Sarkar, A.K., 1984. Ecological studies on the amphibians of Gujarat. *Bull. Zool. Soc. India*, **6**: 87-93.

Shah, K.B. and Tiwari, S., 2004. *Herpetofauna of Nepal.* In: World conservation congress 2004: Bangkok, Thailand. IUCN, Nepal.

Shi, Y.B., 2000. *Amphibian metamorphosis: From morphology to molecular biology*. New York, NY, USA: John Wiley.

Smith, M., 1943. *The Fauna of British India, including ceylon and burma*. Reptilia and Amphibia.Vol II: Sauria. Taylor and Francis Ltd, London.

Sparling, D.W., 2010. Water-quality criteria for amphibians. In: (ed. Dodd Jr.) amphibian ecology and conservation: A handbook of techniques. Oxford University Press, New York. https://doi.org/10.1093/oso/9780199541188.003.0007

Stebbins, R.C. and Cohen, N.W., 1995. A natural history of amphibians. Princeton University Press. https://doi.org/10.1515/9780691234618

Watters, J.L., Cummings, S.T., Flanagan, R.L. and Siler, C.D., 2016. Reviw of morphometric used in anurans species descriptions and recommendations for a standardized approach. Sam Noble Oklahoma Museum of Natural History: University of Oklahoma. https://doi.org/10.11646/zootaxa.4072.4.6