



Comparative Assessment of Anuran Diversity and Morphometric Ratios from Aquatic Ecosystem of Sub Mountainous and Plain Zones of Punjab

Ramanpreet Kaur and Rajwinder Singh*

Department of Zoology, Punjab Agricultural University, Ludhiana-141 004, India
E-mail: ramanpreet0062@yahoo.com

Abstract: Present study investigates four anuran species *Duttaphrynus melanostictus*, *D. stomaticus*, *Euphlyctis cyanophlyctis* and *Hoplobatrachus tigerinus* belonging to families Bufonidae and Dicroglossidae under order anura in aquatic ecosystem (village ponds) of plain zone (Ludhiana) and sub mountainous zone (SBS Nagar) of Punjab (India) during 2022 and 2023. Higher anuran population (2.99 to 5.15 individuals/month/transect) was in sub mountainous zone followed by plain zone (2.02 to 3.98 individuals/month/transect). Seasonal activity of anurans was from May to December and during whole year with higher population during July and August month. A 10.27% increase in population was in sub mountainous zone during 2023 as compared to previous year whereas was only 8.87% in plain zone. Inside pond, *H. tigerinus* showed a significant 46.15-72.22% variation in population in sub mountainous zone as compared to plain zone which was significantly different from other anuran species. However, in outside (periphery) of village ponds, *E. cyanophlyctis* showed highest (47.56-47.61%) population variation in sub mountainous zone as compared to plain zone which was significantly different from other anuran species. Biodiversity indices, like Shannon-Wiener index and Simpson's index improved in 2023 as compared to previous year in plain and sub mountainous zone, respectively. Morphometric analysis revealed significant sexual dimorphism, particularly in *H. tigerinus*, with interspecific variations reflecting adaptations to different microhabitats. Significant seasonal variations and increase in anuran population was in sub mountainous zone consistently supported higher densities than plain zone. Enhanced biodiversity indices and positive correlations with weather parameters indicate improved ecosystem health over time. Morphometric differences, particularly in *H. tigerinus*, highlight species-specific adaptations to environmental niches, advancing our understanding of anuran ecological roles.

Keywords: Anuran, Biodiversity indices, Diversity, Frog, Morphometric ratios, Toad

Anurans (frogs and toads) are one of the most diverse and widespread groups of amphibians, playing vital roles in both terrestrial and aquatic ecosystems. With approximately 7,500 known species worldwide, representing about 88% of all amphibian species (Frost 2023). Their diversity spans continents, with concentrations in tropical regions, particularly Brazil, Colombia and the Indo-Malayan and Afrotropical realms (Hof et al., 2011). In India, approximately 414 anuran species have been recorded, with the Western Ghats being a significant hotspot, harbouring about 159 endemic species (Biju and Bossuyt 2009). However, around 41% of amphibian species, including anurans, are threatened (IUCN 2024), primarily due to habitat loss, climate change, diseases like chytridiomycosis and invasive species. Village ponds are crucial habitats for anurans, providing breeding sites, foraging areas and population connectivity in agricultural landscapes. These ponds are influenced by factors such as water depth, shoreline complexity, vegetation and seasonal variations, which shape anuran diversity and abundance (Jadhav et al., 2023). Larger ponds with richer vegetation typically support more species, while environmental changes and human activities increasingly affect these communities (Kumar et al., 2024). Conservation efforts must integrate local habitat protection

and landscape-level approaches, such as maintaining pond networks, protecting shoreline vegetation and managing water quality (Patel et al., 2023). The use of modern technologies like environmental DNA sampling, automated acoustic monitoring and remote sensing is improving understanding of anuran population dynamics and habitat use (Rana et al., 2023, Sharma et al., 2024;).

Present research was carried out with an objective of comparing the status of anuran diversity in village ponds of sub mountainous and plain zones of Punjab state. By examining these ecosystems, the study seeks to understand how environmental factors influence anuran communities in different ecological settings (Sharma et al., 2024). Future research priorities should focus on long-term monitoring of population trends, assessment of emerging threats and development of effective conservation strategies that consider both ecological requirements and human needs in these important ecosystems.

MATERIAL AND METHODS

Study was carried out during 2022 and 2023 at selected villages (five each) of district Ludhiana and district Shaheed Bhagat Singh (SBS) Nagar which belongs to plain zone and sub mountainous zone, respectively. Ludhiana is located

between the coordinates 30.9010°N,75.8573°E, in the central region of Punjab State, while SBS Nagar lies between the coordinates 31.0913°N, 76.0392°E. The five selected aquatic ecosystems (village ponds) of district Ludhiana included Bhattian, Chak Kalan, Alamgir, Kaind and Jassowal whereas of district SBS Nagar includes Langroya, Amargarh, Ghamour, Rurki Kalan and Hussain Chak.

Sampling of anuran diversity: To study diversity and abundance of anurans, Visual Encounter Survey Method (Heyer 1994) was used for estimating the anuran population in a belt transect of 50×4 m along a survey path at fortnight interval and was pooled at month level from May to December during 2022 and 2023. Each anuran species was captured with hand wearing gloves from selected locations, photographed for identification, checked for any morphological abnormalities and released back in their natural habitat. Anurans were identified from ZSI (Zoological Survey of India), Pune and using keys published by Daniel (2005). Data on atmospheric temperature (°C), relative humidity (%) and rainfall (mm) were taken from Department of Climate Change and Agricultural Meteorology, PAU, Ludhiana and Ballawal Sounkri for Ludhiana and SBS Nagar, respectively.

Calculation of various indices

Simpson index

$$D = \frac{\sum_{i=1}^S n_i(n_i - 1)}{N(N - 1)}$$

Where, D = Simpson index

n_i = Total number of individuals in a particular species

N = Total population of all species

Shannon-Wiener index

$$H' = -\sum (p_i \times \ln p_i)$$

Where,

H' = Shannon-Wiener index

p_i = Total number of individuals in a particular sample

$$J = \frac{H'}{\ln(S)}$$

Pielou's species evenness

Where,

J = Species evenness

H' = Shannon-Wiener index

S = is the number of species in the community.

Statistical analysis: For diversity of anurans, the data was analysed with Tukey's test with p value = 0.05 using SPSS software. Different indices like Shannon-Weiner diversity index, species evenness, species richness and Simpson's index were calculated using different formulas and PAST (paleontological statistics) software. Correlation coefficient was calculated to find relation between anuran diversity and atmospheric temperature (°C), rainfall (mm) and relative humidity (%).

RESULTS AND DISCUSSION

The present study provides significant insights into anuran community dynamics in the aquatic ecosystems (village ponds) of the plain zone (district Ludhiana) and sub-mountainous zone (SBS Nagar). Four anuran species (*Duttaphrynus melanostictus*, *D. stomaticus*, *Euphlyctis cyanophlyctis*, and *Hoplobatrachus tigerinus*) from the families Bufonidae and Dicroglossidae were documented in the village ponds of both zones. These species demonstrated seasonal activity patterns from May to December, with peak abundance during July and August, which coincides with the rainy season in Punjab. This phenological pattern aligns with observations from similar tropical and subtropical regions worldwide (Blaustein et al., 2012, Todd et al., 2011) suggesting consistent breeding strategies across geographical ranges in response to monsoon-dependent ecosystems.

Population in aquatic ecosystem of plain zone and sub mountainous zone: In plain zone (district Ludhiana), from aquatic ecosystem (village ponds) of selected villages, mean anuran population was 2.93 to 3.19 during 2022 and 2023, respectively, being highest in village Jassowal (and lowest in Alamgir) (Table 2). The maximum variation in population

Table 1. Inventory of anuran species from aquatic ecosystem (village ponds) of plain zone (district Ludhiana) and sub mountainous zone (SBS Nagar)

Common name	Scientific name	Family	Order	Ludhiana	SBS Nagar
Common Asian toad	<i>Duttaphrynus melanostictus</i> (Schneider 1799)	Bufonidae	Anura	#	#
Indian marble toad	<i>Duttaphrynus stomaticus</i> (Lütken 1864)			#	#
Indian skittering frog	<i>Euphlyctis cyanophlyctis</i> (Schneider 1799)	Dicroglossidae		#	#
Indian bull frog	<i>Hoplobatrachus tigerinus</i> (Daudin 1802)			#	#

* (#) Indicates presence of species

during 2023 was in Bhattian with increase of 30.55% followed by Kaind (18.40%), while decrease in population was recorded in Alamgir (9.82%) as compared to 2022. The overall increase of 8.87% anuran population was in plain zone (district Ludhiana) during 2023 as compared to 2022 (Table 2). Similarly, in sub mountainous zone (district SBS Nagar), from aquatic ecosystem (village ponds) of selected villages, anuran mean population was 3.70 to 4.08 during 2022 and 2023, respectively, being highest in Hussain Chak and lowest in Amargarh (Table 2). The maximum variation in population during 2023 was in Rurki Kalan with increase of 16.53% followed by Amargarh (10.36%), Hussain Chak (9.80%), Ghamour (8.99%) and Langroya (4.95%) as compared to 2022. overall increase of 10.27% anuran population was in sub mountainous zone (district SBS Nagar) during 2023 as compared to 2022.

Comparison of anuran population in aquatic ecosystem of plain zone and sub mountainous zone: Higher mean anuran population was in village ponds of district SBS Nagar (sub mountainous zone) as compared to district Ludhiana (plain zone). During 2022 and 2023, among outside (periphery) of all the selected aquatic ecosystems (village ponds), *E. cyanophlyctis* showed highest (47.56-47.61%) variation in population at SBS Nagar as compared to district Ludhiana which was significantly different from other anuran

Table 2. Anuran population in aquatic ecosystem (village ponds) of plain zone (district Ludhiana) and sub mountainous zone (SBS Nagar) during 2022 and 2023

Villages	Mean population (2022)	Mean population (2023)	Per cent population variation in 2023 over 2022
Plain zone (district Ludhiana)			
Chak Kalan	3.14 ^a	3.27 ^a	+4.14
Bhattian	2.52 ^a	3.29 ^b	+30.55
Kaind	2.88 ^a	3.41 ^b	+18.4
Alamgir	2.24 ^a	2.02 ^a	-9.82
Jassowal	3.87 ^a	3.98 ^a	+2.84
Total	2.93 ^a	3.19 ^b	+8.87
Sub mountainous (SBS Nagar)			
Hussain Chak	4.69 ^a	5.15 ^a	+9.80
Rurki Kalan	3.75 ^a	4.37 ^b	+16.53
Ghamour	3.67 ^a	4.00 ^a	+8.99
Langroya	3.43 ^a	3.60 ^a	+4.95
Amargarh	2.99 ^a	3.30 ^a	+10.36
Total	3.70 ^a	4.08 ^b	+10.27

*Statistical analysis (showed significant result with p value = 0.05

*Mean value followed by same letter (a,b) in the given table above are not significantly different as per t-Test

species. *D. stomaticus* and *D. melanostictus* showed 28.57-35.91% and 19.64-22.12% variation in population at SBS Nagar as compared to district Ludhiana and was statistically at par with each other. However, *H. tigerinus* showed only 1.47-14.10% variation in population at SBS Nagar as compared to district Ludhiana. Population trend of anurans inside the aquatic ecosystems were more pronounced. *H. tigerinus* showed significant 72.22% variation in population at SBS Nagar as compared to district Ludhiana. However, in 2023, *H. tigerinus* showed a significant 46.15% variation in population at SBS Nagar as compared to district Ludhiana which was significantly different from *E. cyanophlyctis* (7.48%) (Fig. 1, 2). The observed population dynamics revealed spatial and temporal variations between the two selected zones, with the sub-mountainous zone showing a higher population increase (10.27%) compared to the plain zone (8.87%) from 2022 to 2023. This difference in population can primarily be attributed to variations in environmental parameters, particularly rainfall (1396 mm and 1032 mm in sub-mountainous and plain zones, respectively) and relative humidity (0.83% higher in the sub-mountainous zone than in the plain zone). These findings correspond with global studies highlighting the crucial role of precipitation patterns in amphibian population dynamics (Grant et al., 2016, Hopkins, 2007).

Diversity indices in aquatic ecosystem of plain zone and sub mountainous zone: In aquatic ecosystem (village ponds) of district Ludhiana, the Shannon-Wiener index, Pielou's index and Simpson's index increased from 0.901, 0.567 and 0.335 to 0.924, 0.884 and 0.349, respectively in 2023 as compared to 2022 (Table 4). Likewise, in aquatic ecosystem (village ponds) of district SBS Nagar, the

Table 3. Diversity indices for anuran population in selected aquatic ecosystems (village ponds) of plain zone (district Ludhiana) and sub mountainous zone (SBS Nagar)

Indices	Aquatic ecosystem	
	2022	2023
Ludhiana		
Shannon- Wiener's index	0.901	0.924
Pielou's evenness index	0.567	0.884
Simpson's index	0.335	0.349
Species richness	4.00	4.00
SBS nagar		
Shannon- Wiener's 3 index	0.932	0.951
Pielou's evenness index	0.892	0.906
Simpson's index	0.780	0.799
Species richness	4.00	4.00

Shannon-Wiener index, Pielou's index and Simpson's index increased from 0.932, 0.892 and 0.780 to 0.951, 0.906 and 0.799, respectively in 2023 as compared to 2022. However, species richness (four) remained constant during 2022 and 2023 for both the zones under study.

Correlation between anuran population and weather parameters: Strong positive correlations were observed between anuran populations and mean atmospheric temperature and relative humidity in aquatic ecosystem (village ponds) of district Ludhiana and SBS Nagar (Fig. 3 to 6). The stronger correlation between environmental parameters and anuran abundance in 2023 (temperature: $r=+0.394$ to 0.533 ; relative humidity: $r=0.536$ to 0.820) suggests increasing environmental dependency, which possibly reflects adaptive responses to changing climatic conditions, a phenomenon also noted in other regions (Carey and Alexander 2003, Walls et al., 2013). The improvement in biodiversity indices from 2022 to 2023 (Shannon-Wiener index: 0.901 to 0.924 and 0.932 to 0.951 in Ludhiana and SBS Nagar, respectively) indicates enhanced community stability and evenness, despite constant species richness. This pattern mirrors findings from long-term studies in other agricultural landscapes (Cushman 2006, Khatiwada et al., 2016), suggesting that well-maintained village ponds can serve as effective refugia for anuran populations. The observed stability in species composition, coupled with increasing diversity indices, may indicate successful habitat utilization strategies and resource partitioning among species, as documented in similar agroecosystems globally (Ramesh et al., 2013, Dodd 2010). The stronger correlations

between environmental parameters and population dynamics in 2023 raise important considerations about climate change impacts on amphibian communities. This increasing environmental dependency aligns with global observations of amphibian responses to climate change (Urban et al., 2016, Pounds et al., 2006), suggesting potential vulnerability to future climatic variations. The maintenance of stable populations despite these pressures indicates some level of resilience in these agricultural wetland systems, possibly due to the availability of suitable microhabitats and breeding sites (Semlitsch, 2008). However, several factors warrant careful consideration for long-term conservation. First, the constant species richness despite improving diversity indices suggests a possible ceiling effect in these modified landscapes, highlighting the need for habitat enhancement strategies (Gardner et al., 2007). Second, the strong environmental correlations indicate potential vulnerability to climate change, necessitating adaptive management approaches (Shoo et al., 2011). Third, the pronounced sexual dimorphism and morphological variations suggest complex ecological relationships that need preservation through habitat conservation (Stuart et al., 2004).

Morphometric ratios of male and female anuran species: Morphometric analysis of four anuran species revealed variations in body proportions across species and between males and females. The overall mean morphometric variation across all parameters and in significant differences observed in key ratios. The head length to snout-vent length (HL: SVL) ratio exhibited sexual dimorphism, particularly in

Table 4. Per cent increase in morphometric parameters (pooled mean of 2022 and 2023) of anuran species in aquatic ecosystem (village ponds) of sub mountainous zone (SBS Nagar) over plain zone (Ludhiana)

Morphometric parameters	<i>D. stomaticus</i>		<i>D. melanosticus</i>		<i>E. cyanophlyctis</i>		<i>H. tigrinus</i>		Mean
	Male	Female	Male	Female	Male	Female	Male	Female	
HL: SVL	7.41	14.71	7.14	12.9	3.85	17.14	34.45	20.13	14.72
HL: HW	9.23	10.00	7.46	2.74	11.59	11.27	5.56	34.57	11.55
HL: HD	10.28	6.96	6.25	1.68	7.02	4.96	5.47	0.71	5.42
SL: HL	4.76	4.55	5.13	8.89	12.82	8.16	7.02	11.29	7.83
SL: SVL	7.14	5.88	10.53	9.52	7.14	6.25	12.00	18.75	9.65
EN: NS	3.25	3.76	1.55	5.47	6.96	3.85	3.64	30.17	7.33
EN: HL	5.26	3.85	5.88	26.09	11.76	19.05	21.43	14.29	13.45
ED: HL	9.76	2.08	12.9	14.29	5.88	8.89	22.22	12.00	11.00
ED:SL	32.79	15.38	2.50	1.16	1.12	33.78	5.00	24.49	14.53
ED: SVL	9.09	6.25	8.33	35.71	27.78	24.00	20.00	75.00	25.77
ED: EN	6.2	2.01	0	1.61	4.26	0	5.83	4.20	3.01
Mean	9.56	6.86	6.15	10.91	9.11	12.49	12.97	22.33	11.3

H. tigerinus (males: 34.45%, females: 20.13%) and *E. cyanophlyctis* females (17.14%), with a mean ratio of 14.72% across species. Head proportions, measured through HL:

HW ratio, showed high variation (11.55%), with *H. tigerinus* females displaying the higher value (34.57%) as compared to *D. melanostictus* females (2.74%). The head length to head

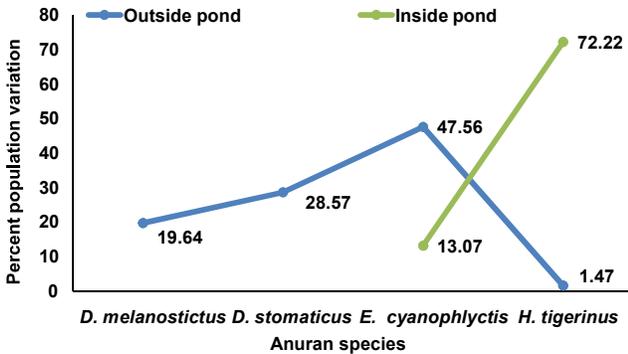


Fig. 1. Comparison of anuran population in aquatic ecosystem (village ponds) of plain zone (district Ludhiana) and sub mountainous zone (SBS Nagar) during 2022

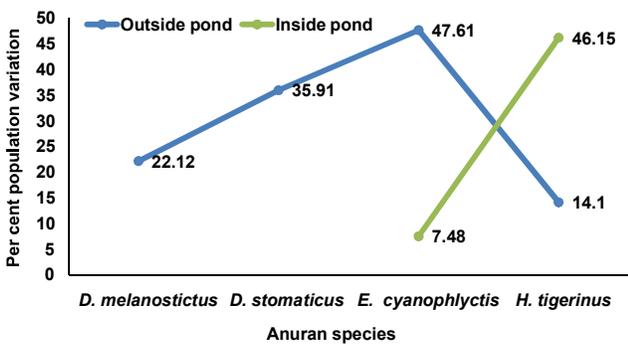


Fig. 2. Comparison of anuran population in aquatic ecosystem (village ponds) of plain zone (district Ludhiana) and sub mountainous zone (SBS Nagar) during 2023

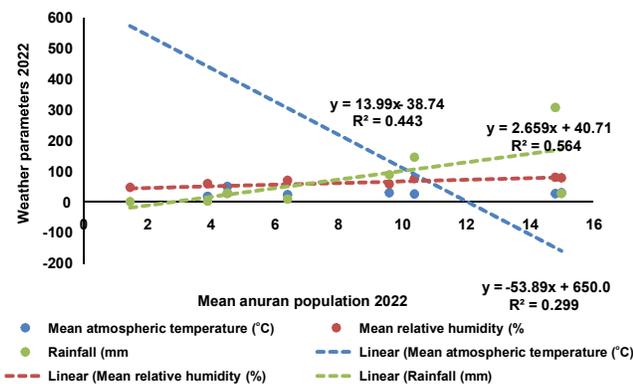


Fig. 5. Correlation between mean anuran population and weather parameters in aquatic ecosystem (village ponds) of sub mountainous zone (SBS Nagar) during 2022

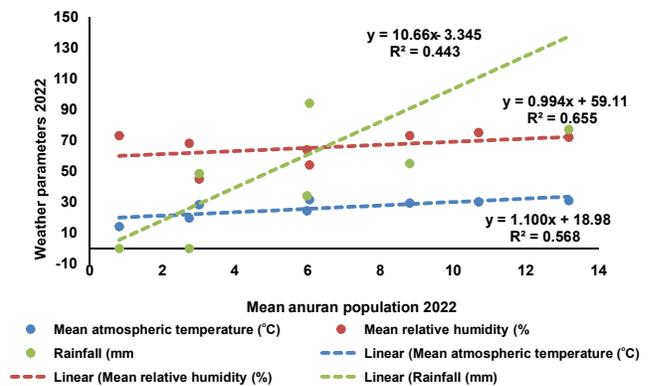


Fig. 3. Correlation between mean anuran population and weather parameters in aquatic ecosystem (village ponds) of plain zone (district Ludhiana) during 2022

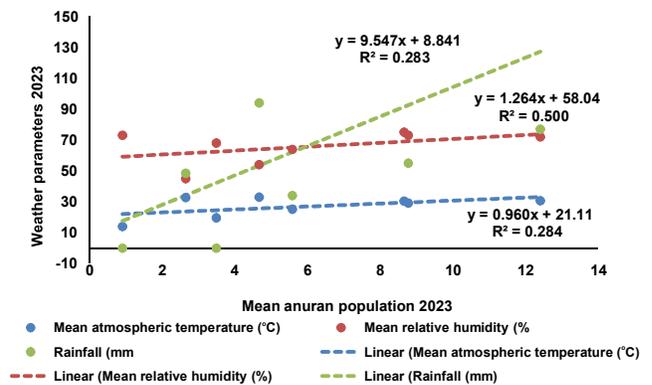


Fig. 4. Correlation between mean anuran population and weather parameters in aquatic ecosystem (village ponds) of plain zone (district Ludhiana) during 2023

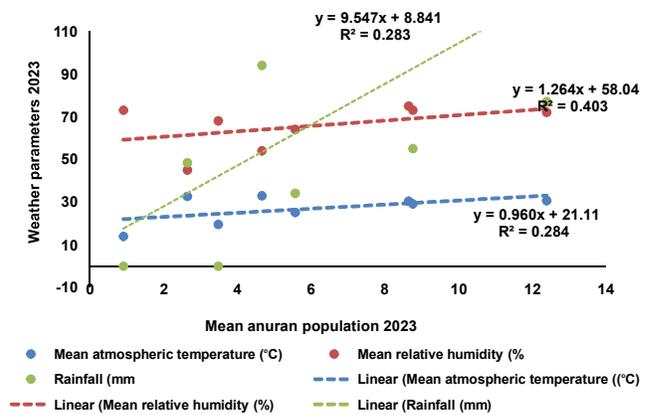


Fig. 6. Correlation between mean anuran population and weather parameters in aquatic ecosystem (village ponds) of sub mountainous zone (SBS Nagar) during 2023

depth ratio (HL: HD) ranged from 10.28% in *D. stomaticus* males to 0.71% in *H. tigerinus* females. SL:HL and SL:SVL ratios increased between 7.83% and 9.65% respectively. For ED: SVL ratios (mean 25.77%), *H. tigerinus* females exhibited higher value (75.0%). Species-specific analysis revealed *H. tigerinus* as the most morphometrically variable species (male: 12.97%, female: 22.33%), followed by *E. cyanophlyctis* (male: 9.11%, female: 12.49%), while *D. melanosticus* (male: 6.15%, female: 10.91%) and *D. stomaticus* (male: 9.56%, female: 6.86%) showed low variations. Sexual dimorphism was most pronounced in *H. tigerinus*, particularly in HL: SVL, HL:HW and ED:SVL ratios (Table 4). The observed interspecific and intersexual variations in morphometric parameters suggest species-specific adaptations to different microhabitats and ecological niches within the study area. These morphological differences likely reflect evolutionary adaptations to different microhabitat utilization and reproductive strategies, consistent with patterns observed in other anuran communities worldwide (Wells 2007). The variation in head and body proportions across species (mean morphometric variation: 11.3%) suggests niche differentiation and resource partitioning strategies, similar to findings reported in other tropical anuran assemblages (Richter-Boix et al., 2007, Vitt and Caldwell 2014;). These findings have important implications for amphibian conservation in village ponds globally. The successful maintenance of anuran populations in village ponds demonstrates the potential of these anthropogenic habitats as conservation units, provided they are properly managed (Hazell et al., 2004). However, continued monitoring is essential to understand long-term population trends and their responses to environmental changes (Egea-Serrano et al., 2012), particularly in the context of global climate change and agricultural intensification.

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