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RESEARCH ARTICLE

A Comparative Study of Morphology and Ecology of *Bufo stomaticus* (Lutken, 1864), (Anura: Bufonidae) from District Larkana and Shikarpur, Pakistan

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In present investigation, characteristics of Morphology and Ecology of *Bufo stomaticus* (Lutken, 1864), (Anura: Bufonidae) populations in two adjacent Districts viz Larkana and Shikarpur of Pakistan have been studied comparatively from March to September 2012 to record morphological and ecological difference between them and also to know which district provides better environmental conditions to support their survival. Literature related with Taxonomy and water quality helped in determination of present study. Physical appearance of both populations observed to be significantly different from each other but morphometric was measured to be relatively same. Habitats of *B. stomaticus* were analyzed by Physico-chemical parameters viz: pH, conductivity, Total dissolved solids, Chloride, Calcium, Magnesium, Potassium and Iron. Most of the parameters were analyzed to be significantly different but within favorable level except Conductivity in both Districts.

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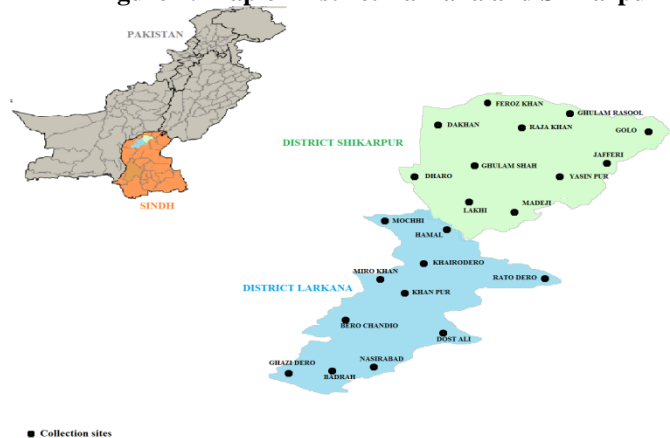
Introduction

Bufo stomaticus (Lutken, 1864), (Anura: Bufonidae) first discovered by Lutken in 1864 from Assam India, is most distributed toad species in Pakistan reported by several researchers including Khan and Mufti, 1994; Khan and Ahmed, 1987; Khan, 1987 and 1968; Daniel, 1963 as light brown, grey or olive to almost black in dorsal body color with small ring like dark mottling reticulation. The parotid gland is longer than broad and a distinct tibial gland is also present. It lacks cranial crests. Inter-orbital space is little broader than upper eyelid and tympanum is distinct and round.

Larkana (7,423 km²) and Shikarpur (2,512 km²) are two main Districts of Sindh Province sharing a border line between them (Fig. 1). The morphology and ecology of *B. stomaticus* populations in these adjacent areas were studied comparatively to evaluate and record possible similarities and dissimilarities between them.

District Larkana is studied previously by Kalsoom, et al. 2013 that recorded different amphibian species including *B. stomaticus* but District Shikarpur is delved for the first time for present study.

B. stomaticus was found and captured into or near the water during all the surveys and being anuran lay their eggs in water that are greatly affected by water quality. Water texture effects their growth, development and morphology, hence analyzed for present study through some main Physico-chemical parameters that have great influence on survival of all amphibians as Gillooly and Dodson, 2000 and Angilletta, et al. 2004 proved acidification to be a major threat to amphibians along with Conductivity and Total dissolved solids (TDS) when increase above normal level may lead eggs and larvae of amphibians to mortality (Gloset al., 2003 and Tattersall and wright, 1996). Calcium, Magnesium, Potassium and Iron in water are also necessary in certain concentration for normal growth and development of all amphibians (Horne and Dunson, 1994).

Figure 1: Map of District Larkana and Shikarpur**Material and method:**

Altogether 1500 adult specimens of *B. stomaticus* were collected (750 adult specimens from Larkana and 750 adult specimens from Shikarpur) manually with scoop net from eleven sites of each District (names of sampling sites and their locations are indicated in Figure 1). Specimens were identified by using authentic literature (Khan and Mufti, 1994; Khan, 1968; Lutken, 1864; Daniel, 1963).

All specimens were examined morphologically and then released in wild. The morphological parameters include body weight, dorsal body color, pattern of patches on dorsum and legs, body length from snout to vent, fore limbs length, hind limbs length, Parotid gland distinct or indistinct (measured for determination of morphological variations), eye diameter and tympanum diameter (measured to identify sex of specimens). All the morphometric and sex identification of specimens was examined using methods by Kalsoom, et al. 2012.

For Physico-chemical analysis, Water samples were collected from the capture sites of *B. stomaticus* using the method (Establier, et al. 1985). pH and Conductivity were measured by using pH meter (Orion. 420) and Conductivity Bridge (Orion. 115). Total dissolved solids concentration was calculated by formula (LeBlond and Duffy, 2001). Chloride was analyzed by titration method (Sunita, 2002).

Water samples were filtered through 0.45 μm filters and concentrated prior to direct an Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 800) for analysis of Calcium, Magnesium, Potassium and Iron. All Glassware was cleaned by the procedure described by Laxen and Horison 1981, and chemicals and reagents used were of analytical or equivalent grade.

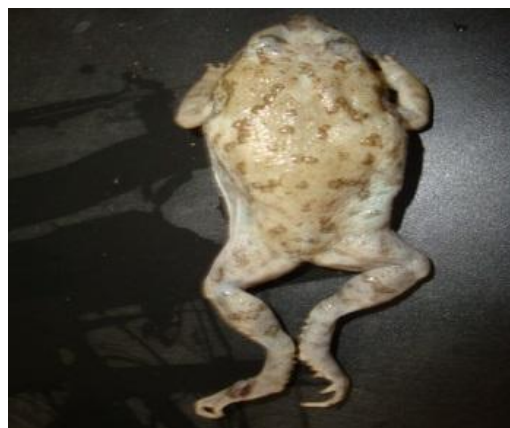
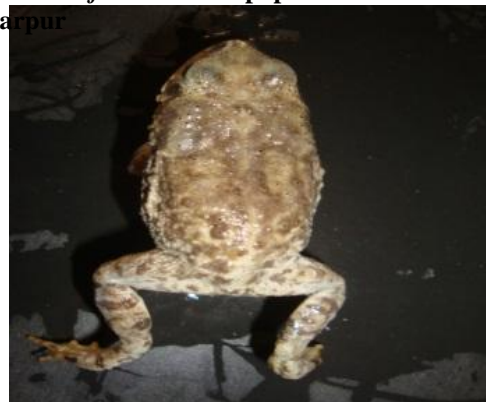
The literature (Beattie, et al. 1992; Boyer, et al. 1995; Mount, et al. 1997; Ketola, et al. 1988;

LeBlond and Duffy, 2001 and EPA, 2001, 1986, 1976 and Dux, 1983 was referred to understand water quality of *B. stomaticus* habitats. Morphological and Physico-chemical parameters were compared by using Student's *t*-statistics to have definite knowledge about difference between them whether significant or insignificant at 99 % confidence level (Daniel, 1948).

Results and discussion:

750 adult specimens of *Bufo stomaticus* collected from Larkana were differentiated into 350 male and 400 female. However 378 male and 372 female specimens were identified from 750 collected specimens of Shikarpur.

Population of *B. stomaticus* collected from district Larkana was light brown in color with rare and slightly dark ring like marks on the dorsum and legs of body with indistinct parotid gland (Fig. 2). Whereas population from Shikarpur was dark brown dorsally with dark patches scattered on all over the body surface, legs with dark strips like bands and distinct parotid gland was present (Fig. 3). Both populations looked noticeably different from each other.

Figure 2: *Bufo stomaticus* population from District Larkana**Figure 3: *Bufo stomaticus* population from District Shikarpur**

B. stomaticus population collected from Larkana was measured to contain members of short body length with lightweight, however longer body height and heavier body weight was examined from population of Shikarpur (Fig. 4-5). Their morphometric difference was calculated comparatively insignificant (Table 1).

Figure 4: Comparative morphometric of male specimens recorded from studied areas

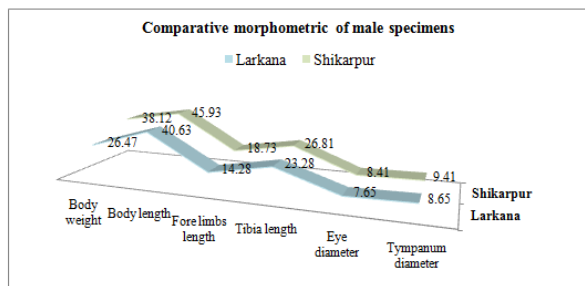


Figure 5: Comparative morphometric of female specimens recorded from studied areas

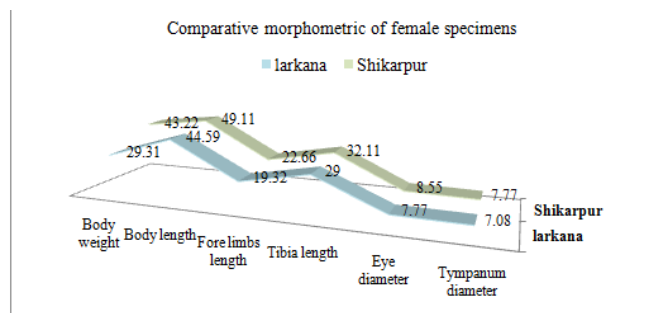


Table 1: Morphological Difference between *B. stomaticus* populations from Larkana and Shikarpur by help of t-statistics

Parameters	t-calculated	t-table	Remarks
Body weight	0.805	2.576	Insignificant
Body length	0.172	2.576	Insignificant
Fore limbs length	0.367	2.576	Insignificant
Hind limbs length	0.191	2.576	Insignificant
Eye diameter	0.148	2.576	Insignificant
Tympanum diameter	0.137	2.576	Insignificant

Table 2: Physico-chemical study of aquatic habitat of studied areas

Parameters	District Larkana	District Shikarpur
pH	7.68±0.42 (6.96-8.67)	7.33±0.39 (6.7-7.88)
Conductivity (µS/cm)	1064.2±1510.171 (121.4-5090)	1886±1331.78 (611-4430)
TDS (mg/L)	713.01±1011.81 (81.33-3410.3)	1263.62±892.29 (530.64-2968.1)
Chloride (mg/L)	178.63±111.25 (75-390.5)	308.52±190.89 (756.15-923)
Calcium (mg/L)	91.35±75.50 (11.45-219.5)	217.87±96.98 (4.99-334.64)
Potassium (mg/L)	50.02±37.99 (12-130.1)	317.15±24.27 (309.04-390.32)
Magnesium (mg/L)	99.69±37.71 (42-166.66)	97.66±15.30 (74.8-131.68)
Iron (mg/L)	6.39±5.97 (0.99-17.48)	13.13±1.89 (11.11-17)

Table 3: Difference between Physico-chemical parameters of studied areas

Parameters	t-calculated	t-table	Remarks
pH	0.36	3.169	Not significant
Conductivity ($\mu\text{S/cm}$)	7.90	3.169	Significant
TDS (mg/L)	7.90	3.169	Significant
Chloride (mg/L)	7.34	3.169	Significant
Calcium (mg/L)	13.55	3.169	Significant
Potassium (mg/L)	12.57	3.169	Significant
Magnesium (mg/L)	0.16	3.169	Not Significant
Iron (mg/L)	11.13	3.169	Significant

Physico-chemical parameters were analyzed to determine environmental conditions of District Larkana and Shikarpur that they provide to *B. stomaticus* (Table 2). Water quality of both habitats was compared through t-statistics (Table 3).

Bufo stomaticus habitat in both Districts was evaluated to provide significantly different but favorable environment as all the Physico-chemical parameters were analyzed to be within normal range except conductivity (Table 2-3) that should be between 150 - 500 $\mu\text{S/cm}$ to support aquatic life including amphibians as suggested by Boyer, et al. 1995.

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