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DIVERSITY, ECOLOGY AND CONSERVATION OF AMPHIBIANS (FAMILY RANIDAE: RAFINESQUE, 1814 AND BUFONIDAE: GRAY, 1825) IN PAKISTAN

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Abstract

Amphibians are amongst most neglected animals in Pakistan. Their ecology and conservation status has been abandoned so far, hence amphibians are found dealing with numerous threats, meanwhile, taxonomic status of amphibians in Pakistan is of great concern due to morphological variation. Sindh (province of Pakistan) is one of the most important abodes of amphibians where wetlands and agricultural fields offer them abundance of food and shelter. For the study of ignored amphibian fauna, we conducted a comprehensive survey to elucidate the distribution of different amphibian species in different habitats. The physical characteristics of ecology such as types of vegetation and predators were observed, while water samples were collected from all aquatic habitats for analysis of parameters i.e. pH, EC, TDS, T. Hard. T. Alk, Cl^-, SO_4^{2-}, PO_4^{3-}, NO_2^- NO_3^-, and CO_2 by using analytical instruments and standard procedures. All the habitats were highly contaminated and unfavorable especially for spawns and larvae and their conservation status was found entirely deteriorated. It was determined that species exhibited minor morphological variation devoid of divergence form taxonomic grade. it was obvious that the diversity of amphibian fauna was very poor, however systematic of each species was arranged methodically.

Keywords*: True frogs; True toads; Taxonomy; Diversity; Habitats; Pollution; Protection; Pakistan*

Introduction

Class Amphibia is a group of more than 6,500 species represented by three orders (Anura, Apoda and Urodela). Order Anura is only order that exist in Pakistan. Ranidae is one of 33 families of order Anura representing 750 species contained by 61 Genera. According to an assessment, most of the amphibian species are threatened [1]. Different species of class amphibia existing in different regions of world exhibit morphological variation and these morphologically variable species sometimes evolve into new species placed in divergent taxonomic categories [2]. For the determination of diversity, morphology plays crucial role for proper identification of species. The pattern of distribution of amphibians varies on the basis of their ecology. Their distribution and abundance also vary even in adjacent areas that share same border [3]. It is prominently established that amphibian decline is rapid in the areas where

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pollution is high. Pollution in aquatic habitats affects growth and physiological development depends on the quality of their aquatic ambient [4].

Amphibians are economically valued animals as they benefit food web, agriculture, medicine, ecosystem, etc. [5-6]. They are exploited as satiating source of food at the local and even at international level. Amphibians are traded as export in some countries of world. In agricultural feeds, they are introduced reduce the population of crop pest such as insects [3]. Tadpoles are herbivorous in feeding nature and they reduce the unnecessary herbs and shrubs in water bodies. Amphibians are also valued for wound healing efficiency, pharmaceutical and therapeutic potential of their skin [7]. Because of their economic importance, amphibians have gained much importance for their problematic status worldwide since past two decades. It is evident that the ecological problems destroy habitats of wild animals especially due to anthropogenic activities and lack of interest of wildlife agencies [6].

Their decline indicates problematic status of natural processes because amphibians are highly sensitive to environmental changes [8]. Decline in amphibian diversity and their reduced population are issues of great concern worldwide. Amphibians are considered to have undergone decline significantly since 1950. It is estimated that there are about more than one third threatened species of class amphibia and over one hundred and twenty species have declined so far. Water pollution is a main reason of amphibian decline and mortality [6]. Dreadful ecological changes affect amphibians considerably and are greatly considered as a major reason for the decline of amphibians in most regions of the world. Existence of predators may also lessen the population of amphibians and may lead them towards mortality. Predators mainly feed on tadpoles and even adult amphibians which eventually become food of potential predators [8-10].

Considering the threatened status of amphibian fauna and their habitats, present study was proposed to explore diverse forms of amphibians with deep concentration on their systematic status based on morphological variation. This study was mainly focused on divulging into problems that amphibians face and revealing their status as protected or unprotected. Furthermore, this study was carried out to bring a novel, distinguished and comprehensive knowledge about amphibians of Pakistan.

Experimental part

Sindh province offers different types of habitats to different group of animals. Amphibians are special group of vertebrates that have adapted to survive in many different types of habitats of Sindh (Fig. 1). The study sites, their geographical area, Global positioning System (GPS) and types of habitats are detailed in table 1.

The physical characteristics of each study site were observed, while gross water samples were collected from aquatic habitats into in stoppered polyethylene plastic bottles which were analyzed by following systematic and scientific methods [11-14]. pH was analyzed using pH meter (Orion, 420), however electric conductivity (EC) and total dissolved solids (TDS) were evaluated using conductivity meter (Orion 115). Titration procedures were adopted for analysis of total hardness (T-Hard), total alkalinity (T-Alk), chloride (Cl) and carbon dioxide (CO_2). Ultra-violet spectrophotometer (Hitachi 200) was used for analysis of sulphate (SO_4^{2-}), phosphate (PO_4^{3-}), nitrite (NO_2^-) and nitrate (NO_3^-). Status of amphibian habitats was identified via scientifically approved water quality criteria [11]. The species were identified using morphometry; identification keys and catalogues [15-17].

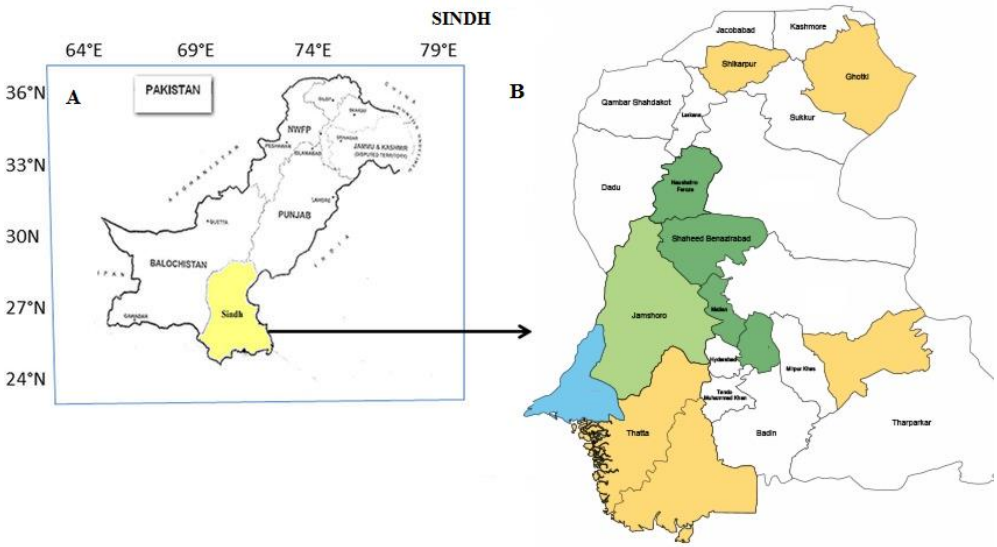

Fig. 1. (A) Map of Pakistan, highlighting location of Sindh province. (B) Map of Sindh with indication of study sites where presence of amphibians was recorded and ecological conditions were analyzed

Table 1. Details about study sites and habitats where live amphibian specimens were sighted. Name of study sites is followed by their geographical area. Global positioning System (GPS) of each site is given along different types of habitats that harbored amphibians

Study Sites	Area (2^{km})	GPS coordinates	Types of habitats
Badin	6,726	24°40′N 69°00′E	Ponds, marshes, bogs
Dadu	19,070	26°20′N 67°35′E	Ponds, marshes, bogs, springs, rough grassland
Ghotki	6975	27°49′N 69°39′E	Ponds
Hyderabad	3,198	25°15′N 68°45′E	River, Ponds, marshes, springs
Jamshoro	11,517	25.43212°N68.263171°E	Bogs, River, Ponds, marshes, springs
Jacobabad	5278	28°16′48″N 68°25′48″E	Ponds
Kashmor	2592	28°26′00″N 69°35′06″E	Ponds, shrub land, marshes, bogs, springs, rough grassland
Larkana	7423	27°33′36″N 68°13′35″E	Lakes, river, ponds, bogs, heathland
Matiari	3035.142	25°36′00″N 68°26′24″E	Ponds
Mirpurkas	2991	25°33′02N 069°00′11E	Shrub land, heathland, rough grassland, Ponds, marshes, bogs
Naushahro Feroze	2,945	26.8463° N, 68.1253° E	Ponds, marshes, shrub land, scrub land
Qambar Shahdadkot	5,599	27.5859° N, 68.0060° E	Lakes, streams, ponds, meadows, scrubland, shurb land, springs
Shaheed Benazirabad	4,502	26°35′N 68°10′E	Ponds, shrub land
Shikarpur	2512	28°00′N 68°40′E	Ponds, shrub land, bogs
Sukkur	5165	27°40′N 69°30′E	Ponds, shrubland
Tharparkar	19,638	24°44′24″N 69°48′00″E	Desert, shrub land, scrub land
Thatta	7,705	24.7475° N, 67.9106° E	Lakes, streams, ponds, springs, shrub land, scrubland

Results and discussion

Among collected specimens of amphibians, immature forms and juveniles could not be identified up to species level, whereas adult specimens were identified as *Hoplobatrachus tigerinus* (Daudin, 1802), *Euphlyctis cyanophlyctis* (Schneider, 1799), *Allopa hazarensis* (Dubois and khan, 1979) and *Duttaphrynus stomaticus* (Lutken, 1864) (Fig. 2).

Fig. 2. Diverse species of amphibians in Sindh. (A) *Hoplobatrachus tigerinus*, (B) *Euphlyctis cyanophlyctis*, (C) *Allopa hazarensis*, (D) *Duttaphrynus stomaticus.*

Species of family Ranidae were found to possess distinct characteristics due to their highly aquatic nature as compared to *D. stomaticus* that belongs to family Duttaphrynusnidae. *H. tigerinus* was observed adopting variable body colors in different habitats (Fig. 1); however, *E. cyanophlyctis* and *A. hazarensis* were yellowish brown and grey respectively (Fig. 2). *D. stomaticus* was light brown with numerous pores and dark blotches all over the dorsum. *H. tigerinus* was characterized with longitudinal folds and mid-dorsal line with numerous dark blotches on dorsum and limbs. Other species did not exhibit latero-dorsal folds and mid-dorsal line. However dorsal side of *E. cyanophlyctis* was observed devoid of any spackles with exception of few specimens. Skin of *D. stomaticus* was highly rough consisting of numerous pores; parotid glands were visible. Head was moderate in *H. tigerinus* and *E. cyanophlyctis*; though it was slightly longer in *A. hazarensis*. Snout was round and pointed in *E. cyanophlyctis* and it was projecting beyond the mouth in *A. hazarensis*. Canthus rostral was found obtuse in *H. tigerinus*, indistinct in *E. cyanophlyctis* and round in *A. hazarensis*. Tympanic membrane was distinct in all ranids and *D. stomaticus*.

H. tigerinus possessed moderate sub-articular tubercles; inner metatarsal tubercle was strongly compressed. *H. tigerinus and E. cyanophlyctis* possessed narrow inter-orbital space. *H. tigerinus* and *E. cyanophlyctis* were characterized with short and pointed fingers that were slender in *A. hazarensis*. Sub-articular articulation and inner metatarsal tubercle were small sized in *E. cyanophlyctis* that was found devoid of nuptial spines. Loreal region of *A. hazarensis* was strongly concave and it also possessed a small vestige of pineal ocellus. Extensively webbed feet were observed in all the species of family Ranidae, except *D. stomaticus* because of their less aquatic nature. Distinct characteristics of *D. stomaticus* included lack of cranial crests and smooth skin, however tibial and parotid glands were present. Tympanic membrane was distinct and two-thirds the diameter of the eye of *D. stomaticus*. Loreal space was broader than the upper eyelid and a spiny ridge on the tarsus was also observed. First and second fingers were approximately equal with a single sub-articular tubercle. Population of *D. stomaticus* had previously been recorded from District Larkana to possess a distinct characteristic of red round dot in mid of palm of forelimbs and red claws [8-9]. Present investigation confirmed the prevalence of same variable characteristic in population of *D. stomaticus* in same area (Fig. 3).

Findings of present study show that among about 6,500 amphibian species worldwide (Frost, et al. 2006) and 24 species reported from some areas of Pakistan (Khan, 2008), only four species exist (Fig. 2) in heavily agricultural area of Sindh province where a high number of aquatic and terrestrial habitats are available for biodiversity. Body weight of male and female specimens was determined different as followed: *A. hazarensis* 28.6±2.7 (♀) and 23.0±1.9 (♂), *H. tigerinus* 254.6±3.91 (♀) and 172.4±61.2 (♂), *E. cyanophlyctis* 36.8±1.9 (♀) and 34.1±2.0

(♂), *D. stomaticus* 29.31±0.95 (♀) and 26.47±1.12 (♂). Body size of both sexes was also recorded different i.e. *A. hazarensis* 49.3±0.7 (♀) and 46.8±1.7 (♂), *H. tigerinus* 136.2±3.0 (♀), 111.7±10.3 (♂), *Euphlyctis cyanophlyctis* 55.1±3.5 (♀), 48.6±1.7 (♂), *D. stomaticus* 44.59±0.77 (♀) and 40.63±0.93 (♂).

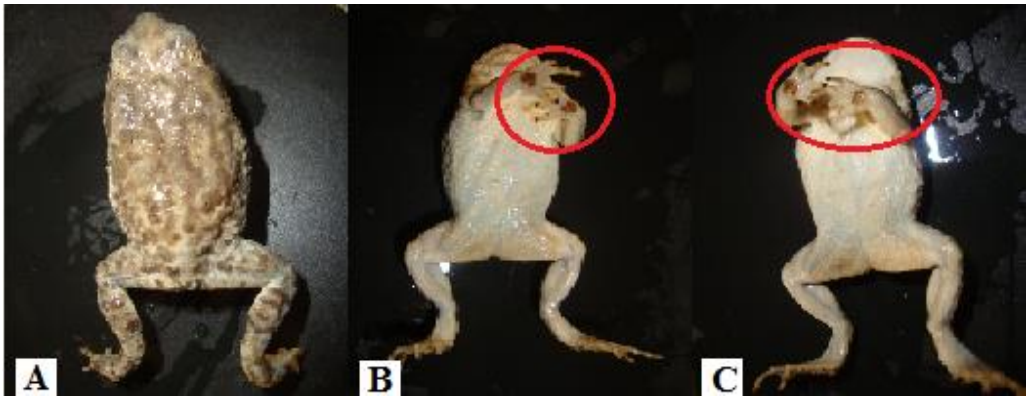

Fig. 3. *Duttaphrynus stomaticus* collected from district Larkana, (A) Dorsal image (B), ventral view highlighting palm of forelimbs having a round red blotch in mid of palm and red colored claws (C), forelimbs view of another specimen with red colored patch in mid of palm and red claws.

Amphibians were found to survive in different habitats such as bogs, grassland, lakes, marshes, ponds, rivers, scrub land, shrub land, springs, streams, swamps. Among so many types of habitats, ponds especially in agricultural areas were found having abundant population of amphibians. Vegetation present in premises of amphibian habitat in Sindh included *Salvadora oleoides* (Khabar), *Prosopis cineraria* (Kandi), *Tamarix dioica* (Laya*), Tamarix aphylla* (Lai), *Typha latifolia* (Pan), *Typha elephantiana* (Elephant grass), *Typha domingensis* (Cattail), *Phragmites karka* (Common Reed), *Ipomoea aquatic* (Leaf vegetable) *and Salvinia molesta* (kariba weed). All ranids were observed dwelling near aquatic bodies, whereas *D. stomaticus* was mostly found in dry habitats especially in desert area of Thar. Amphibians were found settled near terrestrial area for the vegetation appropriate for the concerned **species**. A Habitat is an ecological or environmental area that is inhabited by a particular Species of Animal, Plant or other type of organism. Ecological condition of study area was determined as unstable for amphibians due to presence of potential predators. Amphibians prayed by predators in aquatic environment include *Anastomus oscitans* (Asian openbill), *Catla catla* (Thaili), *Ciconia ciconia* (whilte strok), *Ciconia nigra* (black stork), *Labeo rohita* (Rohu), *Labeo calbas* (Dahi), (*Osteobarma catio (*Makhni), *Puntius ticto* (Popri), *Cirrhinus mirgala* (Morakhi), *Psammophis leithi* (Ribbon snake), *Ptyas mucosus* (Common rate snake), and *Typhlopes porrects* (Slender blind snake). Terrestrial predators of amphibians include: *Corvus splendens* (house crow), *Python Molurus* (Indian Python), *Naja Naja* (Indian or Spectacled Cobra), *Echis Carinatus* (Saw Scaled Viper or Carpet Viper), *Vipera Russelli* (Russell's viper), *Bungares Caeruleus Sindanus* (Indian or Sind Krait), *Lutra lutra* (Eurasian or common otter). Analysis of amphibian primary "aquatic" habitats revealed poor quality as shown in Table 2.

Study of physico-chemical parameters showed an unstable condition of amphibian habitats. Value of almost all parameters except pH and CO_2 was recorded extremely high than recommended level. Hydrogen ion concentration indicated by pH (6.5-9.2) and carbon dioxide value (12.0-26.0mg $L^{-1)}$ were within normal limit [18]. Meanwhile value of EC (1180.5-4769.0 µS cm^{-1}) was entirely above the recommended limit (150.0-500.0µs/cm). EC shows presence of dissolved electrolytes in form of total dissolved solids and total hardness that conduct heat efficiently across the water [19-20]. In this context, concentration of TDS and T. Hard were also

as high as 788.8-3280.5mg·L^{-1} and 200.5-960.3mg·L^{-1} respectively. Total alkalinity consists of Total Dissolved Solids (TDS) and total concentration of bases and in water. It plays significant role in resisting changes in pH and its value into amphibian habitats was found from 175.2 to 470.0mg·L^{-1} regarded as dreadful especially for eggs and larvae [12]. Cl concentration (200.2-690.2mg·L^{-1}) was also beyond favorable limit alike sulphate that was measured within 200.8 to 817.9mg·L^{-1}, considered as extremely above the normal level which is strongly recommended to be maintained within 50-100mg/L for survival of aquatic animals [11-12]. Other non-metallic parameter PO_4^{3-} was analyzed to occur in habitats from 200.0 to 673.5mg·L^{-1} that also did not meet the standard criteria for water quality for amphibians [21]. Minimum and maximum values of NO_2^- and NO_3^- were evaluated respectively within 0.9-19.5mg·L^{-1} and 1.3-20.6mg·L^{-1}.

Table 2. Physico-chemical analysis of amphibian habitats

Parameters	Value
pH	7.8±0.7
EC µS cm^{-1}	2559.4±1087.0
TDS mg L^{-1}	1759.6±677.1
T-Hard mg L^{-1}	1759.6±677.1
T-Alk mg L^{-1}	1759.6±677.1
Cl mg L^{-1}	447.9±120.7
CO_2 mg L^{-1}	8.6±4.0
SO_4 mg L^{-1}	475.6±162.0
PO_4 mg L^{-1}	475.6±162.0
NO_2 mg L^{-1}	5.7±3.5
NO_3 mg L^{-1}	8.6±4.0

Concentration of physico-chemical parameters make water either safe or hazardous according to their level. High EC, TDS and T. Hard are harmful for spawns and tadpoles of amphibians that can be severally damaged [19]. The eggs and larvae and juveniles also appear to be very sensitive to high EC and TDS levels which can gradually kill them through desiccation or dehydration [20]. Exceeded level of T-Hard may also cause negative impacts on physiological functions of amphibians [22]. The eggs and larvae also appear to be very sensitive to too high or too low alkalinity that leads to failure to resist changes in pH [23]. Range of T. Alk was recorded from 145.5 to 537mg, whwereas Cl^- was dissolved in habitats from 175.3 to 745.1mg. High quantity of Cl^- is also toxic to amphibians as it threatens the health, survival, growth, and reproduction mainly by damaging their osmoregulation. SO_4^{2-} and PO_4^{3-} are also very influential parameters as they equally increase the eutrophication and increases population of algae that creates oxygen shortage for all the aquatic animals inhabiting same water body [24]. Sindh offered amphibian habitats consisting of 200-817.9mg/L of SO_4 and 150.8-800mg of $PO4^{3-}$. Nitrogen fertilizers may also contribute to pollute water of amphibian habitats. Value of NO_2^- 0.1-19.5mg/L and NO_3^- 1.0 to 20.6mg/L may induce behavioral and morphologic changes into them [25].

High value of analyzed physico-chemical parameters may occur as a result of atmospheric deposition, surface and ground water runoff or biological degradation of organic matter. Therefore, throw of unhygienic things such as garbage, plastic bottles and excessive use of toxic fertilizers, insecticides, pesticides and herbicides should be avoided to keep water bodies clean especially of those areas where wild animals inhabit.

Conclusions

Amphibians face major threats due to negligence towards saving wildlife. Habitat destruction, potential predators and water contamination have altogether deteriorated the amphibian diversity and have limited their distribution. Due to large number of threats in form

of predators and pollution, population of species is decreasing rapidly. Sindh offers one of the most important abodes to amphibian species, where it is very important to take efforts for saving amphibians. Unfortunately, wildlife of Sindh is poorly studied, and therefore, most ignored species get lost even without being recognized. In this context, there is need of conducting surveys to establish checklist of species for better understanding of their existence and to elucidate their distribution, status, ecology, threats, and conservation. Based on our observations and analysis, we recommend for the conservation of amphibian fauna and their habitats in wild environment of Sindh province.

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