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Demographic insights for the endangered Apennine yellow-bellied toad in the Pollino National Park (Southern Italy). A five-year Capture-Mark-Recapture study

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A R T I C L E I N F O

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A B S T R A C T

Investigating population structure, parameters, and growth over time is vital for conserving endangered taxa like the Apennine yellow-bellied toad (*Bombina variegata pachypus*) in Italy. The Apennine yellow-bellied toad faces habitat loss or degradation, disease, and climate change threats. Our study delves into the population dynamics of this endemic amphibian by monitoring populations in two breeding sites within the Pollino National Park (Southern Italy) using a five-year Capture-Mark-Recapture (CMR) approach. With a particular emphasis on demographic parameters such as population size, detectability, survival, temporary emigration probability, and body condition index, the research aims to contribute to understanding population dynamics and inform conservation efforts. We used two CMR models to estimate these parameters: Jolly-Seber with POPAN parametrisation and Pollock's robust design with Huggins' estimator. Results showed variation in body size between males and females, challenging previous findings in south-central Italy. For both populations, demographic data indicate high annual adult survival, fidelity to the breeding areas, support for no movement, and minimal trap response. Abundance over the years reveals a slight decline in one site and a gradual increase in the other. The estimated maximum population size indicated ~ 92 animals in one population and ~ 70 in the other one. Despite the high conservation concern for the Apennine yellow-bellied toad, there remains a dearth of quantitative information on demographic aspects in Southern Italy. The findings underscore the importance of protected areas in biodiversity conservation and contribute to understanding the *B. v. pachypus* conservation status, suggesting that small, isolated populations may be vulnerable to unpredictable fluctuations, inbreeding depression, and extinction risks. Additionally, the research emphasises the importance of long-term CMR studies to gain insights into population dynamics and develop effective conservation strategies and management practices, particularly in the face of climate changes that may impact both the endemic taxon and its habitat.

1. Introduction

Demographic monitoring of wild populations is a crucial aspect of ecological research and conservation efforts (e.g., Danielsen et al., 2005). It involves systematically collecting and analysing data related to species' population dynamics in their natural habitats. This monitoring provides valuable information for understanding wild populations' health, viability, and resilience. In particular, for taxa of high conservation concern, monitoring population abundance and demographic rates is essential to identify potential challenges to population viability and implement valid conservation and management actions. The amphibians are the most threatened vertebrate class (40.7 % of species are globally threatened; Luedtke et al., 2023). The global decline of amphibians is an ongoing phenomenon determined by the combined effects

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of multiple threats that jointly impact population trends (Falaschi et al., 2019; Luedtke et al., 2023). However, the demographic processes driving the declines remain inadequately understood and have displayed heterogeneous responses to broad-scale environmental stressors (Muths et al., 2018).

Capture-mark-recapture (CMR) models are effective for estimating population demography and testing ecological hypotheses (Jolly, 1965; Lebreton et al., 1992; Sandercock, 2020; Seber, 1965; Williams et al., 2002). Also, for amphibians, CMR methods are widely used tools (e.g., Cayuela et al., 2014, 2016a,b; Muths et al., 2018; Schmidt, 2004). In particular, photographic mark-recapture has become an increasingly popular non-invasive technique used on a wide range of taxa with unique natural marking patterns (Angelini et al., 2018; Romano et al., 2018; Sannolo et al., 2016; Wagner et al., 2011).

Italy is the most species-rich European country for amphibians, and many of the 41 species are endemic (Cox & Temple, 2009, Fig. 3 in Sillero et al., 2014, Sindaco & Razzetti, 2021). This study focused on an endemic pond-breeding amphibian, the Apennine yellow-bellied toad *Bombina variegata pachypus* (Bonaparte, 1838), which is distributed from the Northern (south of the Valley of the River Po) to Southern Apennines from a few meters above sea level up to 1700 m a.s.l. (Sindaco et al., 2006). At a specific rank, this endemic anuran is protected by Appendix II of the Bern Convention and Annex II and IV of the EU Habitat Directive (92/43/EEC). The Apennine yellow-bellied toad appears to have vanished from 50 to 80 % of the surveyed locations between 1996 and 2004 (Barbieri et al., 2004). For these reasons and the contraction of the area of occupancy and a significant reduction of mature individuals in the last ten years, this toad is among the most threatened Italian amphibians and is considered "Endangered" by the Italian IUCN assessment (Rondinini et al., 2022). Although the causes of this decline are still a matter of debate, the disease caused by the pathogenic fungus *Batrachochytrium dendrobatidis*, climate changes, introduction of predatory fish, habitat loss and degradation due to overgrazing or disappearance of suitable aquatic habitats, have all been linked to population crashes of the Apennine yellow-bellied toad (Bernabò et al., 2022a; Canessa et al., 2013; Canestrelli et al., 2013; D'Amen & Bombi, 2009; Fiacchini, 2007; Romano et al., 2012, 2023a; Stagni et al., 2004). Over the past few years, several effective conservation actions on this endemic taxon, at the local scale, aimed at protecting the natural breeding habitats, restoring artificial aquatic habitats, and reintroducing captive-bred animals in historical and new sites (Arillo et al., 2022, Brusco et al., 2022; Vignoli et al., 2021; LIFE WetFlyAmphibia, 2019). Despite the high conservation concern, there is a need for more quantitative information from long-term studies on the abundance, structure and dynamics of populations of *B. v. pachypus*, particularly in the southernmost portion of its range. This is a significant gap in research, considering that Southernmost populations harbours the highest genetic diversity (Canestrelli et al., 2006; Zampiglia et al., 2019) and, therefore, Southern Apennine represents a crucial area where conservation is (and will be) the most important in preserving this taxon (Bernabò et al., 2022b). Moreover, in recent research, Southern Italy's suitability is predicted to remain stable over time in a scenario of a worsening in *B. v. pachypus'* future suitable areas (Bernabò et al., 2022b). While many conservationists claim to safeguard "species," the practical focus of conservation efforts often lies in managing individual populations. This is even more true when dealing with the Evolutionary Significant Unit (ESU), a concept that allows for a disconnect between taxonomic and conservation value (e.g., Casacci et al., 2014; Fraser & Bernatchez, 2001).

We used data from five years of CMR sampling in two breeding sites of *B. v. pachypus* in the Pollino National Park with different aquatic environments and disturbance grades. Our aim was threefold: first, to highlight demographic parameters, including population size, detectability, survival, temporary emigration probabilities, and population gains; second, to evaluate the inter-annual variation of body condition between the two populations, one occurring in a disturbed site and the other in an undisturbed site; third, to plan specific measures for conservation, thus ensuring early management of this endangered taxon.

2. Materials and methods

2.1. Study taxon

The taxonomic rank of the Apennine yellow-bellied toads has sparked controversy over the years. In alignment with Sindaco and Razzetti's perspective (2021), we considered it at the subspecies level. *Bombina variegata pachypus* is a heliophilous anuran and usually breeds in small water bodies, such as shallow and unshaded ponds, including ephemeral puddles formed in farmland or pasture land as well as small temporary streams and rocky pools (Lanza et al., 2007). The Apennine yellow-bellied toads also colonise artificial aquatic sites in Mediterranean rural landscapes (Bernabò et al., 2022a; Canessa et al., 2013; Martino et al., 2022; Romano et al., 2023a, 2023b). Throughout the long breeding season (from May to October), females can lay multiple clutches due to postvitellogenic follicles, and males show prolonged spermiation (Guarino et al., 1998).

2.2. Study area

The study was conducted in two breeding areas in the Pollino National Park (Southern Italy), the largest of Italy's protected areas straddling two regions, Calabria and Basilicata (Fig. 1). Both areas were chosen within Special Areas of Conservation (SACs) of the Natura 2000 network, the largest coordinated network of protected areas in the world, extending across all 27 European Member States. The initiation of this study was integral to a broader monitoring project focusing on some amphibian species, a collaborative effort with the Park Authority. The two study areas are "Pianicello di Ruggio" (PRUG: 39°54′27,8′'N, 16°7′19′'E, 1550 m a.s.l; SAC IT9210125 Timpa dell'Orso – Serra del Prete) and "Piano di Tavolara" (PTAV: 39°46′18′'N, 16°1′33′'E, 1200 m a.s.l.; SAC IT9310020 Fonte Cardillo) (Fig. 1).

PRUG (Fig. 1B) is a flooded meadow (about 1.4 ha) with a peat bog and a small stream that branches off from a spring, localised in a small clearing in a microtherm beech forest (*Fagus sylvatica*) represented by natural habitat type "Apennine beech forests with *Abies alba* and beech forests with *Abies nebrodensis*" (habitat code 9220* of the EU Habitats Directive). PRUG is located in an environmental matrix dominated by the habitats "Lowland hay meadows (*Alopecurus pratensis*, *Sanguisorba officinalis*)" (habitat code 6510) and "Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia) (important orchid sites)" (habitat code 6210*). PRUG is not subjected to anthropogenic pressures and is located approximately 1.5 km linearly from another historically known population of *B. v. pachypus*.

PTAV (Fig. 1C) is a closely spaced water body system in an area of about 1.2 ha, situated in a clearing, characterised by habitat type code 6210* and surrounded by habitat type "Apennine beech forests with Taxus and Ilex" (habitat code 9210*). In PTAV, there are four water bodies. The larger one is an artificial elliptical pond (PTAV1, about 30 x 40 m) where alien fish (*Carassius auratus*) has been introduced; the second is a close smaller pond (PTAV2, about 4 x 10 m); the third is a flooded meadow with a brook in the N-E direction (PTAV3); finally, adjacent to the ponds, temporary pools form yearly in the deepest incisions along the forest track (PTAV4). We assumed that PTAV is an isolated population since no observations were reported from the surrounding areas (i.e., in a radius of about 2 km).

2.3. Sampling methods

We used visual encounter surveys to capture animals by hand or using a dipnet. The presence of eggs and tadpoles was recorded at each breeding site, but no targeted efforts at estimating their numbers were undertaken. We performed primary capture occasions for the two *B. v.*

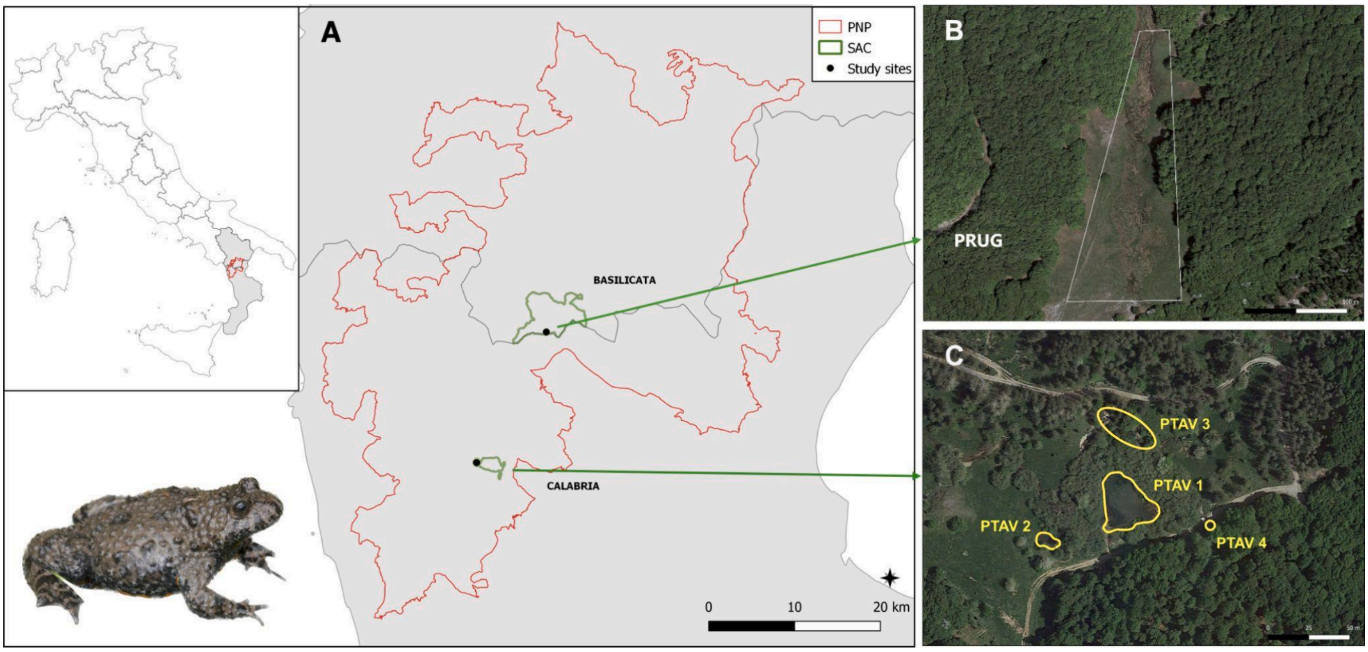

Fig. 1. (A) Location of the study sites and Special Areas of Conservation (SAC) in the Pollino National Park (PNP) in Southern Italy. (B) Satellite image of Pianicello di Ruggio (PRUG) and (C) Piano di Tavolara (PTAV). The investigated area and aquatic habitats are highlighted by white polygon and pond perimeter outlines, respectively.

pachypus populations during the breeding peak (May-July) of five consecutive years (2018–2022). These primary occasions consisted of three secondary sampling events separated by 5–10 days, except for 2021, when only two secondary occasions were conducted. The resulting encounter history consists of 14 live capture occasions with unequal time spacing. Each sampling occasion, carried out by 3–5 surveyors, entailed three capture sessions of 60 min in PRUG and 30 min for each waterbody in PTAV. Sampling was conducted during daylight (12:00 – 6:00 pm).

2.4. Marking procedure and morphometrics

Since colouration and spot patterns in Yellow-bellied toads become permanent after the first year and do not change over time (example provided in Supplementary Fig. S1) (Zakharova et al., 2022 and reference therein), pictures of the ventral pattern are widely used for individual identification in multi-year studies (e.g., Angelini et al., 2018; Cayuela et al., 2014). Photo-identification was performed using the program I3S Pattern+ (Interactive Individual Identification System, available at https://www.reijns.com/i3s), which has been proven to be efficient for amphibians characterised by unique natural mark patterns (Sannolo et al., 2016). A number code was assigned to Apennine yellow-bellied toads, and a photographic database was assembled and updated after each sampling occasion. Recently metamorphosed with ventral markings not yet wholly developed were not incorporated in CMR analyses. Definitive sex was retroactively assigned to juveniles or subadults upon recapture in subsequent years, provided they had reached sexual maturity.

Each captured individual was also measured for snout-to-vent length (SVL) by the same person (ensuring that any operator-dependent measurement error remained constant), using a calliper with a precision of $\pm$ 1 mm. Additionally, they were weighted (to the nearest 0.01 g using digital scales) and sexed based on the presence of nuptial pads on the forelimbs. Toads were released at the capture point at the end of each session. Four age classes for Apennine yellow-bellied toads were defined based on SVL: recently metamorphosed (SVL $\leq$ 19 mm), juveniles (SVL $>$ 19 and $<$ 30), subadults (SVL $\geq$ 30 and $<$ 35), and adults with SVL equal or over 35 mm (Cayuela et al., 2016a, 2016b).

2.5. Capture-mark-recapture models and demographic parameters

We used Program CloseTest (Stanley & Richards, 2005) to investigate population closure between years and within each year. The demographics of both populations were found to be open between primary occasions (all P $<$ 0.001) and closed between secondary ones (P $>$ 0.5 for both populations). Encounter histories were thus analysed using two open population CMR models to estimate several demographic parameters detailed in Table 1. First, we modelled the encounter histories using a POPAN formulation of the Jolly-Seber model (POPAN) (Schwarz & Arnason, 1996). POPAN does not condition on the first capture and uses the assumptions of equal capture probability to both marked and unmarked individuals. Moreover, the POPAN model assumes that all emigration from the sampling area is permanent (Pollock et al., 1990). POPAN enables joint estimation of four parameters: apparent survival (Φ), probability of capture (p), superpopulation size (i.e., the total number of individuals available to enter the sampled population) during the whole study period (N_{super}), recruitment or probability of entry from the superpopulation (*pent*). In addition, the POPAN model allows estimating the population size at each sample occasion *i* (N_{popan}) through the formula: $N_{popant+1} = (N_{popant} \times \Phi_t) + (N_{super} \times pent_t)$ (Table 1). Second, data were analysed with Pollock's robust design (PRD) with Huggins' estimator model (Pollock, 1982; Williams et al., 2002), which accommodates to a hierarchical structure of the data organised in secondary occasions (sampling sessions) nested within primary occasions (the annual sampling periods). Under this framework, populations are assumed to be open during the time interval between primary occasions and closed within secondary occasions. PRD provides unbiased estimates of annual population size (N_i), true survival probability (S), and two detection parameters, the probability of capture (p) and recapture (c), which describe the average probability of capturing each unmarked and marked individual, respectively, in a given secondary sampling occasion. In addition, PRD analysis allows to decompose apparent survival φ into true survival (S) and migration rates (γ). The probabilities of temporary emigration γ_i'' and γ_i', which are the probabilities that an individual in period *i* is unavailable for detection (e.g., outside the study area), given that it was available or unavailable, respectively, for

Table 1
List of parameters estimated and derived from the POPAN Jolly-Seber (POPAN) and Pollock's robust design (PRD) with Huggins' estimator models.

	Parameter	Symbol	Description
	Population size	N_{super}	Total number of animals, both observed and unobserved, in the study area and available for capture.
		N_{popan}	Single-occasion estimates of population size.
		N_i	Estimation of population size for each primary sampling period.
POPAN	Apparent survival	Φ	Probability of an individual surviving between sampling occasions from time *t* to time $t+1$ and that it is available for recapture in the study area.
	Probability of capture	*p*	Probability that an individual is available for capture at time *t*, given that it is alive and in the observable population.
	Recruitment	*pent*	Probability that new individuals from N_{super} enter the population between *t* and $t+1$.
PRD	Survival	*S*	Probability that an individual survives in the study area between primary sessions.
	Probability of capture	*p*	*p* refers to the probability that an animal is captured on secondary occasion *j* of primary period *i*, given that it is in the population on that occasion.
	Probability of recapture	*c*	Probability that an individual previously marked in primary period *i* is available for capture and is recaptured in sampling secondary occasion *j*, given that it is in the population on that occasion.
	Probability of temporary emigration	γ_i	Probability that an animal is out of the study area and unavailable for capture during the primary period *i*, given that it was present (γ'') or was not present (γ') in the previous primary sampling period ($i-1$) and survives to capture occasion. Temporary emigration can be modelled for no movement, as random, where the individual's presence or absence during a previous sampling period has no bearing on their status in the next sampling period, or Markovian movement, where an individual's status in the present sampling period is influenced by their status in the previous one.

detection in the period i–1 (Kendall et al., 1997). Temporary emigration (γ", γ') can be modelled for no movement ($\gamma' = \gamma'' = 0$; all animals available for capture), as random ($\gamma' = \gamma''$) or Markovian movement ($\gamma' \neq \gamma''$) (Table 1). The movements of animals in the form of temporary emigration from the study area (i.e., shifting to an unobservable state) were considered since a previous study on *B. variegata* has shown that breeding can be facultative in a given year and thus skipping breeding in a given year can be regarded as a temporary emigrant (Cayuela et al., 2014, 2015). For a subsequent comparison of population size estimates from the models, we used the mean of secondary occasion estimates as a measure of annual synthesis for POPAN.

2.6. Model selection and statistical analysis

Main effect models were built in order to evaluate the variations in demographic parameters as a function of several factors, specifically whether they are assumed to be constant (.), or vary with time (t) or sex-dependent (sex). Before restricting to above-cited models also additive and interaction effect were also considered. We constructed 18 candidate models for the POPAN models; we tested Φ and p to remain constant or vary across time and by sex and recruitment (*pent*) without accounting for gender dependency (Supplementary Table S1). For the PRD analysis, 54 candidate models were constructed, running biologically plausible parameter settings combinations: *S* and p were modelled as constant, time-varying or sex-dependent, whereas c is constrained equal to p; γ", γ' were tested null, random or Markovian (Table S2).

We ranked candidate models based on Akaike's Information Criterion adjusted for small sample sizes (AICc), and normalised Akaike weights (w); we considered models with ΔAICc of less than two from the best model as showing substantial support (Burnham & Anderson, 2002). If the Akaike weight w of the best supported model was < 0.90 (i.e., more than one model is supported), we used model-averaging to obtain parameter estimates (Burnham & Anderson, 2002). Thus, we report model-averaged survival, entry and capture probabilities, temporary emigration, and abundance estimates. All models were built and parameterized according to observed data using the 'RMark' package v.3.0 (Laake, 2013), through the R software (version 4.2.1; R Core Team, 2022), which provides a formula-based interface for the analysis of CMR data with the program MARK (White & Burnham, 1999). Transience and trap-dependence tests were applied to the CMR dataset using R2ucare, an R package to perform goodness-of-fit-tests (Gimenez et al., 2022).

The operational sex ratio was calculated as the proportion of mature males to the total number of adults [males/(males + females)] (Wilson & Hardy, 2002). The deviations from the equality were assessed by a two-tailed binomial test. Variations between sites and sex in body size and body condition index were investigated only for adults and subadults; if individuals were recorded more than once, only size data from the last capture were included in our analyses.

$$\widehat{M}i = Mi\left[\frac{L0}{Li}\right]^{bSMA}$$

We computed the body condition index as the Scaled Mass Index (SMI) according to Peig and Green (2009); it provides a comparable measure among individuals and populations in amphibians (Brodeur et al., 2020; Landler et al., 2022; MacCracken & Stebbings, 2012). Accounting for allometric growth, this index is computed by rescaling body mass measures to SVL (Peig & Green, 2009) using the formula:

We calculated the scaling exponent bSMA in R on log-transformed data using the "smatr" package (Warton et al., 2012). The slope of the regression was used during the SMI calculations, where M_i and L_i were the observed values, L_0 was the average length value for all individuals, and $\widehat{M}_i$ was the predicted value for mass (Peig & Green, 2009). For sex comparisons, we separately calculated the SMI for females and males using the bSMA derived from the entire sample.

Since morphometric data were not normally distributed based on Shapiro–Wilk tests, we used Wilcoxon rank-sum tests to determine whether there was a significant difference in body size and condition between sex intra- and inter-populations. To test if SMI displayed sex- and site-specific variations, we performed a Linear Mixed-Effects Model with sex, site, and their interaction as covariates and year as random effect using "lme4" package (Bates et al., 2015) in R4.2.1. We considered differences significant at $P < 0.05$.

3. Results

The reproductive contingents of both populations of the Apennine yellow-bellied toads are relatively small, each consisting of a few dozen individuals. In PRUG, over the period 2018–2022, a total of 281 toads were captured, with 64 unique individuals marked (30 males, 27 females, and 7 juveniles); the total number of captures varied among years, with a minimum of 41 and a maximum of 74 (Table 2 and Fig. 2). In PTAV, a total of 137 capture events of Apennine yellow-bellied toads were recorded, from which 46 different animals were identified (22

Table 2
Summary of *Bombina variegata pachypus* capture data and *sex* ratio in annual primary sampling period in Pianicello di Ruggio (PRUG) and Piano di Tavolara (PTAV). Asterisks mark a significant deviation from evenness, * P < 0.05, *** P < 0.001 (Fisher's exact probability test).

Year	CMR occasions		N captures		N recaptures		N new captures		*Sex ratio*	
	PRUG	PTAV	PRUG	PTAV	PRUG	PTAV	PRUG	PTAV	PRUG	PTAV
2018	3	3	67	25	31	9	36	16	0.59	0.62
2019	3	3	74	18	61	15	13	3	0.50	0.59
2020	3	3	46	34	44	25	2	9	0.61	0.66*
2021	2	2	41	27	37	20	4	7	0.50	0.55
2022	3	3	53	33	44	22	9	11	0.56	0.76***
Total	14	14	281	137	217	91	64	46		

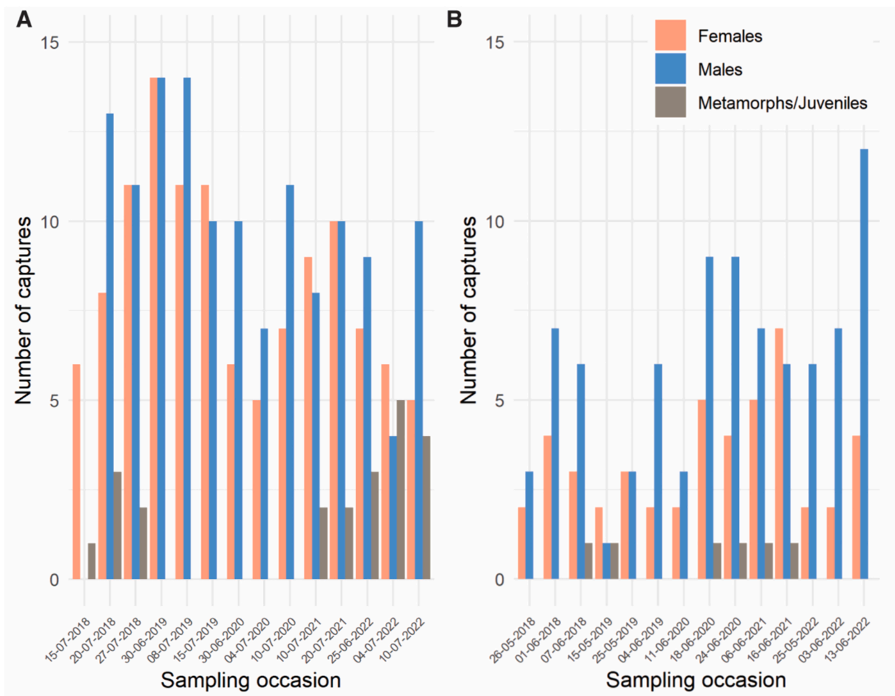

Fig. 2. Number of males, females, recently metamorphed and juveniles of *Bombina variegata pachypus* captured in each sampling occasion over the study period in (A) Pianicello di Ruggio and (B) Piano di Tavolara.

males, 21 females, and 3 juveniles); the total number of individuals captured per year ranged from 18 to 34 (Table 2 and Fig. 2). In PTAV, during the study period, 66 % of the total capture occurred in the same water body. The number of captures in the four water bodies was as follows: 11 in PATV1 (the larger pond, with alien fish), 91 in PTAV2 (the smaller pond), 5 in PTAV3 (the brook), and 30 in PTAV4 (the temporary pond) (Fig. 1). Movement among water bodies was recorded for ten animals (6 females and 4 males) who moved between PTAV1, PTAV2 and PTAV4, while one female moved from PTAV3 to PTAV2. The movement distances among water bodies range from 50 to a maximum of 110 m. No eggs were observed in the fish-invaded pond (PTAV1) over the years. In PRUG and PTAV, respectively, 76.6 % and 67.4 % of the animals were recaptured more than once across the primary periods, whereas 9.4 % and 10.9 % all years. Throughout the 14 secondary occasions, individual capturing frequency ranged from 1 to 11 (PRUG: mean = 4.4, SE = 0.38; PTAV: mean = 3, SE = 0.35), with 23.4 % in PRUG and 32.6 % in PTAV of individuals captured only once and 12.5 % and 4.3 % at least ten times, respectively. Recently metamorphosed were rarely captured in both sites (2.2 % of all captures). During the single annual season, the male captures outnumbered the female captures in PTAV with a statistically significant sex bias in 2020 and 2022 (binomial test: P = 0.03 and P = 0.0002, respectively), whereas in PRUG, male predominance at the breeding site was less marked and *sex ratio* almost balanced (Table 2 and Fig. 2).

3.1. Morphometrics

Overall, the body size and condition index of females and males were not similar. Females were significantly larger than males in both populations (mean value ± SE; SVL = 50.50 mm ± 0.57 range: 35–55 mm, and 47.75 mm ± 0.59, range: 33–53 mm, respectively; F = 16.23, df = 1.98, P < 0.001). Females were also significantly heavier than males (11.05 g ± 0.34, range: 3.40–15.15 g, and 9.51 g ± 0.27, range: 3.40–12.76 g, respectively; F = 15.37, df = 1.98, P < 0.001). The SMI highlighted significant differences between sexes (10.94 ± 0.23, range: 8.13–14.64 for females, and 9.43 ± 0.16, range: 7.46–12.03 for males; F = 27.6, df = 1.98, P < 0.001). Therefore, in both areas, statistically significant differences were found between sexes in all morphometrics variables at the intra-population level, whereas no differences were found comparing the same sex at the inter-population level (Table 3). The annual body size and condition of captured individuals in the two populations are shown in Fig. 3. Results from the linear model indicated a significant effect of sex on SMI; females had better SMI than males within populations, estimated with either the bSMA from the entire sample or population-specific coefficient (Table S3 and Fig. S2).

Table 3
Descriptive statistics of biometrics of *Bombina variegata pachypus* captured at PRUG and PTAV. SVL = snout-vent length; SMI = scaled mass index; N = number of individuals; SE = Standard Error; Range (min–max).

Site	Sex	N	Body mass (g)	SVL (mm)	SMI
			Mean ± SE(Min-max)	Mean ± SE (Min-max)	Mean ± SE(Min-max)
PRUG	Females	27	11.09 ± 0.50 (3.40–14.93)	50.04 ± 0.89(35–55)	10.85 ± 0.31 (8.35–14.27)
	Males	30	9.82 ± 0.40 (5.23–12.76)	47.43 ± 0.77(37–52)	9.70 ± 0.17 (8.08–11.81)
	F *vs* M		P < 0.05	P < 0.01	P < 0.01
PTAV	Females	21	11.01 ± 0.45 (7.76–15.15)	51.10 ± 0.63(46–55)	10.97 ± 0.33 (8.40–14.32)
	Males	22	9.08 ± 0.34 (3.40–11.18)	48.18 ± 0.93(33–53)	9.06 ± 0.23 (7.65—11.93)
	F *vs* M		P < 0.01	P < 0.05	P < 0.001

3.2. Survival, probability of capture, recruitment and temporary emigration

We did not detect "transience" or "trap-dependence" effects in any dataset (P-values ranged from 0.243 to 1.000, in both sites), thus providing evidence in support of the null hypothesis of test 3.SR and 2.CT, respectively.

In PRUG, the time-dependent survival probability (Φ and *S*) received a reasonable level of support in our set of POPAN and PRD models (Table 4). Model-averaged apparent survival probability Φ between consecutive sampling occasions varied from 0.60 (95 % CI = 0.43–0.74) to a maximum of 1 (95 % CI = 0.99–1.00) in POPAN models (Table S4). Similarly, from PRD true survival S range from 0.57 (95 % CI = 0.42–0.72) to a maximum of 1 (95 % CI = 0.99–1.00) across years. The average estimated probability of catching an animal during a capture event was sex-dependent and similar between POPAN (♀: 0.61, CI = 0.53–0.67; ♂: 0.62, CI = 0.55–0.68) and PRD (♀: 0.60, CI = 0.40–0.76; ♂: 0.62, CI = 0.42–0.78) models (Table S4). The recapture rate was 0.60 (CI = 0.53–0.66). The probability of entry (pent) was very low and not affected by time (0.03, CI = 0.02–0.04).

In PTAV, the time dependent survival probability was less supported while the capture probability resulted sex-dependent from both used approaches (Table 4). Specifically, averaged Φ estimate resulted in 0.93 (95 % CI = 0.87–0.97) from POPAN and S estimate equal to 0.91 (CI = 0.44–0.99) from PRD approach. The sex-dependant capture probability estimate from POPAN was 0.54 (CI = 0.44–0.66) for males and 0.30 (CI = 0.21–0.42) for females (Table S4). Also from PRD, the estimated p resulted higher for males 0.36 (0.36, CI = 0.16–0.59) than females (0.25, CI 0.07–0.58), with a higher estimated recapture (c = 0.62; CI = 0.50–0.72) leading to a positive behavioural response to capture. The pent value was constant (0.06, CI = 0.04–0.07). For both sites, model-averaged temporary emigration probability was null for γ' and approximately close to zero for γ'', even though some models have Markovian movement (Table 4).

3.3. Population size

We derived population size in the study sites for each sampling occasion using estimated parameters from POPAN (N_{popan}) based on the best supported models shown in Table 4. All the total numbers per capture occasion were lower than the per occasion estimates. In PRUG, estimates varied from 40 in 2018 (occasion 2 and 3, 95 % CI = 32–49, and CI = 31–49, respectively) to 21 in 2022 (occ. 2, CI = 13–29), whereas in PTAV, N_{popan} ranged from 14 in 2018 (occ. 1, CI = 6–22) to 29 in 2022 (occ. 3, CI = 20–38) (Table S4). The superpopulation was estimated as ~ 92 for PRUG and ~ 70 for PTAV. Both numbers were higher than the total number of Apennine yellow-bellied toads captured during the study; that is, 30 % of individuals in PRUG and 34 % in PTAV were never observed. In PRUG, the annual population size estimates were consistent between the two analysis and were slightly higher or similar to the maximum number of individuals captured per year; estimates ranged from 23 (CI = 15–31) to 40 (CI = 31–49) with POPAN and from 24 (CI = 20–28) to 42 (CI = 36–48) with PRD (Fig. 4A). At the beginning of our study, PRUG hosted a larger population, but progressively, the population declined, with an apparent recovery in 2021 (Fig. 4A). On the contrary, the population at PTAV showed a slight increase over the five years, except for the estimate in the breeding season of 2022 from the PRD model (Fig. 4B). The yearly estimates of population size from PRD model, except for 2019, exceed the estimated mean annual abundance obtained from POPAN, ranging from 16 (CI = 8–33) to 46 (CI = 24–93) in 2021, and showed wide confidence intervals (Fig. 4B). The estimates of annual population size provided by the POPAN model were more precise, lower than in PRUG and resulted in values slightly superior or similar to the maximum number of individuals captured per year varying from 16 (CI = 8–23) to 29 (CI = 20–38) (Fig. 4B).

4. Discussion

Traditionally considered crucial tools for conservation (Margules & Pressey, 2000), protected areas are viewed as the most likely sanctuaries for biodiversity in landscapes, which are increasingly impacted by human activities (Bruner et al., 2001). Long-term monitoring in protected areas plays a pivotal role for assessing potential threats, establishing connections between conservation measures and species outcomes, and improving overall management practices. Understanding the demographic dynamics of populations over time is crucial for effective conservation efforts, and CMR methods have proven invaluable in achieving this understanding (Schmidt, 2004; Wagner et al., 2011). Like many amphibians (Leudtke et al., 2023), the Apennine yellow-bellied toad faces various threats (habitat loss, disease, climate change effects, invasive alien species). Therefore, population studies are essential to identifying these threats' effects and directing conservation efforts accordingly.

4.1. Morphometrics

Our study revealed sexual size dimorphism (SSD), in contrast to other populations of Apennine yellow-bellied toads in south-central Italy (de Meis et al., 2022; Di Cerbo & Ferri, 2000; Guarino et al., 1995; Mattoccia et al., 2006; Romano et al., 2014; Romano & Carafa, 2022). Unlike previous studies, the toads in the examined populations exhibited SSD, with females being slightly larger than males, as reported for the Italian populations of the nominal subspecies (Caldonazzi et al., 2000; Di Cerbo & Biancardi, 2006) although this pattern is not evident at a specific level (Di Cerbo & Biancardi, 2011). The reasons underlying SSD in amphibians are numerous and remain subjects of ongoing debate (Pincheira-Donoso et al., 2021). At the family level, Bombinatoridae, which includes only two genera, *Bombina* and *Barbourula*, is noted for having a relatively small proportion of SSD (refer to Fig. 1 in Pincheira-Donoso et al., 2021). It is plausible that in taxa where SSD is not pronounced, genetic or ecological factors may operate at a local level, potentially playing a decisive role in either mitigating or amplifying size differences between the sexes. The reliability of using body length as an age index was questioned (Guarino et al., 1995; Plytycz & Bigaj, 1993) and a broader morphometrical study, encompassing more populations and variables, may elucidate whether it is a distinctive trait exhibited by all populations at the edge of the range.

The body condition index, a proxy for physiological state and general health in amphibians, was employed to assess individual fitness (Brodeur et al., 2020; MacCracken & Stebbings, 2012). In *Bombina variegata*, mating success may depend on body condition, and it is correlated with population and habitat quality parameters (Băncilă et al., 2010; Cayuela et al., 2017; Scheele et al., 2014). Our study focused on two sites with

Fig. 3. Body mass (A and B), snout-to-vent length (SVL) (C and D), and Scaled Mass Index (SMI) (E and F) of females and males of *Bombina variegata pachypus* in PRUG and PTAV across 5 years of the study. Box plot expressing median (line inside the box), upper and lower quartiles (top and bottom of the boxes), range minimum and maximum (whisker) and outliers (dots).

differing levels of disturbance: PTAV, impacted by off-road vehicles, alien predatory fish presence and a *Batrachochytrium dendrobatidis* (Bd) infection (Costa et al., 2021) and PRUG, unaffected by human interference and by Bd (unpublished data). We used the scaled mass index (SMI) to compare body conditions between these populations. Although no significant difference in SMI between the populations was observed over the study period, there were consistent differences between males and females in both sites. High values of physical condition do not necessarily indicate good health or strong fitness. In pioneer species that breed in temporary water bodies, reproductive opportunities can differ significantly between populations and seasons. While high body condition values likely reflect favorable feeding conditions, they could also suggest a lack of opportunities to allocate stored resources towards reproduction, in particular for females (Baumgartner & Gollmann, 2017; Landler et al., 2022). This perspective justifies that populations do not differ overall in the body condition index but, simultaneously, justifies a difference in the SMI between males and females at an intrapopulation level. In our populations, females generally exhibited higher indices, with an increasing trend in PRUG over five years while in PTAV, females maintained higher values than males, except in 2022, when values were comparable. The presence of predatory alien fish can lead to direct reductions in amphibian populations (reviewed by Kats & Ferrer, 2003). Contrary to expectations, anthropogenic stress and fish introduction did not affect individual body conditions in PTAV. However, this could be attributed to the quite exclusive use of fishless alternative breeding ponds (PTAV2-4) adjacent to the main one (PTAV1), where fish are

Table 4
Summary of the best fitting models for POPAN Jolly-Seber model (POPAN) and Robust Design (RD) ranked by their Akaike information criterion for small samples values (AIC_c) with $\Delta AIC_c < 2$ (AICc weight < 0.9 for the first model). ΔAIC_c: difference in AIC_c value from the model with the lowest AIC_c; (*k*) number of parameters. (Φ) apparent survival, (*S*) true survival, (*p*) capture probability, (*c*) probability of recapture, (*pent*) probability of entry, (*γ'*,*γ"*) temporary emigration parameters. (.) costant; (*t*) time-dependent parameter; (*sex*) sex-dependent; for PRD *c* is constrained as *c* = *p*.

Site	Model	AIC_c	ΔAIC_c	AIC_c weight	*k*
PRUG					
	(i) POPAN				
	$\Phi(t)p(.)pent(.)$	690.70	0	0.65	16
	$\Phi(t)p(sex)pent(.)$	692.43	1.73	0.27	17
	(ii) RD				
	$S(t)\gamma'_0(.)\gamma''_0(.)p(.)c()$	710.38	0.00	0.17	6
	$S(t)\gamma'_0(.)\gamma''_0(.)p(t)c()$	710.97	0.60	0.12	8
	$S(t)\gamma'_0(.)\gamma''_{rand}(.)p(.)c()$	711.06	0.68	0.12	7
	$S(t)\gamma'_0(.)\gamma''_{rand}(.)p(sex)c()$	711.59	1.21	0.09	8
	$S(t)\gamma'_0(.)\gamma''_0(.)p(sex)c()$	711.68	1.30	0.09	7
	$S(t)\gamma'_0(.)\gamma''_{rand}(.)p(t)c()$	711.84	1.45	0.08	9
PTAV					
	(i) POPAN				
	$\Phi(.)p(sex)pent(.)$	411.12	0	0.64	5
	$\Phi(sex)p(sex)pent(.)$	412.88	1.75	0.27	6
	(ii) RD				
	$S(.)\gamma'_0(.)\gamma''_0(.)p(sex)c()$	403.38	0	0.14	4
	$S(t)\gamma'_0(.)\gamma''_0(.)p(sex)c()$	404.13	0.75	0.10	7
	$S(.)\gamma'_0(.)\gamma''_{markov}(t)p(t)c()$	404.46	1.09	0.08	9
	$S(.)\gamma'_0(.)\gamma''_{markov}(t)p(sex)c()$	404.93	1.55	0.07	8
	$S(.)\gamma'_0(.)\gamma''_0(.)p(.)c()$	405.02	1.65	0.06	3

present, and in which only 8 % of captures occurred. Further quantifications of the relationship between environmental characteristics, body conditions of individuals and populations, and population abundance are required to elucidate this topic.

4.2. *Survival, probability of capture, recruitment and temporary emigration*

In PRUG, the adult *sex ratio* is balanced, while in PTAV, it appears skewed towards males in some years. As adult males and females are estimated to have similar survival, the skewed *sex ratio* could be attributed to a bias in fidelity to the breeding site or to differences in capture probabilities between sexes, with males having higher capture rates (as we observed in our populations). Similar instances of a *sex ratio* in favour of males have been reported in other populations (Colliva et al., 2007; Di Cerbo & Ferri, 2000) although they contrast with what is typically observed in the nominal subspecies, where males tend to exhibit lower site fidelity compared to females (Barandun & Reyer, 1998).

High global recapture rates were observed in both sites. In PRUG, survival was high and time-varying, with high sex-dependent capture probability. In PTAV, also high but constant survival was noted, with sex-dependent capture probabilities favouring males. Both populations showed no migration and stability, with negligible recruitment, few juveniles, and a predominance of adults. Demographic parameters were similar to other populations in central Italy (Angelini et al., 2018). The low number of juveniles presents a challenge to explain, potentially stemming from various factors like poor reproductive success, low detectability or a high juvenile mortality rate. Long-term studies on *B. v. variegata* have revealed a decreased survival probability for juveniles in comparison to adults (Cayuela et al., 2016a), although this finding is accompanied by considerable uncertainty due to the scarcity of juvenile recaptures (Spitzen-van der Sluijs et al., 2017), mirroring the situation we have encountered. PRD models indicate the lack of temporary emigration, thereby supporting the notion of high site fidelity in *B. v. pachypus*. This observation aligns with the general pattern observed in *B. variegata*, as documented in previous studies (Płytycz & Bigaj, 1984, Hartel, 2008). According to this general rule, individuals of *B. variegata* are less prone to dispersal in stable habitats and breeding dispersal is strongly related to the predictability of breeding resources (Cayuela et al., 2016b, 2020).

4.3. *Population size*

We used two reliable methods to estimate population sizes of an anuran with prolonged breeding, whose members are asynchronously present at the breeding site: the POPAN model and the robust design (e. g., Bailey et al., 2004, Pellet et al., 2007, Wagner et al., 2011). As expected, the POPAN model performed best when both survival and capture probabilities were high (Schwarz & Arnason, 1996, Wagner et al., 2011). In the PRUG breeding site, population size estimates were consistent (23 to 40 individuals with POPAN, 24 to 42 with PRD model), while in PTAV, POPAN produced lower estimates (16 to 29) than the PRD model (16 to 46). The 2021 estimate may be less reliable due to limited secondary sampling occasions. In PRUG, both models provided robust population estimates, higher than those based on simple captures, despite a slight decrease in the toad population over five years. The decline or disappearance of Appenine yellow-bellied toad populations in aquatic sites where fish actively prey on eggs and larvae has been reported (Fiacchini, 2007). This harmful effect is also observed in

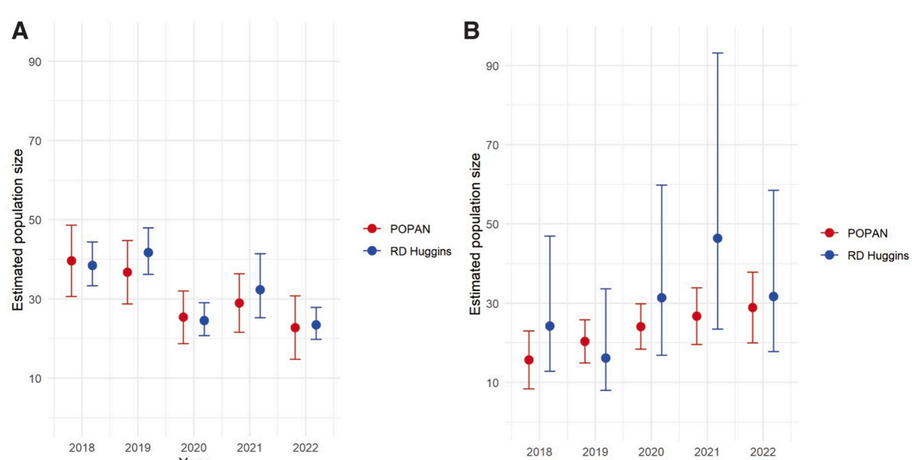

Fig. 4. Estimated abundance of *Bombina variegata pachypus* in the two study populations: (A) PRUG and (B) PTAV derived from Jolly-Seber with the POPAN parametrisation (POPAN) and Pollock's robust design with Huggins' estimator (PRD) models in Table 4. Bars represent 95% confidence intervals.

many other populations of amphibian species facing pressure, both directly through predation and indirectly through factors such as chemical cues of predators that reduce larval swimming activity (e.g., Bernabò et al., 2023, Marquis et al., 2004, Šunje et al., 2021). In the PTAV, a gradual increase in the Appenine yellow-bellied toad population has been observed, although the population remains critically small. This increase coincides with a notable reduction in the population of Carassius auratus (goldfish) in PTAV. This positive demographic trend provides further evidence of how amphibian population densities can significantly increase following the removal of predatory fish (Knapp et al., 2007).

The POPAN method allows also estimation of the cumulative number of mature individuals over five seasons, with PRUG having a slightly higher adult population size (~92) than PTAV (~70). Despite the Apennine yellow-bellied toad being gregarious, populations are typically small, and our results align with existing demographic studies that report populations of only a few dozen individuals (e.g., Angelini et al., 2018, Mattoccia et al., 2006, Romano et al., 2014, Romano & Carafa, 2022), just occasionally reaching a few hundred (Angelini et al., 2018, Colliva et al., 2007, Martino et al., 2022).

5. Conclusions and conservation implications

We provide a comprehensive analysis of the ecology and dynamics of two populations of the Apennine yellow-bellied toad, *Bombina variegata pachypus*, within the Pollino National Park. Considering the small size of these populations, concerns arise about unpredictable population fluctuations, inbreeding depression, stochastic genetic drift, and extinction risk. Climate change and disease are also identified as potential threats, suggesting the likelihood of significant declines (Bernabò et al., 2022b, Costa et al., 2021). Tailored strategies are needed to address these threats factors. Our research emphasizes the importance of long-term monitoring in this protected area, to have a deeper understanding of the Apennine yellow-bellied toad demographic trends, as tools for effective conservation. Despite having the highest abundance, the population in Pianicello di Ruggio (PRUG) shows a slight decline. The causes of this decline are challenging to understand, but it is known that weather fluctuations may affect demographic traits in *Bombina variegata* populations, leading to modifications in resource allocation schedules between survival and reproductive efforts (Cayuela et al., 2016a, 2016b). A conservation perspective involves thoroughly investigating suitable neighbouring areas and assessing dispersion patterns to PRUG; these knowledges could be crucial to guarantee the long-term persistence of this population. In Piano di Tavolara (PTAV), the apparent coexistence of toad populations and introduced fish is probably due to the use of few alternative reproductive sites adjacent to the main fish-invaded pond. Therefore, ongoing habitat restoration efforts (i.e., ponds creation and strict regulations of motor vehicles) are decisive for countering the impact of alien predatory fish and the limited availability of suitable breeding habitats.

The Park Authority's efforts in habitat restoration and disease monitoring are crucial for preserving not only this toad species but also other amphibians and unique habitats within Pollino National Park. Overall, the research provides a foundation for informed management conservation and highlights the multifaceted implications for the long-term persistence of amphibians and their habitats.

CRediT authorship contribution statement

Ilaria Bernabò: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Viviana Cittadino:** Writing – original draft, Visualization, Validation, Investigation, Data curation. **Vittoria Marchianò:** Visualization, Validation, Investigation, Conceptualization. **Lea Anna Cozzucoli:** Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Giuseppe De Bonis:** Investigation, Data curation. **Roberto Macrì Demartino:** Methodology, Formal analysis. **Antonio Romano:** Writing – review & editing, Writing – original draft, Visualization, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnc.2024.126656.

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