

Abundance and Morphometric Variation of Amphibians Across a Riverine Wetland Habitat Mosaic of River Indus

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ABSTRACT

We studied abundance, snout-vent length, head length, and body weight of amphibians among habitat types along a riverine wetland mosaic of the River Indus (Mianwali, Punjab, Pakistan). We surveyed 19 sites representing six habitat types (flood plains, marshy areas, man-made wetlands, seasonal ponds connected, seasonal ponds unconnected, and permanent wetlands) during May–August 2024 and 2025 using visual encounter surveys. Species occurrence was significantly associated with habitat. The bull frog (*Hoplobatrachus tigerinus*) was most abundant in flood plains, man-made wetlands, and seasonal ponds unconnected; the skittering frog (*Euphlyctis adolfi*) in marshy areas and man-made wetlands; and the Indus Valley toad (*Firouzophrynus stomaticus*) in seasonal ponds connected to wetlands. Morphometric analyses revealed habitat-specific differences in body size and weight. Indus Valley toad individuals from seasonal ponds connected had the largest snout-vent length, head length, and body weight, whereas skittering frogs from marshy areas were larger than those from man-made wetlands. Bull frogs from flood plains and seasonal ponds unconnected were the largest. Head length of Indus Valley toad differed significantly between sexes and among habitats, while body weight of bull frog varied among habitats but not between sexes. Small sample sizes in certain habitats–sex groups and the cross-sectional nature of the study limited statistical power and confidence interval calculations. We recommend future long-term studies incorporating environmental variables such as prey availability, water quality, and vegetation structure to understand habitat influences on amphibian morphometry and performance.

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Authors' Contribution

ASH: Conceptualization, methodology, investigation, field surveys, sample collection, morphometric measurements, software (GIS analysis), data curation, formal analysis, visualization, mapping, and writing original draft. AA: Supervision, methodology, project administration, and writing review and editing. SM: software (GIS analysis), data curation, and visualization. TA: Writing review and editing, and formatting and manuscript preparation. MR: Supervision, methodology, formal analysis, statistical analysis, validation, and writing review and editing.

Key words

Bull frog, Croplands, Dicroglossidae, Snout-vent length, Skittering frog, Indus valley toad, Manmade wetlands

INTRODUCTION

Amphibians play an important role in the ecosystem. They serve as bioindicators of ecosystem health, control pests, and help in nutrient cycling (Kanagavel *et al.*, 2017). Floodplains associated with riverine wetlands include an array of habitat types such as main river channels, side channels, oxbow lakes, ponds, agricultural wetlands, and seasonally inundated depressions. Habitat variation within these landscapes is largely influenced by

hydrological processes and land use land cover (LULC) patterns (Tockner and Stanford, 2002). However, riverine wetlands worldwide are increasingly impacted by river regulation, pollution, land-use change, and invasive species, which alter nutrient dynamics, hydroperiods, and habitat structure (Holgerson *et al.*, 2019; Pereyra *et al.*, 2021). Categorizing habitats within riverine landscapes is therefore essential for understanding amphibian distribution and their ecological responses. Habitat characteristics such as water availability, vegetation cover, substrate type, and surrounding land use directly influence amphibian growth, survival, and physiological condition (Paudel *et al.*, 2022).

Twenty-one amphibian species are reported from Pakistan (Rais *et al.*, 2021b) which are associated with freshwater wetlands and agricultural landscapes (Sheraz *et al.*, 2024). Previous research on sexual dimorphism, morphometric variation, and habitat influences in bullfrog (*Hoplobatrachus tigerinus*) and skittering frog (*Euphlyctis adolfi*), has shown that there are a few sex-based

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differences (Kandel, 2024). Similarly, several amphibian species, including Indus Valley toad *Firouzophrynus stomaticus*, Asian common toad *Duttaphrynus melanostictus*, skittering frog *Euphlyctis cyanophlyctis*, Indian bullfrog *Hoplobatrachus tigerinus*, Kashmir paa frog/Hazara frog *Allopaa hazarensis*, and Hazara torrent frog *Nanorana hazarensis* were found to have ecological and morphological variation associated with water quality and environmental conditions in Pakistan (Tagar *et al.*, 2022; Sheraz *et al.*, 2024).

Studies on bull frog tadpoles also revealed adaptive morphological changes under predation and cannibalism stress (Phuge *et al.*, 2023). However, morphometry of amphibians from across a riverine wetland habitat mosaic is seldom compared. We compared abundance, snout-vent length, head length, and body weight of amphibians from different habitats along a riverine wetland.

MATERIALS AND METHODS

Study area

We conducted this study along the riverine wetland-the River Indus (32.43585°N, 71.38017°E. Average elevation is approximately 190 to 200 meters (about 620 to 650 feet) above sea level, near Dera Ismail Khan Road, Tehsil Piplan, District Mianwali, Punjab, Pakistan.

Fertile land and abundant water from the Indus make this habitat ideal for aquatic organisms (Khan *et al.*, 2023). In the Chashma region, temperatures range from 18–22 °C in winter to 35–45 °C in summer, with a dry subtropical climate characterized by hot summers and cool winters (Habib *et al.*, 2024). The lake has a pH of 6.5–7.2 (Ahmad *et al.*, 2016). Aquatic vegetation includes *Hydrilla verticillata*, *Nelumbium speciosum*, water supports a diverse fauna of invertebrates, fish, amphibians, reptiles, and birds (Latif *et al.*, 2010).

Study sites, habitats and species system

We downloaded satellite imagery (Landsat 8–9 OLI/TIRS collection 2 Level-2) for the District Mianwali from the USGS Earth Explorer. Images acquired on 18 June 2025 (path/row 151/038) and 30 September 2025 (path/row 151/037) were selected. Although multiple images were available for the same dates, only those covering all sampling sites within District Mianwali and having cloud cover between 0–10% were used in the analysis. We extracted the shapefile of the study area and conducted land use and land cover (LULC) classification in ArcMap 10.5.1. We selected 19 sites for data collection. These sites represent six amphibian habitat types. (i) Flood Plains (FP): low-lying areas adjacent to the Indus River that are seasonally inundated during river floods. These areas

support temporary aquatic habitats and vegetation adapted to periodic flooding, (ii) Marshy Areas (MA): Permanently or seasonally waterlogged areas with dense emergent vegetation, often at the margins of the river or small streams, (iii) Man-Made Wetland (MMW): Artificially created water bodies, such as reservoirs, irrigation ponds, or farm ponds, that retain water for extended periods, (iv) Seasonal pond connected (SPC): Temporary ponds that form during the rainy season and are hydrologically connected to other wetlands or water bodies; (v) Seasonal pond unconnected (SPU): Temporary ponds that form seasonally but are isolated from other wetlands; (vi) Permanent wetland (PW): Long-lasting water bodies, such as lakes, large ponds, or oxbow lakes that retain water year-round. We made 38 visits to these sites during May–August 2024 and May–August 2025 using the visual encounter survey (VES) method (Dupler *et al.*, 2019).

The Indus Valley toad (*Firouzophrynus stomaticus*) (Fig. 1A) inhabits freshwater wetlands and agricultural fields; it is nocturnal, remaining concealed during the day and emerging at night to feed primarily on insects, with breeding occurring in temporary monsoon pools. The bullfrog (Fig. 1B) is a large semi-aquatic species commonly found in marshes, ponds, and paddy fields; it is mainly nocturnal and breeds during monsoon rains, with tadpoles developing in standing water. The skittering frog (Fig. 1C) is an aquatic species inhabiting ponds, marshes, and rice fields; it is known for skittering across the water surface when disturbed and breeds in standing water during the rainy season, feeding predominantly on insects (Mahmood *et al.*, 2016; Rais *et al.*, 2021a; Sheraz *et al.*, 2024).

Morphometric measurements

We recorded amphibian species, identified those following Rais *et al.* (2021b) and noted the number of individuals of each species from each habitat. We used a dip net to collect amphibians and measured their snout-vent length (mm) and head length (mm) using a digital Vernier caliper (0.01 mm with liquid crystal display) and weighed them using a digital weighing balance (version SF-400, 0.01g accuracy) (Boswald *et al.*, 2022; Majtyka *et al.*, 2021; Jilani *et al.*, 2018).

Statistical analysis

We calculated mean, standard error, range (for $n > 2$) and confidence interval (95%) (for $n > 2$) for each sex habitat-wise in R 4.3.1 (R Core Team, 2022). To examine patterns of species occurrence across habitats, we compiled count data for the three studied amphibian species recorded from the six distinct habitat types (FP, MA, MMW, PW, SPC, and SPU). For each habitat, the number of individuals of each species was tallied to create a contingency

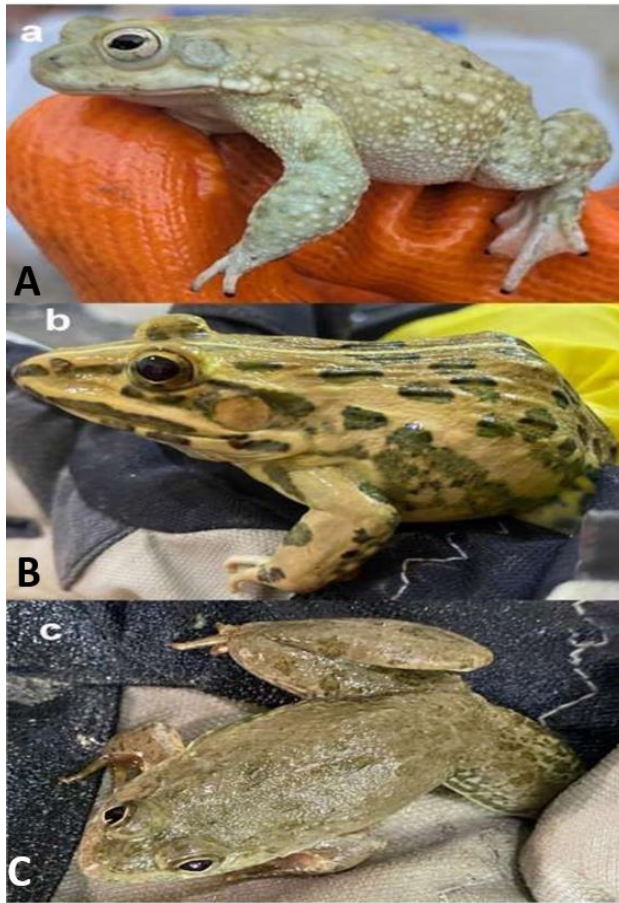


Fig. 1. Amphibian species recorded during the current study from the study area, Indus Valley toad (*Firouzophrynus stomaticus*) (A), bull frog (*Hoplobatrachus tigerinus*) (B), and skittering frog (*Euphlyctis adolfi*) (C).

table representing species–habitat occurrence. To test whether species distribution differed significantly among habitats, we conducted a Chi-square test of independence. Because several cells had low expected counts or zeros, we applied a Monte Carlo simulation with 10,000 replicates to generate a more reliable p-value. Following a significant overall Chi-square result, we calculated adjusted residuals for each species–habitat combination to identify which cells contributed most strongly to the association. Cells with adjusted residuals greater than +2 were interpreted as species occurring more frequently than expected, whereas residuals less than -2 indicated underrepresentation. This approach allowed us to determine not only whether species occurrence was significantly associated with habitat, but also which specific species–habitat combinations were driving the observed patterns. Cramér's V was calculated to quantify the strength of the association between species and habitat.

We fitted a linear model to assess the effects of sex, habitat, and their interaction on snout-vent length (SVL), head length (HL) and weight of each species (Weight ~ Sex * Habitat). Then, we performed ANOVA on each model to test for significance ($P < 0.05$ was considered significant).

RESULTS

Based on the habitat records, the bull frog had the highest number of individuals in flood plains (6), man-made wetland (8), and seasonal pond unconnected (4) habitats; Skittering frog was most abundant in marshy areas (11) and manmade wetland (13) while Indus Valley toad was primarily recorded in seasonal pond connected (8) and seasonal pond unconnected (2). Species occurrence was significantly associated with habitat ($\chi^2 = X$, $df = 10$, $p < 0.001$, Cramér's $V = 0.73$). Post hoc analysis indicated that the bull frog occurred more frequently than expected in floodplain habitats, suggesting a preference for these areas; the skittering frog was more frequent in marshy areas, while the Indus Valley toad in seasonal ponds connected to wetlands. In contrast, the skittering frog and Indus Valley toad were less frequent than expected in floodplains and marshy areas, while the bullfrog and skittering frog were less frequent in seasonal ponds connected to wetlands, indicating these habitats were less favorable for them.

The Indus Valley toad showed variation in body size. Individuals from seasonal pond connected exhibited the largest mean SVL, HL, and weighed heavy when compared with individuals from flood plains. Individuals of the skittering frog from marshy areas showed relatively larger mean SVL, HL, and body mass than those from man-made wetland. Individuals from man-made wetland were generally smaller and weighed less. The largest individuals of the bull frog were reported from seasonal pond unconnected and flood plains. Individuals from marshy areas and man-made wetland were generally smaller, with man-made wetland females showing especially high variability in all traits (Table I). Head length of Indus Valley toad differed significantly between sexes (ANOVA: $F_{1, 49} = 5.88$, $p = 0.019$) and among habitat types ($F_{3, 49} = 3.24$, $p = 0.030$). However, the interaction between sex and habitat was not significant ($F_{2, 49} = 0.31$, $p = 0.735$), indicating that habitat-related variation in head length was consistent across sexes. Body weight of bull frog varied significantly among habitat types ($F_{4, 18} = 5.21$, $p = 0.0058$). In contrast, neither sex ($F_{1, 18} = 2.08$, $p = 0.166$) nor the interaction between sex and habitat ($F_{3, 18} = 2.27$, $p = 0.116$) had a significant effect on body weight. Several habitats–sex groups were represented by very small sample sizes ($n = 1-2$), and therefore the absence of confidence intervals and the wide ranges were observed.

Table I. Snout-vent length (mm), head length (mm) and body weight of Indus Valley Toad (*Firouzophrynus stomaticus*), common skittering frog (*Euphlyctis adolfi*) and bull frog (*Hoplobatrachus tigerinus*) recorded from different habitats along a riverine wetland (River Indus, District Mianwali, Punjab, Pakistan).

Habitat	Sex	n	Snout-vent length (mm)		Head length (mm)		Weight (g)	
			Mean $\pm$ SE (Range)	CI (low–upper)	Mean $\pm$ SE (Range)	CI (low–upper)	Mean $\pm$ SE (Range)	CI (low–upper)
Indus Valley toad (<i>Firouzophrynus stomaticus</i>)								
FP	M	33	49.70 $\pm$ 1.25 (34.05–65.67)	47.16–52.24	13.78 $\pm$ 0.42 (8.67–18.63)	12.92–14.64	13.14 $\pm$ 1.05 (4–30)	11.00–15.29
	F	11	53.61 $\pm$ 1.74 (46.34–66.94)	49.74–57.48	15.40 $\pm$ 0.38 (14.28–18.53)	14.55–16.24	17.36 $\pm$ 3.68 (9–53)	9.17–25.55
MMW	F	2	54.35 $\pm$ 2.99 (51.36–57.34)	NA	16.48 $\pm$ 1.03 (15.45–17.50)	NA	20.50 $\pm$ 6.50 (14–27)	NA
SPC	M	5	55.01 $\pm$ 1.23 (51.00–57.80)	51.60–58.41	16.37 $\pm$ 0.91 (13.83–18.22)	13.84–18.91	18.00 $\pm$ 2.35 (12–25)	11.49–24.51
	F	3	56.68 $\pm$ 1.78 (54.56–60.21)	49.03–64.33	16.82 $\pm$ 0.45 (15.94–17.45)	14.87–18.78	26.00 $\pm$ 4.04 (18–31)	8.61–43.39
SPU	M	1	51.16	NA	14.63	NA	16	NA
	F	1	51.78	NA	17.26	NA	13	NA
Common skittering frog (<i>Euphlyctis adolfi</i>)								
MA	M	7	53.03 $\pm$ 4.37 (38.75–69.32)	42.35–63.71	15.95 $\pm$ 0.94 (12.85–18.57)	13.66–18.24	20.00 $\pm$ 3.41 (9–36)	11.66–28.34
	F	4	55.51 $\pm$ 6.99 (41.75–70.10)	33.26–77.77	18.87 $\pm$ 3.22 (13.00–25.17)	8.64–29.10	21.25 $\pm$ 5.34 (12–31)	4.24–38.26
MMW	M	7	50.32 $\pm$ 1.60 (41.75–70.10)	46.41–54.24	17.43 $\pm$ 0.54 (16.13–19.26)	16.12–18.74	16.71 $\pm$ 2.26 (11–25)	11.17–22.26
	F	6	41.14 $\pm$ 4.54 (41.75–70.10)	29.48–52.81	15.20 $\pm$ 1.00 (13.00–18.46)	12.62–17.78	14.50 $\pm$ 2.22 (10–24)	8.80–20.20
SPC	F	1	54.15	NA	14.42	NA	13	NA
Bull frog (<i>Hoplobatrachus tigerinus</i>)								
FP	M	8	87.19 $\pm$ 3.22 (68.18–98.42)	79.57–94.82	29.83 $\pm$ 1.39 (20.73–33.46)	26.54–33.12	60.63 $\pm$ 5.13 (25–68)	48.50–72.75
	M	2	109.94 $\pm$ 22.18 (87.76–132.12)	NA	31.06 $\pm$ 9.13 (21.93–40.19)	NA	128.00 $\pm$ 76.00 (52–204)	NA
MA	M	3	74.86 $\pm$ 3.80 (67.30–79.29)	58.51–91.21	27.51 $\pm$ 1.87 (24.62–31.00)	19.48–35.54	35.00 $\pm$ 4.36 (27–42)	16.25–53.75
MMW	M	2	69.97 $\pm$ 29.96 (40.01–99.93)	NA	23.08 $\pm$ 8.54 (14.54–31.61)	NA	46.50 $\pm$ 37.50 (9–84)	NA
	F	6	83.55 $\pm$ 9.07 (41.03–100.18)	60.24–106.85	26.16 $\pm$ 2.62 (14.94–32.69)	19.42–32.90	59.50 $\pm$ 12.83 (10–98)	26.52–92.48
PW	M	1	80	NA	26	NA	50	NA
PW	F	1	68.55	NA	27.5	NA	28	NA
SPU	M	2	97.29 $\pm$ 2.72 (94.57–100.01)	NA	34.53 $\pm$ 1.03 (33.50–35.56)	NA	66.00 $\pm$ 5.00 (61–71)	NA
	F	2	114.07 $\pm$ 6.44 (107.63–120.51)	NA	33.81 $\pm$ 8.52 (25.29–42.32)	NA	169.00 $\pm$ 3.00 (166–172)	NA

FP, flood plains; MA, marshy areas; MMW, man-made wetland; SPC, seasonal Pond connected with other wetlands; SPU, seasonal Pond unconnected; PW, permanent wetland; SE, standard error, CI, confidence interval, SVL, snout–vent length, HL, head length; NA, not applicable; confidence intervals were not calculated for sample sizes ≤ 2

DISCUSSION

Our study generated data on habitat-specific variation in abundance, body size, and body condition of three anuran species: bull frog, skittering frog and Indus Valley toad across diverse habitats along the riverine wetland. Our results indicate that habitat type strongly influences both species occurrence and morphometric traits. The bull frog was most abundant in larger, natural water bodies such as floodplains and seasonal ponds, the skittering frog was frequently observed in marshes and man-made wetlands, while the Indus Valley toad was largely restricted to natural, hydrologically connected ponds. Statistical analyses also confirmed a significant association between species occurrence and habitat.

Morphometric analyses revealed that individuals of all three species attained the largest body sizes and highest weights in natural, less-disturbed habitats. Bull frogs reached exceptionally high SVL (females: 114.07 ± 6.44 mm; males: 109.94 ± 22.18 mm), markedly exceeding previous reports from Abbottabad, Khyber Pakhtunkhwa (Sheraz *et al.*, 2024; 48.5 ± 11.2 mm), suggesting that resource-rich, productive wetlands support enhanced growth. Similarly, skittering frog and Indus Valley toad exhibited larger body size and better body condition in natural wetlands compared to artificial or degraded habitats, highlighting the influence of habitat quality on growth and development. Head length of the Indus Valley toad differed significantly between sexes and among habitat types, indicating that both environmental and biological factors shape morphometric traits. These findings align with previous studies, which documented the importance of wetland quality for amphibian growth and survival, and natural wetlands with stable hydrology, adequate prey availability, and minimal anthropogenic disturbance facilitate larger body size and improved condition in amphibians (Tagar *et al.*, 2022; Kandel, 2024). Rais *et al.* (2021a) reported similar patterns in Margalla Hills National Park, Islamabad, where amphibian assemblages differed along habitat gradients, with some species preferring large, permanent water bodies and others tolerating disturbed or artificial habitats. The differential abundance and morphometric variation highlight the ecological significance of heterogeneous wetland mosaics, where hydroperiod duration, vegetation complexity, and prey availability collectively influence growth rates and body condition (Wilbur, 1987; Semlitsch, 2000; Wells, 2007).

Despite these insights, the study has several limitations. Sample size for certain habitat–sex groups was very small ($n = 1–2$), limiting statistical power and preventing the calculation of confidence intervals for some traits. The study focused on short-term, cross-sectional

field surveys; seasonal or interannual variation in habitat conditions and amphibian growth was not captured. Due to lack of resources, environmental variables such as water quality, prey abundance, or predation pressure were not quantified, which could provide mechanistic explanations for the observed patterns.

We suggest that conservation efforts should prioritize the protection and restoration of natural wetlands and hydrologically connected pond systems, which support optimal growth and body condition in amphibians. Future studies should incorporate long-term monitoring and include detailed environmental metrics, such as prey availability, water quality, and vegetation structure, to better understand relationships between habitat characteristics and amphibians.

DECLARATION

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Ethical statement

All animals were handled ethically, and after recording morphometric measurements, they were safely released back into their natural habitats.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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