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Anuran responses to urbanization: evaluating life history traits of *Rhinella arenarum* in urban wetlands

Pollo Favio[a,b], Bionda Clarisa[a,b], Baraquet Mariana[a,b], Otero Manuel[a,b], Martino Adolfo[a,b], and Grenat Pablo[a,b,*]

[a]Ecología, Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional N° 36-km 601, X5804BYA, Río Cuarto, Córdoba, Argentina
[b]Instituto de Ciencias de la Tierra, Biodiversidad y Ambiente (ICBIA), UNRC-CONICET, Ruta Nacional N° 36-km 601, X5804BYA, Río Cuarto, Argentina

*Address correspondence to Grenat Pablo. E-mail: pgrenat@exa.unrc.edu.ar
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Abstract

Wetlands are unique ecological environments capable of harboring high biodiversity. However, urbanization can degrade, eliminate, or transform these habitats. Although amphibians utilize habitats created by humans in urban landscapes, few studies have investigated the influence of the habitat quality on the life history of anurans. We assessed life history traits such as snout-vent length (SVL), body condition, and reproductive investment in the South American common toad *Rhinella arenarum*, to determine whether urbanization is harmful or beneficial to this species. We sampled wetlands with different levels of urbanization in Río Cuarto city, Córdoba, Argentina. We recorded males with lower SVL in medium urbanized wetlands and those with the highest SVL in both low and high urbanization categories, similar to what was found for body conditions with males with low body conditions inhabiting wetlands with a medium degree of urbanization. In females, lower SVL was recorded in medium urbanization and highest SVL in high and low urbanization. It is observed that females recorded in highly urbanized wetlands have a very low body condition. The reproductive investment parameters were not significantly different, but we observed an association between a greater number of eggs and clutch size with wetlands of low urbanization. These results show a variability of responses of *R. arenarum* to urbanization, which could be due to phenotypic plasticity in its life history parameters, allowing it to inhabit urban areas. Continuous monitoring of the species in these wetlands is needed to determine if these biological responses are temporary or persistent.

Key words: pond, reproductive investment, South American common toad, urbanization.

Wetlands are unique ecological environments serving as vital components in climate change mitigation and water management (European Pond Conservation Network 2008), as well as hosting rich biodiversity (Foley et al. 2005; Lougheed et al. 2008; Hu et al. 2017; Reis et al. 2017). However, its degradation is more rapid than other ecosystems due primarily to major changes in land use (Hamer and McDonnell 2008; Hamer 2022). Urbanization is one of the main forces involving land use conversion and has led to substantial wetland loss globally, particularly in Asia, Europe, and South America (Hu et al. 2017).

Several studies have demonstrated that urbanization affects wildlife by altering hydrology (Paul and Meyer 2001; Miltner et al. 2004), soil composition (Effland and Pouyat 1997), introducing exotic species (Riley et al. 2005), increasing vehicular traffic (Bautista et al. 2004; Grenat et al. 2019, 2023), releasing pollutants (Burger et al. 2004; Bionda et al. 2012), and introducing non-native predators (Koenig et al. 2002). These changes can negatively affect their life history traits (Rytwinski and Fahrig 2012; Meillère et al. 2015; Iglesias-Carrasco et al. 2017) and sometimes even reduce species richness and abundance in urban areas (McKinney 2008; Rytwinski and Fahrig 2012; Pereyra et al. 2021). On

the other hand, as urban areas expand, new aquatic habitats emerge, such as public parks, community gardens, sports recreation areas, flooded roadside ditches, and others, that can serve as substitute habitats for many native species (Nielsen et al. 2014; Hutto and Barrett 2021). Thus, some more tolerant species, which do not require specialized abilities or habitat conditions, can persist and even increase in abundance in urban wetlands due to the combination of ecological traits that can make it easier to live in extremely disturbed areas such as urban places (Brand and Snodgrass 2010; Francis and Chadwick 2012; Nielsen et al. 2014; Lourenço-de-Moraes et al. 2018; Ordóñez-Delgado et al. 2022).

Research on the impact of urbanization on biodiversity primarily focuses on birds and plants in the Paleoarctic and Neoarctic regions (Rega-Brodsky et al. 2022), whereas studies on amphibians in urban environments remain limited (Jennette et al. 2019). Changes in habitat architecture caused by urbanization may lead to the loss of anuran species due to the presence of environmental filters, resulting in species, which either avoid or tolerate this disturbance (Collins and Crump 2009; Collins and Fahrig 2017; Cogălniceanu et al. 2021; Pereyra et al. 2021). Consequently, studying the life history traits of these organisms, such as body size and

reproductive parameters, contributes to understanding the species dynamics and the causes of population instability (Driscoll 1999; Sinsch 2015; Bionda et al. 2018; Otero et al. 2021; Pereyra et al. 2021), and can be a useful approach to elucidate the effect of urbanization at different life stages (Stearns 1992; Jennette et al. 2019).

Amphibians are excellent indicators for studying the quality of urban habitats because, despite the numerous threats they face in these substitute habitats, some species thrive whereas others decline (Pope et al. 2000; Fuyuki et al. 2014; Kruger et al. 2015; Li et al. 2018; Hutto and Barrett 2021; Pereyra et al. 2021; Valdez et al. 2021; Hamer 2022). The prediction of life history theory, suggests that organisms will optimize their fitness by allocating energy optimally among growth, reproduction, and maintenance requirements (van Noordwijk and de Jong 1986). However, some life history traits such as body size, fecundity, and size-assortative mating tend to evolve in response to environmental conditions and often interact via trade-offs and their measurement can serve as an indicator of the population stability if they show unfavorable dynamics (Sinsch et al. 2007a, b; Spear et al. 2009; Bionda et al. 2018; Babini et al. 2024).

The common South American toad has a wide distribution in South America and inhabits a large number of habitat types (IUCN, 2024). The reproduction period is approximately 1 month. However, most mating occurs in the short period of the main breeding season, which can last about a week after significant rains. The toad is therefore regarded as a typical explosive breeder. Adult toads congregate to breed in both temporary and permanent ponds, encompassing natural and anthropogenically disturbed environments. Thus, habitat diversity and large population size make this toad a particularly conspicuous species (Bionda et al. 2011; Sinsch et al. 2022).

Under the hypothesis that the disturbances caused by different levels of urbanization could produce a variation in size and body condition of individuals and a differentiation in their reproductive investment, we evaluated life history traits related to the fitness of common South American toad, *Rhinella arenarum*. We aimed to determine whether urbanization is harmful or beneficial to this species. In particular, we examined the body size, body and somatic condition (SC), parameters of reproductive investment, and size-assortative mating.

Materials and Methods

Study site and classification

The study area is characterized by a semi-dry climate, which tends toward semi-humid conditions. The highest rainfall typically occurs between October and March, with an annual average ranging from 800 to 1,000 mm (Cabido et al. 2003). The physiography of the study area consists of gently undulating plains with a slight slope that facilitates water stagnation. We selected 6 temporary and permanent wetlands of Río Cuarto (33º07′55″18″S—64º21′08″′96″W; Figure 1), located in the province of Córdoba, Argentina: 1) "Charca de las Brujas" (CB) is a temporary, shallow peri-urban wetland surrounded by a relict of native forest. 2) "Villa Dalcar" (VD), is a permanent recreational urban wetland with shallow margins; 3) "PR-30," is a temporary peri-urban wetland formed on the ditches of the Provincial Road Nº 30 (annual average daily traffic (AADT): medium to low with

significant peaks during the summer, 3,000–4,000 vehicles/day); 4) "NR-A005," is a temporary urban wetland formed in flooded ditches on National Road A005 (AADT: high, 10,000 to 15,000 v/d), surrounded by urban development on both sides, including residences and shops; 5) "NR-36," is a temporary peri-urban wetland located on the margin of National Road Nº 36 (AADT: medium-high, +7,000 v/d); 6) "CR," is a permanent wetland formed in an abandoned meander on Chocancharava river, a major watercourse that crosses the city, surrounded by the typical vegetation of the Espinal biogeographic district (xeric deciduous forest, Morrone 2014; Natale et al. 2019). Each wetland was previously known to have the presence of the species (Bionda et al. 2011; Babini et al. 2015; Grenat et al. 2023).

Wetlands were categorized according to the surrounding urbanization using Google Earth Pro™ and ArcGIS 10.5-ESRI. Wetlands were classified into 3 landscape categories according to the percentage of impervious surface within the 1,000 m buffer zone: high urbanization (≥50%), medium urbanization (≥30 < 50%), low urbanization (≥10 < 30%), and urban open space (<10) (Figure 1), after Hutto and Barrett (2021) and Grenat et al. (2023).

Data collection of *R. arenarum*

The wetlands were visited on the same day after 12 h of a rainfall event greater than 20 mm, during the 2017 breeding season. Breeding season began in October when the first amplexus was recorded. For each wetland, we collected between 5 and 11 amplexed pairs that had not yet initiated oviposition. We utilized the visual encounter survey method, which involves conducting surveys along the wetland's perimeter to locate these pairs (Heyer et al. 1994). Each captured pair was transported to the laboratory in a 10 L bucket filled with 5 cm of water to collect their eggs.

We measured the snout-vent length (SVL; mm) of each individual using a Vernier caliper with 0.01 mm precision and their weight (g) using a digital balance with 0.01 g precision. To determine reproductive effort (RE), we weighed amplexed females before and after spawning, after Bionda et al. (2011).

After spawning, we recorded the mass of each clutch by placing them in a strainer to drip dry and then weighing them using a digital balance with a precision of 0.01 g. Approximately 5% of the total mass of each oviposition was then removed and stored in Formalin Buffer (Bionda et al. 2011; Babini et al. 2018). To estimate the number of eggs per clutch, we counted the number of eggs in a 5% portion of the total weight and extrapolated this data to the total weight of the clutch. Finally, all adult individuals and eggs were released back into their original habitats.

The body condition index (BCI) of each individual and the somatic condition (SC) of the females after spawning were calculated as the residual of the SVL-mass (Băncilă et al. 2010). Given the sexual dimorphism in *R. arenarum*, with females being larger and heavier (Bionda et al. 2011), we calculated the BCI separately for each sex. Individuals with positive residuals are considered to be in good condition, whereas individuals with negative residuals are regarded as having low energy reserves (Brodeur et al. 2020).

Attributes of wetlands

We recorded habitat attributes related to water quality, morphometric structure, and vegetation coverage in each of the wetlands where amplexed pairs of *R. arenarum* were

Figure 1. Map of the location of the wetlands sampled in the city of Río Cuarto, Córdoba Argentina (geographic coordinates—WGS84). The maps were edited and illustrated using Google Earth Pro™ and ArcGIS 10.5-ESRI. Service Layer Credits: Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community.

collected. Water quality parameters, such as water and air temperature, pH, electrical conductivity, total dissolved solids, and salinity were measured in situ using a digital Oakton® Multi-Parameter Testr™ 35-Series 35425-10. Morphometry involved measuring the wetland depth, using a ruler, at the specific location where each amplexus was collected. Furthermore, we estimated the area of each wetland by measuring its length and width, as well as its temporality as permanent or temporary. Both submerged and emergent vegetation cover was visually recorded as the percentage within a 5-m strip surrounding the wetland.

Data analyses

Analysis of variance (ANOVA) was used to compare SVL between males and females. The body condition of each individual was calculated as a linear regression using log10 transformed mass as the dependent variable and log10 transformed SVL as the independent variable (Băncilă et al. 2010). Body and SC indexes were evaluated using ANOVA between wetlands. The DCG post hoc test (Test Di Rienzo, Guzmán, and Casanoves) was applied in all comparisons. The nonparametric Kruskal–Wallis test was employed when the assumptions of the ANOVA were not met.

A multivariate approach was conducted using principal component analysis to observe how the habitat variables are associated by the site. Subsequently, we extracted the first 2 PCs (which explained 62.3% and 21.3% of the variation). PC1 was strongly associated with the local attributes of wetlands (temporality, submerged vegetation, and water depth), whereas PC2 was associated with the percentage of surrounding urbanization (Table

1; Figure 2). The PCs were used as uncorrelated variables and included in a generalized linear model, to explore if the habitat/urban PCs can predict variations in any of the toad life history traits (WC, NEC, RE, and SC). We included the PC1 (habitat), PC2 (urbanization), and the interaction between them as fixed predictor variables. To control for potential pseudoreplication, the sampling site was included as a random effect in all models. We used Akaike's information criterion (AIC) to determine the model that better fits the data. Furthermore, we performed linear regression analysis to investigate: 1) size-assortative mating using the SVL of males and females in each amplexus; 2) the relationship between each female's body length and the number of eggs or clutch weight that it produces, using SVL data, number of eggs and total clutch weight; 3) the relationship between the number of eggs per clutch and the RE (weight loss) made by the female. All analyses were conducted using the software InfoStat (Di Rienzo et al. 2020) and R 3.4.1 (R Core Team, 2017). Statistical significance was considered to be reached at a P-value < 0.05.

Results

Biometric parameters

On average, the SVL of females was greater than that of males at all wetlands (Table 2). GLMs (Table 3A) showed differences among male and female SVL between urbanization categories, but the body condition index (BCI) was not statistically significant (Table 3A). We recorded in both males and females that individuals with smaller SVL also presented less BCI (Table 3; Figure 3). Only the VD wetland was the females with higher SVL but with poor BCI.

The relationship between the SVL of males and females (simple regression) collected in amplexus was positive but not significant in most wetlands (Table 4).

Reproductive investment parameters

The average weight and number of eggs showed high variability among wetlands, as did RE (Figure 4). However, they

Table 1. Eigenvectors of PCA of the habitat variables

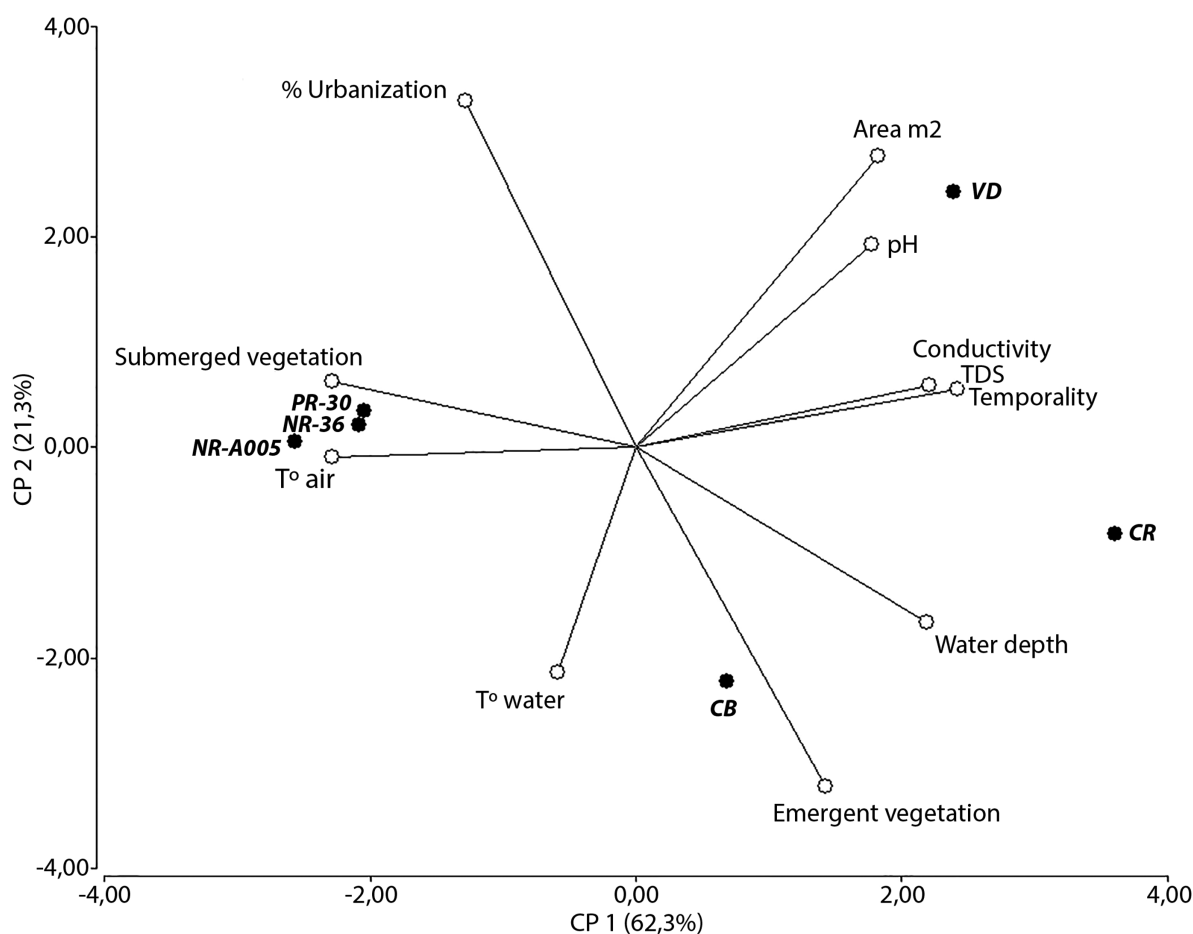|	Eigenvectors	
	PC1 (62.3)	PC2 (21.3)
$T°$ water (temperature of water)	–0.09	–0.33
$T°$ air (temperature of air)	–0.36	–0.01
Water depth	0.34	–0.26
pH	0.28	0.30
TDS (total dissolved solids)	0.34	0.09
Area	0.28	0.43
Urbanization percentage	–0.20	0.51
Emergent vegetation	0.22	–0.48
Submerged vegetation	–0.36	0.10
Temporality	0.38	0.09

Figure 2. Biplot (PCA) shows the differentiation of sampling wetlands based on habitat variables.

were not statistically significant, except for the SC ($P < 0.05$) (Table 3 B). It is observed that less urbanized wetlands (CB and CR) have heavier clutches a greater number of eggs and better RE.

The relationship between the SVL of females and clutch size was positive but not significant ($P > 0.05$). Additionally, we did not find a relationship between the number of eggs and RE for any wetlands ($P > 0.05$).

Discussion

Our study provides evidence that life history traits are modified according to the degree of urbanization of wetlands in the South American common toad *R. arenarum*. Males that inhabit wetlands with a medium degree of urbanization exhibit smaller SVLs. In contrast, other males have SVLs similar to those in highly urbanized wetlands and open spaces. This corresponds to body condition because males with low body condition index (BIC) are those who live in wetlands with a medium degree of urbanization. Females with higher SVLs were recorded in urban wetlands (both high and low urbanization), whereas those with lower SVLs were found in open space wetlands and with medium urbanization. Regarding the body condition of these females, it is observed

that those recorded in highly urbanized wetlands have a very low body condition. These very different results between wetlands may be due to attributed to phenotypic plasticity, local adaptation of the specie (Acosta 2009; Babini et al. 2018), or multiple factors such as survival, food availability, higher temperatures, behavioral changes, or simply older age, suggesting they have had more time to grow (Iglesias-Carrasco et al. 2017; Cogălniceanu et al. 2021). Because of this, determining the age of these individuals of *R. arenarum* would be interesting to corroborate this hypothesis. Low body condition in amphibians inhabiting urban wetlands, as also observed in the common spadefoot toad, *Pelobates fuscus*, has also been reported (Cogălniceanu et al. 2021) and may be attributed to the profound ecological changes associated with urbanization. These changes inevitably impact the trophic resources available to amphibians (Cogălniceanu et al. 2021). While some authors argue that urban environments have a greater abundance of resources (McIntyre 2000; Deichsel 2006), a habitat richer in nutrient availability does not necessarily mean an adequate and quality habitat (Polo-Cavia et al. 2010).

Reproductive investment parameters, such as clutch weight, number of eggs, and RE, were higher in wetlands categorized as low urbanization (CB), urban open spaces (CR), and high

Table 2. Mean and standard deviations of SVL in the studied *R. arenarum* (*n* = sample size) per sex and wetland. Different letters show statistically significant differences ($P > 0.005$) using DGC posterior tests

Wetland	Urbanization categories	SVL (mm)			
		Female	*n*	Male	*n*
CB	Low	114.61 ± 7.16 B	7	103.05 ± 6.79 B	7
VD	High	113.59 ± 12.43 B	6	107.33 ± 7.50 B	6
PR-30	Medium	104.49 ± 11.36 A	9	71.44 ± 20.38 A	9
NR-A005	High	110.98 ± 10.26 B	9	107.01 ± 16.46 B	9
NR-36	Medium	97.62 ± 7.44 A	4	91.46 ± 9.95 B	4
CR	Open space	104.53 ± 7.33 A	11	101.77 ± 9.65 B	11

Table 3. Results from the best GLM fit for the (A) biometric parameters by sex and (B) reproductive investment parameters analyzed between wetlands. Models include urbanization categories, PC1 (local attributes of wetlands), and PC2 (% of urbanization) as fixed factors and sampling sites, as random factors. AIC = Akaike information criterion. *P*-values significant at the 0.05 level are in bold

(A) Biometric parameter

	Male				Female			
Factor	SVL		BCI		SVL		BCI	
	AIC = 361.28		AIC = −30.08		AIC = 328.16		AIC = −202.52	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
	11.58	**<0.0001**	0.6	0.67	4.01	**0.01**	0.21	0.88

(B) Reproductive investment parameters

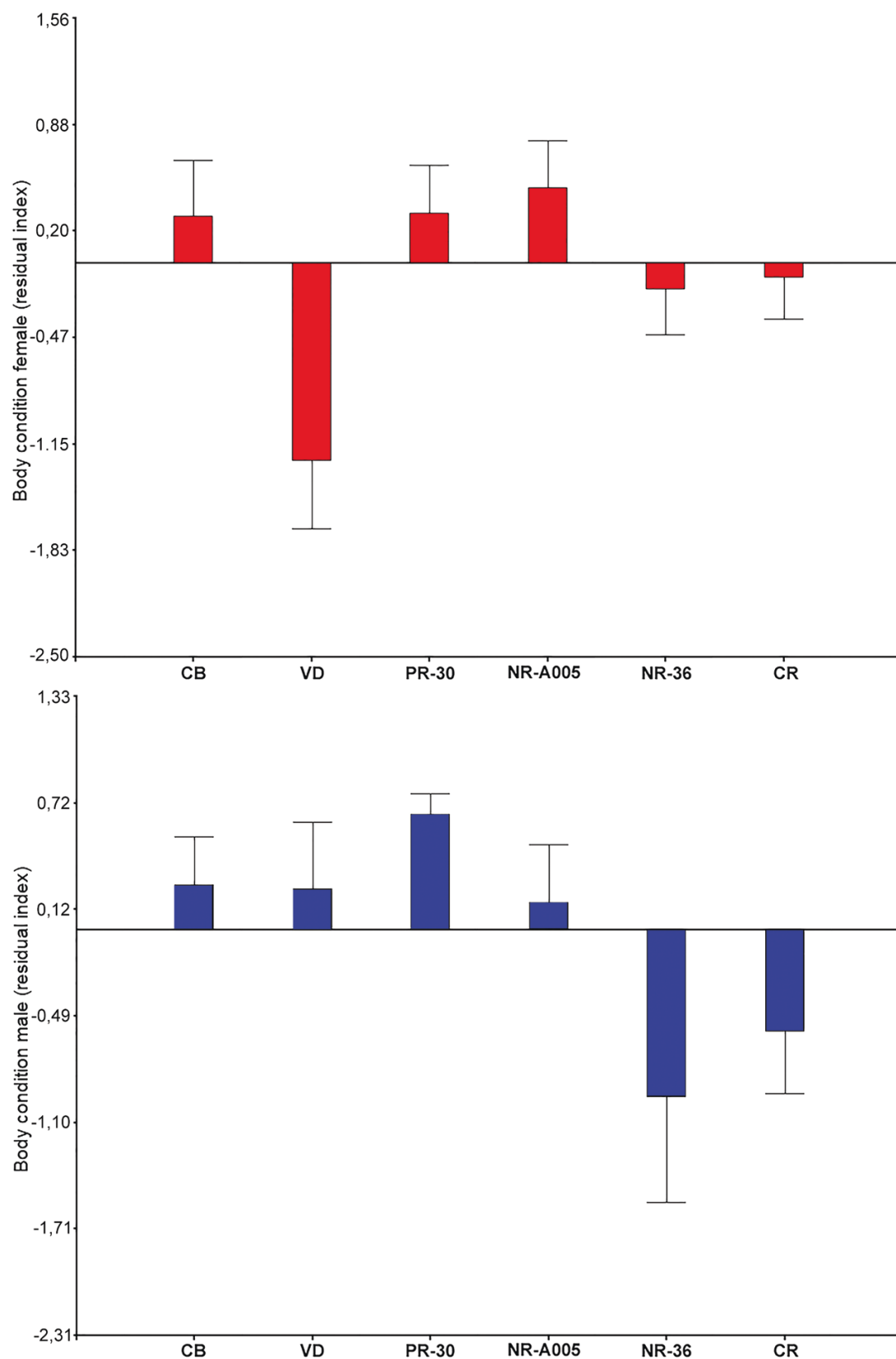| Factor	WC		NEC		RE		SC	
	AIC = 553.36		AIC = 621.05		AIC = 430.65		AIC = -426.03	
	F	P	F	P	F	P	F	P
PC1	0.06	0.91	0.38	0.79	0.005	0.82	4.41	0.042
PC2	1.48	0.69	0.004	0.96	1.62	0.21	6.99	0.01
PC1 × PC2	2.14	0.55	0.005	0.97	0.04	0.85	2.28	0.14

Figure 3. Body condition (mean ± S.E.) by the residual index of *R. arenarum* of different wetlands for females (above) and males (below).

urbanization (NR-A005) with values close to those reported for environments with little impact (Sanabria et al. 2007; Bionda et al. 2011). While most highly urbanized wetlands have a lower number of eggs (NEC, Figure 4), they show a

large variation in clutch weight (WC, Figure 4), with values that even overlap those of the sites with a greater number of eggs. This could reflect a compensation in reproductive traits, such as egg number and clutch weight, due to the protective

purpose of the gelatinous covering (Duellman and Trueb 1994; Méndez-Tepepa et al. 2023). Other highly urbanized sites, such as NR-A005, present reproductive parameters similar to the less urbanized ones, whereas in VD, a wetland with high urbanization, females remain in low SC. Alternatively, it could be attributed to the high phenotypic plasticity of the species (Acosta 2009), which suggests it could be considered a species tolerant to urbanization, potentially involving changes in life history traits (Cogălniceanu et al. 2021). This adaptive capacity aligns with life cycle theory, suggesting organisms optimize their fitness by allocating energy optimally between growth, reproduction, and maintenance requirements (van Noordwijk and de Jong 1986; Roff 2002). Size-selective mating was found in many amphibians, where females prefer larger males (e.g., Morris 1989; Tárano and Herrera 2003). Preference for large mates has presumably evolved because large females produce more eggs per clutch and large males have larger testes that contain more sperm (Halliday and Verrell 1988; Reiss 1989; Heino and Kaitala 1999; Bionda et

al. 2011). In our study, particularly in urbanized wetlands, we recorded a negative correlation in body size between males and females, with males considerably smaller. This bias in body sizes among populations during reproduction may be associated with the species' explosive reproductive behavior (Sinsch et al. 2022). The smaller size of males could reduce fertilization success, as the distance between cloacae increases, potentially hindering egg deposition signals (Chajma and Vojar 2016). Therefore, it would be interesting to evaluate the eggs' viability in these populations. Additionally, small males in heavily urbanized wetlands may result from carry-over effects from earlier life stages (larvae and metamorphs) due to factors such as food scarcity, water quality, temperature, and larval density (Snodgrass et al. 2008; Scheffers and Paszkowski 2016). Generally, larger females tend to lay more eggs (Duellman and Trueb 1994; Prado and Uetanabaro 2000; Chajma and Vojar 2016), and smaller maternal sizes could lead to reduced reproductive potential (Semlitsch et al. 1988), potentially increasing vulnerability to environmental disturbances (Jennette et al. 2019). The size and body condition of female anurans are recognized factors that significantly impact the allocation of energy to their offspring. However, in our study, we did not find a correlation between reproductive aspects and SVL of females. Similar results were reported for *R. arenarum* (Bionda et al. 2011) and other anuran species (Prado and Uetanabaro 2000). The absence of a correlation between female SVLs and the number of eggs per clutch could be due to the presence of multiple egg clutches during the reproductive season, impacting the number of eggs within each clutch (Prado and Uetanabaro 2000).

While urban wetlands might support amphibians' persistence in urban landscapes (Brand and Snodgrass 2010) by providing requirements such as submerged and floating

Table 4. Simple regression between the snout-vent length of females and males by wetland

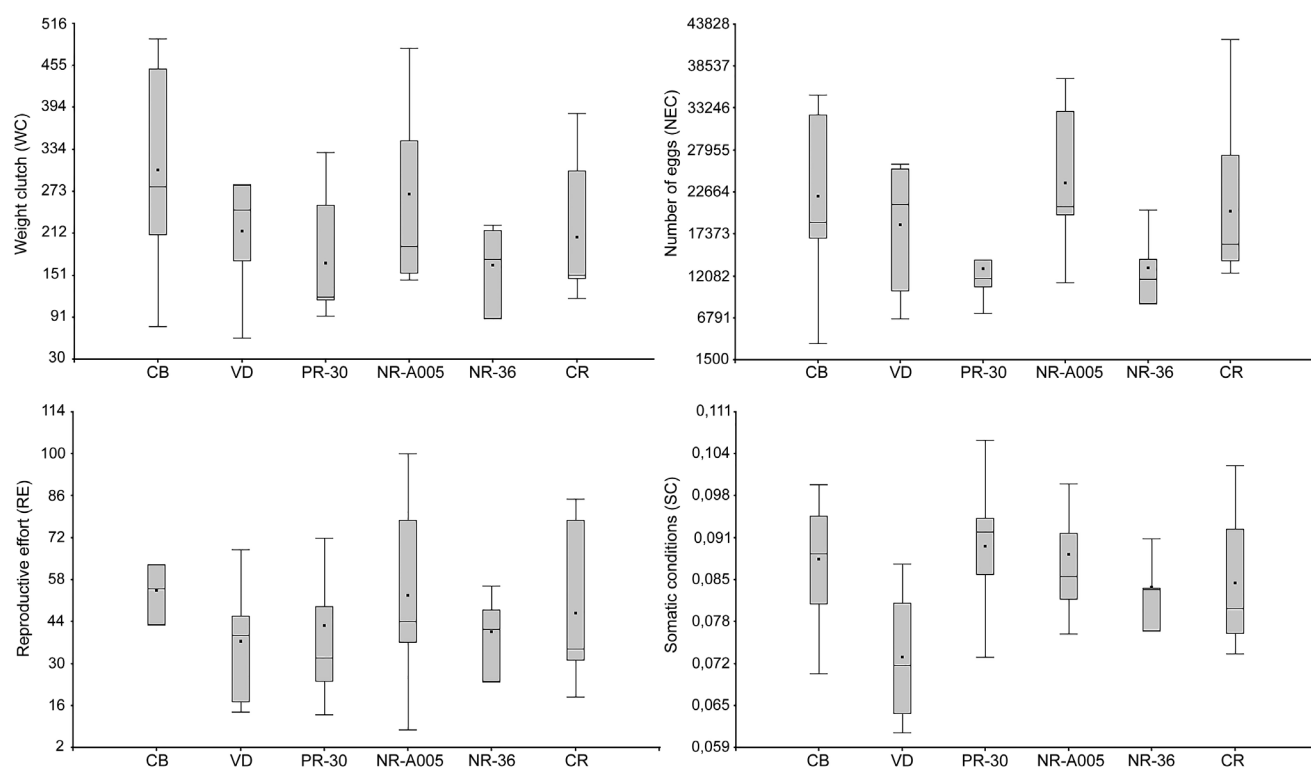| Wetland	R²	*P-values*
CB	0.15	0.39
VD	0.69	0.04
RP-30	0.13	0.35
RP-A005	0.09	0.44
RN-36	0.73	0.14
CR	0.1	0.8

Figure 4. Reproductive investment of *R. arenarum*.

aquatic vegetation and suitable water temperatures for spawning (Sanabria et al. 2007; Bionda et al. 2011), these habitats could act as ecological traps. An ecological trap occurs when environmental cues inaccurately represent a habitat's suitability for reproduction and survival (e.g., Schlaepfer et al. 2002; Battin 2004; Robertson and Hutto 2006). Therefore, we recommend evaluating egg viability (fertilized and abnormal eggs), in addition to the size of the clutches and the number of eggs in them. This is important because most urban and peri-urban wetlands receive run-off from impermeable surfaces that may contain contaminants, (Jennette et al. 2019) affecting the habitat quality for *R. arenarum* and with it, success in the reproduction event. Further studies are necessary to assess the long-term viability of the toad in urban wetlands, understand the variation in habitat quality between wetlands based on urbanization levels, and explore other populations and temporal variations to test whether our findings are general trends. Additionally, incorporating physiological methods (e.g., leukocyte profile and corticosterone level) would provide valuable insights into assessing the health status of wildlife populations.

Acknowledgments

Authors' Contributions

F.P., P.G., and A.M. conceived the ideas and study. F.P., C.B., M.B., M.O., and P.G. collected the data; F.P., C.B., and P.G. analyzed the data; P.G. and A.M. directed the working group. All authors contributed critically to the draft. All authors read and approved the final manuscript.

Conflict of Interest statement

The authors declare no conflict of interest.

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