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The amphibians of the Circeo National Park, central Italy: distribution and aquatic habitat use

(Amphibia: Caudata: Salamandridae; Anura: Bufonidae, Hylidae, Ranidae)

Die Amphibien des Circeo Nationalparks (Mittelitalien):
Verbreitung und Nutzung des aquatischen Lebensraumes
(Amphibia: Caudata: Salamandridae; Anura: Bufonidae, Hylidae, Ranidae)

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KURZFASSUNG

Abhängig von Entwicklungsstadium (Larve, Jungtier, Erwachsener) und phänologischer Phase (terrestrisch, aquatisch) bewohnen Amphibien gewöhnlich deutlich unterschiediche Lebensräume, wobei sie jeweils nur Teile ihres gesamten Aktionsraumes nutzen. In der vorliegenden Arbeit untersuchten die Autoren die Verbreitung und aquatische Habitatnutzung der sieben Amphibienarten des Circeo Nationalparks in Mitteitalien. Dazu wurden die aquatischen Lebensräume vierzehntäglich in Tages- bzw. Nachtexkursionen von September 2009 bis August 2010 begangen. Amphibien waren in 15 Wasserkörpern (drei künstliche, 12 natürliche) vertreten, die sich in ihrer Hydromorphologie (Größe, Tiefe, Bodensubstrat, Trübe), Vegetation (aquatische, Ufer- und Umgebungsvegetation) und Lage (see- und ufernahe Lagen, Felder oder Waldgebiete) unterschieden. Die Analyse der Amphibienfunde umfaßte die Abschätzung der Verläßlichkeit der festgestellte An- und Abwesenheit von Arten sowie eine Hauptkomponentenanalyse (PCA) zur Reduktion der Datendimensionalität und Redundanzverringerung bei den als Kovariaten fungierenden Habitatparametern. Logistische Regressionsanalysen ergaben signifikante Habitatmodelle für die untersuchten Anurenarten (*Bufo bufo*, *Bufotes balearicus*, *Hyla intermedia*, zwei gemeinsam bewertete *Pelophylax*-Taxa (*lessonae* und *esculentus* und *Rana dalmatina*), während für die beiden Urodelenarten (*Lissotriton vulgaris* und *Triturus carnifex*) die Ableitung von statistisch signifikanten Modellen nicht möglich war. Es wird diskutiert wie in geeigneter Weise ausgewertete faunistische und ökologische Daten auf lokaler Ebene im praktischen Amphibienschutz zur Feststellung von prioritär zu schützenden Arten und Biotopen und deren Management verwendet werden können.

ABSTRACT

Amphibians usually show pronounced variety in habitat use at different periods, selecting only part of the habitats available from their home range depending on the specific stage of the life cycle (larval, juvenile, adult) and the phenological phase (terrestrial, aquatic). The authors investigated the distribution and aquatic habitat use of seven species of amphibians occurring in Circeo National Park, central Italy. Aquatic habitats were inspected fortnightly in diurnal and nocturnal surveys from September 2009 to August 2010. Amphibians were found in 15 water bodies (three artificial and 12 natural) that differed in terms of hydro-morphology (size, depth, bottom substrate, turbidity), vegetation (aquatic, riparian and surrounding), and location (near lakes and riparian areas, fields or wooded areas). Analysis of the individuals detected was done to assess the reliability of presence/absence data and a PCA was performed for reducing dimensionality and decreasing redundancy of the environmental covariate data set. Logistic regression analysis produced significant habitat models for the anuran species (*Bufo bufo*, *Bufotes balearicus*, *Hyla intermedia*, two collectively evaluated *Pelophylax* taxa [*lessonae* and *esculentus*] and *Rana dalmatina*), whereas for both urodelan species (*Lissotriton vulgaris* and *Triturus carnifex*) it was not possible to derive statistically significant models. It is discussed how faunistic and ecological data appropriately analyzed could provide practical instruments for the amphibian conservation at a local scale by identifying priority species and biotopes that deserve strict management.

KEY WORDS

Amphibia: Caudata, Anura; *Lissotriton vulgaris*, *Triturus carnifex*; *Bufo bufo*, *Bufotes balearicus*, *Hyla intermedia*, *Pelophylax lessonae*, *Pelophylax esculentus*, *Rana dalmatina*, ecology, habitat use, Circeo National Park, Italy

INTRODUCTION

Habitat selection strategies are of particular concern because of their effect on reproductive success (ROUNTREE & ABLE 2007), in particular in species with complex life cycles because of the life stages' different ecological requirements (WIND 2000). In amphibians, terrestrial and aquatic habitats are required during different phases of their development (POUGH et. al. 2004) and, as in all other organisms, the distribution is strongly determined by the extent of suitable habitat features (HUTCHINSON 1957).

Various statistical tools can be used to model amphibian-habitat relationships by relating species distribution, richness and/or abundance to a range of environmental features (CUSHMAN 2006). Nevertheless, detecting amphibians during a survey may be difficult, e.g., with cryptic species (e.g., DODD & DORAZIO 2004; DURSO et al. 2011) and a species may remain undetected even though it is present (MACKENZIE et al. 2002), thus resulting in an underestimation of its real range (MACKENZIE & ROYLE 2005). An unaccounted bias in species detection may affect the evaluation of state variables such as abundance or occupancy directly but also indirectly by influencing the estimates of survival and extinction probabilities (WILLIAMS et al. 2002; REFSNIDER et al. 2011). Unaccounted detection probability could produce misleading habitat-use models (TYRE et al. 2003; MOILANEN 2002; GU & SWIHART 2004) and failing to account for imperfect detectability will result in underestimates of site occupancy and biased values of local colonization and extinction probabilities (MACKENZIE et al. 2003; MACKENZIE & ROYLE 2005). Species distribution models try to minimize this kind of error by attempting to avoid erroneous conclusions about species-environment relationships (GU & SWIHART 2004; MAZEROLLE et al. 2005).

In this study, the authors investigated by means of occupancy models (MACKENZIE 2006) the influence of environmental characteristics on the spatial and habitat use of seven species of amphibians in the Circeo National Park located in Central Italy. Detection probability was estimated for each species, since absence of detectability analyses may lead to inaccurate inferences (e.g., BAILEY et al. 2007; WEIR et al. 2005). The main aim of this study was to contribute to the knowledge of the distribution of amphibians in the National Park, based on presence/absence data collected during the periods of breeding (adults) and larval development (larvae and tadpoles). The sampled water bodies represented a very significant part (if not all) of the breeding sites in the Park. Another objective was to determine the environmental factors influencing amphibian distribution in the study area to produce an effective tool for their productive management.

MATERIALS AND METHODS

Study area (Fig. 1).– The research was carried out in the Circeo National Park, located in southern Latium, Central Italy (geographic center of the park at 41°N, 13°E). This area belongs to the Mediterranean Bioclimatic Region, in particular the sub-humid meso-Mediterranean climate. Mean annual rainfall is 960 mm and mean annual temperature 16 °C, with a minimum (8 °C) in January and a maximum (25 °C) in July. A summer drought lasts from June to August and alternates with a period of moderate cold from November to April when the average temperature is about 5°C. Holocene dunes, of an average width of 250 m and an average altitude of ca. 15 m, occupy a 30-km strip along the seashore, from a small sand promontory in the north to the Circeo limestone promontory in the south. This Circeo dune system is bounded by the Tyrrhenian Sea to the west and four coastal lagoons to the east. The plain forest covers about 3190 hectares and consists mainly of deciduous mesophytic woods on soils that are mainly formed by Würmian sand with pyroclastic material, referred to as the Vulcano Laziale activity (MANES et al. 1997).

Sampling design.– Data collection was done from September 2009 to August 2010. The authors sampled a total of 15 water bodies (three artificial and 12 natural) suitable for amphibians, identified by means of digital topographical maps, and different in their hydro-morphology (size, depth, bottom substrate, turbidity), vegetation (aquatic, riparian and surrounding), and location (near lake and riparian areas, fields or wooded areas). Sampling sites were surveyed during monthly diurnal and nocturnal

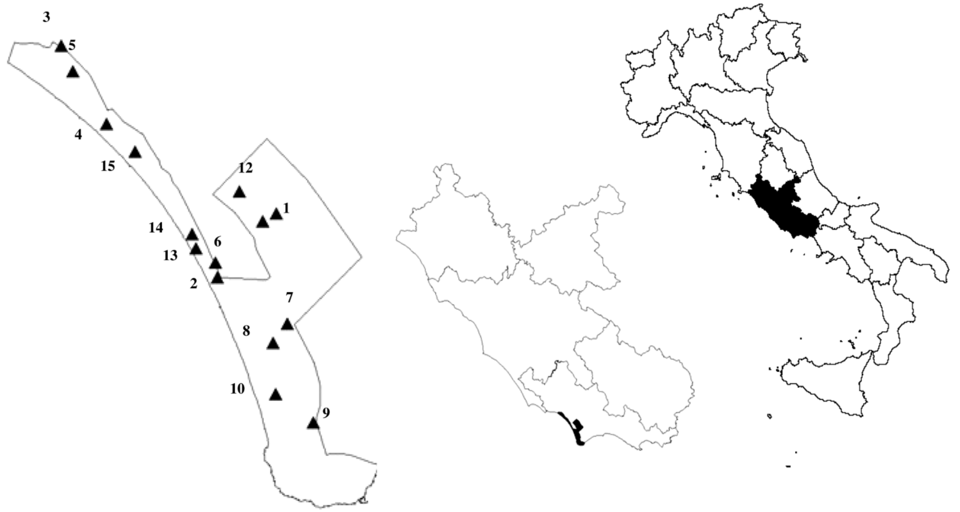

Fig. 1: Study area and sampling sites. Left map: triangles represent the 15 sampling sites within the Circeo National Park (numbered according to Table 1); central map: placement of the Circeo National Park (black area) in the Latium Region; right map: placement of the Latium Region (black area) in Italy.

Abb 1: Untersuchungsgebiet und Untersuchungsstellen. Linke Karte: Dreiecke repräsentieren die 15 Untersuchungsstellen im Circeo Nationalpark (Numerierung entsprechend Tab. 1); mittlere Karte: Die Lage des Circeo Nationalparks (schwarz) in der Region Latium; rechte Karte: Lage der Region Latium (schwarz) in Italien.

excursions. Each water body was visited fourteen times (one diurnal and nocturnal survey per month, except for a hibernal sampling pause between November and early March) to encounter both early and late breeding species.

A standardized sampling event lasting 45 minutes, for both diurnal and nocturnal samplings, was carried out. Diurnal surveys took place from 6:01 a.m. - 6:00 p.m., nocturnal between 6:01 p.m. and 6:00 a.m. On the whole, a field effort of approximately 80 hours was prorated among sites.

The presence of amphibians (metamorphs, larvae and eggs) within and around the ponds was assessed by visual encounter survey (VES) (Campbell & Christman 1982; Heyer et al. 1994). To increase sampling efficiency for newts, traps were used temporarily, consisting of cylindrical PVC tubes one meter in length and 15 cm in diameter, provided with five openings inwardly tapering into funnels (Vignoli et al. 2007). One trap was placed in each water body every month (except from November to the beginning of March) and removed after three consecutive days, resulting in a total of 21 days per funnel trap per water body. Traps were checked every morning. Although the literature is ambivalent about the relative performance of funnel trapping, it can be an efficient sampling technique, especially whenever is associated with active sampling techniques (e.g., VES) (Greenberg et al. 1994; Jorgensen et al. 1998; Kronshage & Glandt 2014). However, Ribeiro-Jùnior et al. (2008) showed that funnel traps may not provide any additional value over pitfall traps and diurnal time-constrained searches (DTCS), and Jorgensen et al. (1998) did not find any species in funnel traps that were not also caught by pitfall traps. Nevertheless, despite not being recommended as main trapping technique for sampling in Neotropical forest (Ribeiro-Jùnior et al. 2008), this method proved to be particularly efficient in other ecosystems, such as temperate and Mediterranean habitats, especially for non-vocalizing amphibians (e.g., salamanders) (Griffiths & Mylotte 1986; Enge 2001; Vignoli et al. 2007a, 2007b, 2009).

Species were identified in the field and then released at the sampling site. Larval stages, when necessary, were temporarily taken to the lab and identified under a microscope according to LANZA et al. (2007). Water frogs were considered a single taxonomic unit in the analyses rather than discriminating between parental (*Pelophylax lessonae* [CAMERANO, 1882]) and hybridogenetic (*Pelophylax* kl *esculentus* [LINNAEUS, 1758]) species, because they were not unambiguously identified in the field (VIGNOLI et al. 2007).

The majority of the observed species spend most of their annual cycle in terrestrial habitats; however, the authors restricted their observations to the aquatic environment and its immediate surroundings due to the inefficiency of sampling amphibians outside the aquatic reproductive sites. For each site, the authors recorded nine parameters related to the aquatic environment, which are supposed to influence the presence of amphibians (e.g., SEMLITSCH 2000): riparian vegetation (five ranked classes of coverage), aquatic vegetation (five ranked classes of coverage), slope of the shoreline (five ranked classes of inclination), type of water body (lentic/lotic and permanent/seasonal), bottom substratum (four ranked classes of granulometry), water turbidity (five ranked classes), water depth (four ranked classes), and presence of leaf litter (yes/no). In addition, the presence of the following potential predators of amphibians was assessed (four ranked classes of abundance): crayfish (*Procambarus clarkii*), mosquitofish (*Gambusia holbrooki*), Odonata larvae, reptiles [*Natrix natrix* (LINNAEUS, 1758), *Natrix tessellata* (LAURENTI, 1768)] and birds (e.g., herons). Classes of predator abundance were roughly estimated by VES from 1 (no threat) to 4 (high threat). Thus, four ranked values were assigned to the aquatic habitats relative to their threat potential to the amphibian population. Moreover, the authors estimated the levels of threat looming over the study sites, determined by factors known to have a negative effect on amphibian populations: presence of cattle, tourism, run-off of public and/or agricultural wastewater (RODRÍGUEZ-PRIETO & FERNÁNDEZ-JURICIC 2005; HAMER & MCDONNELL 2008; KING et al. 2010).

Statistical analyses.– Site-specific detection probability (p) for seven species of amphibians was estimated using the software package PRESENCE (www.mbr-pwrc.usgs.gov/software/presence.html). Detection probabilities were used to estimate the number of visits necessary to assert that a species is definitely absent from a site with a specified degree of confidence (MCARDLE 1990; REED 1996; KÈRY 2002). The probability F of not seeing a species after N visits was calculated as $F = (1 - p)^N$, where p is the detection probability, under the assumption that samplings are comparable and independent. If the probability for the absence of a given species is 95 per cent, then F = 0.05. Thus, the previous equation can be solved for N_{min}, that is the minimum number of visits necessary to be 95 % certain that a species is absent, $N_{min} = \log(0.05)/\log(1 - p)$. Misdetection rate was calculated, assuming that a species was reliably detected if misdetection rate was < 5 %.

A Principal Component Analysis (PCA) was performed on the variables that showed collinearity in order to reduce the dimensionality and the redundancy of the environmental covariate data set. Downstream analyses were performed on the PCA scores in preparation of the data for further investigations. The PCA was based on the correlation matrix retaining only factors with eigenvalues greater than one (DU TOIT & CILLIERS 2011). Variable loadings on a factor of above 0.71 are considered excellent, as that variable can be seen as a pure measure of that factor (TABACHNICK & FIDELL 1996). The environmental variables were not normally distributed, and were $\log_{10}$-transformed when necessary to achieve normal distribution. Logistic regression analyses (forward stepwise approach) were performed to model the distribution of the amphibian species in relation to the environmental factors recorded in the various habitats. The presence/absence of each species was entered as dependent variable and the environmental variables and the scores of the PCA factors with eigenvalues > 1 as independent variables. All above statistical analyses were performed using the statistical software package SPSS 17.0 (SPSS Inc., USA).

RESULTS

In the study area six anuran taxa were observed: *Bufo bufo* (LINNAEUS, 1758), *Bufotes balearicus* (BOETTGER, 1880), *Rana dalmatina* FITZINGER in BONAPARTE, 1839, *Hyla intermedia* BOULENGER, 1882, two forms of *Pelophylax*, and two urodeles, *Lissotriton vulgaris* (LINNAEUS, 1758) and *Triturus carnifex* (LAURENTI, 1768) (Table 1). *Bufotes balearicus*, *B. bufo*, and *R dalmatina* occurred in 20 % of the sampled breeding sites, *H. intermedia* in 53 %, *Pelophylax* spp. in 67 %, *L. vulgaris* in 20 %, and *T. carnifex* in 13 %.

Amphibians were found in all the 15 surveyed sites, both in artificial and natural water bodies. For all taxa, the number of performed visits (N = 14) exceeded the minimum number of visits necessary to be 95 % certain that a species was absent (Table 2). As far as the detectability is concerned, only two species showed a detection probability greater than 0.3 and a misdetection rate < 5 %, namely *R. dalmatina* and *T. carnifex*. For *B. bufo* the detection probability exceeded 0.3, whereas the misdetection rate slightly exceeded the threshold value of 5 % (Table 2). However, for the remaining species, misdetection rate ranged from 9.2 to 13.7, indicating low probability for these species to remain undetected at some sites.

The relationships among the considered ecological variables are reported in the correlation matrix in Table 3. Principal component analysis extracted one factor with eigenvalues > 1 (PCA Factor1, Table 4). PCA Factor1 alone explained 63.4 % of total variance. The first factor was negatively associated with water permanence (-0.958) and positively with aquatic vegetation (0.796) and aquatic leaf litter (0.796). Therefore, high values of PCA Factor 1 indicate seasonal water bodies, extensive aquatic vegetation and wide presence of aquatic leaf litter.

Logistic regression analyses produced significant models for the anurans (*B. bufo*, *B. balearicus*, *H. intermedia*, *Pelophylax* spp. and *R. dalmatina*), but not for the newts (*L. vulgaris* and *T. carnifex*) (Table 5). There was no more than one independent variable influencing significantly the presence/absence of any of the species models. The distribution of *B. balearicus* and *B. bufo* was influenced by 'riparian vegetation', that of *R. dalmatina* and *H. intermedia* by 'PCA Factor 1', whereas *Pelophylax* spp. was influenced by 'predators'.

DISCUSSION

The sampling design included all the known freshwater bodies in the study area, thus reliably providing a complete update of the amphibian species list of the Circeo National Park. This information allows the chronological comparison with faunistic data recorded four to five decades ago (BRUNO 1981; CARPANETO 1986). The authors found eight amphibian taxa (two salamanders and six anurans, two forms of water frog included) representing more than half (53.3 %) of the batrachofauna of the Latium Region (BOLOGNA et al. 2000). Previous studies by BRUNO (1981) and CARPANETO (1986; this study seems not to include original data from BRUNO 1981) reported three more amphibian species for the study area, *Salamandrina perspicillata* (SAVI, 1821), *Bombina pachypus* (BONAPARTE, 1838) and *Rana italica* DUBOIS, 1987. There are no records of these species in the Circeo National Park from after 1980 (BOLOGNA et al. 2000), probably also because most likely no further data on amphibian presence in the Park was gathered after the anecdotal observations in the 1960s - 1970s by BRUNO (1981). *Bombina pachypus* is now extinct (BOLOGNA et al. 2000), whereas for *S. perspicillata*, and *R. italica* it is not clear whether these species ever inhabited the study area. Indeed, the records by BRUNO (1981) are considered dubious since during several subsequent surveys in the study area, neither was the presence of *S. perspicillata* and *R. italica* ever confirmed (BOLOGNA et al. 2000; this study), nor were suitable habitats identified for these species in terms of water body typology and water

Table 1: Amphibian distribution (presence/absence) in the 15 surveyed aquatic habitat sites of the Circeo National Park (Central Italy).

Tab. 1: Die Verteilung (An-/Abwesenheit) der Amphibien in den 15 untersuchten aquatischen Lebensräumen des Circeo Nationalparks (Mittelitalien).

LV - *Lissotriton vulgaris*, TC - *Triturus carnifex*, BBu - *Bufo bufo*, BBa - *Bufotes balearicus*; HI - *Hyla intermedia*, Pspp - *Pelophylax* spp., RD - *Rana dalmatina*.

	Species / Art						
Site toponym / Fundortname	LV	TC	BBu	BBa	HI	Pspp	RD
1. Piscina della Verdesca	1	1	0	0	0	0	1
2. Sant'Andrea	0	0	1	1	1	1	0
3. Laghetto di Fogliano	0	0	0	0	0	1	0
4. Canale Rio Martino	0	0	0	0	1	1	0
5. Cicerchia	0	0	0	0	1	1	0
6. Diversivo Nocchia	0	0	0	0	1	1	0
7. Centro visitatori	1	0	1	1	1	1	0
8. Fonte degli Arciglioni	0	0	1	0	0	0	0
9. Fonte di Lucullo	0	0	0	0	0	0	0
10. Piscina della Villa di Domiziano	0	0	0	0	0	1	0
11. Piscina delle Bagnature	1	1	0	0	0	0	1
12. Piscina del Carpino	0	0	0	0	0	0	1
13. Pantani dell'Inferno (Integral reserve)	0	0	0	0	1	1	0
14. Pantani dell'Inferno (Lake)	0	0	0	0	1	1	0
15. Lago dei Monaci	0	0	0	1	1	1	0

quality (ANGELINI et al. 2007; PICARIELLO et al. 2007).

Despite that wetland is not evenly and abundantly distributed in the Circeo National Park, comparatively high amphibian species diversity was observed, confirming the Circeo National Park to be a herpetologically rich site as reported in previous studies (BRUNO 1981; CARPANETO 1986; BOLOGNA et al. 2000; ZERUNIAN 2005; VIGNOLI et al. 2013).

Amphibians occurred in most of the water bodies available in the Park, and, in some of them, in diversified assemblages. The water bodies of the Park included various types such as lakes, water channels, natural pools, astatic ponds, artificial basins, and streams used in a species-specific way. Indeed, the logistic regression models showed that the occurrence of the amphibian species was determined by different environmental factors and that the high amphibian diversity of the Circeo National Park is explained by two variables: i) the diversity of water body types available; and ii) the differential habitat use by amphibian species.

The probability of detection varied considerably among species. For three amphibians (*T. carnifex*, *B. bufo*, *R. dalmatina*), the sampling rate used was high enough

Table 2: Detection probabilities (p), misdetection rates (M) and minimum number of visits (N_m) necessary to be 95 % certain that an unrecorded species is in fact absent from a given site.

Tab. 2: Nachweiswahrscheinlichkeit (p), Raten des fälschlichen Nicht-Nachweises (M) und Minimalanzahl (N_m) notwendiger Begehungen, um bei Nicht-Nachweis einer Art ihre tatsächliche Abwesenheit vom Biotop mit 95 prozentiger Wahrscheinlichkeit angeben zu können.

Species /Art	p	M	N_m
Bufo bufo	0.318	6.8 %	7.83
Bufotes balearicus	0.289	9.2 %	8.78
Hyla intermedia	0.251	13.2 %	10.36
Lissotriton vulgaris	0.247	13.7 %	10.56
Pelophylax spp.	0.272	10.8 %	9.47
Rana dalmatina	0.612	0.13 %	3.16
Triturus carnifex	0.856	0.0001 %	1.55

Table 3: Correlation matrix of the ecological variables (ranked in four classes each). In bold the significant ($p < 0.05$) correlations. Rv - Riparian vegetation; Av - Aquatic vegetation; Ss - Shore slope; Bs - Bottom substratum; Wf - Water flow; Wp - Water permanence; De - Depth; Ll - Leaf litter; Wt - Water turbidity; Pr - Predators.

Tab. 3: Korrelationsmatrix der in jeweils vier Klassen kategorisierten ökologischen Variablen. Signifikante ($p < 0.05$) Korrelationen in Fettschrift. Rv - Ufervegetation; Av - Aquatische Vegetation; Ss - Uferneigung; Bs - Bodensubstrat; Wf - Fließgeschwindigkeit; Wp - Gewässerpermanenz; De - Gewässertiefe; Ll - Fallaub; Wt - Wassertrübe; Pr - Freßfeinde.

	Rv	Av	Ss	Bs	Wf	Wp	De	Ll	Wt	Pr
Rv	1	0	0.259	-0.173	0.259	-0.327	0.138	0.474	0.420	0.075
Av	0	1	**-0.588**	-0.258	-0.377	**-0.732**	-0.356	0.464	0.049	-0.327
Ss	0.259	**-0.588**	1	0.507	0.387	0.461	0.478	-0.461	0.366	0.338
Bs	-0.173	-0.258	0.507	1	0.273	**0.517**	0.297	-0.258	-0.142	-0.268
Wf	0.259	-0.377	0.387	0.273	1	0.377	-0.017	-0.377	0.017	0.017
Wp	-0.327	**-0.732**	0.461	**0.517**	0.377	1	0.356	**-0.732**	-0.196	0.179
De	0.138	-0.356	0.478	0.297	-0.017	0.356	1	-0.048	0.490	0.468
Ll	0.474	0.464	-0.461	-0.258	-0.377	**-0.732**	-0.048	1	0.196	-0.327
Wt	0.421	0.049	0.366	-0.142	0.017	-0.196	0.490	0.196	1	0.377
Pr	0.075	-0.327	0.338	-0.268	0.017	0.179	0.468	-0.327	0.377	1

to reliably (> 95 %) diagnose true absences, whereas for *B. balearicus* and *Pelophylax* the probability of correct estimation ranged from 74 to 80 % and was below 70 % for the remaining species, thus suggesting reduced detection probability in the latter. Nevertheless, it should be noted that detection probabilities of certain amphibian species may vary among years (MacKenzie et al. 2003); consequently, the number of searches required to establish the absence of a given species may need to be determined across multiple years. The detection probabilities estimated in this study were based on a single season of field work. To tailor the design of a monitoring program, variation in detection probabilities among years should be assessed.

As for habitat selection by species, the newts were found only in the temporary pools and the channels inside the forest but their preferences did not produce significant models, whereas for *Hyla* neither detection probability nor percentage of correct classification in the regression analyses were significant. The presence of potential predators such as fishes characterizes large ponds which are thus avoided by most amphibians (Morin 1986; McCollum & Leimberger 1997; Relyea 2003; Resetarits 2005)

Table 4: Results of reducing dimensionality and decreasing redundancy by the Principal Component Analysis (Varimax rotation) performed on the structural variables measured in 15 wetlands in the Circeo National Park. In bold the significant ($p < 0.05$) correlations between variable loadings and PCA Factors. Note that only the first factor (eigenvalue > 1) was considered in the downstream analyses. Av - Aquatic vegetation; Bs - Bottom substratum; Wp - Water permanence; Pr - Predators.

Tab. 4: Ergebnisse der Datenreduktion durch die Hauptkomponentenanalyse (PCA, Varimax-Rotation) der in 15 Feuchtbiotopen des Circeo Nationalparks erhobenen Strukturvariablen. Signifikante ($p < 0.05$) Korrelationen zwischen Ladungen und PCA-Faktoren in Fettschrift. Nur Faktor 1 (Eigenvalue > 1) wurde in den nachfolgenden Analysen berücksichtigt. Av - Aquatische Vegetation; Bs - Bodensubstrat; Wp - Wasserpermanenz; Pr - Freßfeinde.

Structural variables / Strukturvariable	Factor 1	Factor 2	Factor 3	Factor 4
Av	**0.796**	0.279	0.517	-0.139
Bs	-0.590	**0.802**	0.000	0.086
Wp	**-0.958**	-0.031	0.000	-0.284
Ll	**0.796**	0.279	-0.517	-0.139
Eigenvalue	2.536	0.801	0.535	0.127
Percentage of explained variance / % der Varianz erklärt	63.419	20.007	13.392	3.181
Cumulated explained variance / Kumulative % der Varianz erklärt	63.419	83.426	96.819	100.000

Table 5: Ecological equations and percentage of cases correctly classified by a logistic regression model (forward stepwise conditional design) for each species of amphibians in the study area. The Model Log Likelihood function tested for significance the model after removing the independent variables included in the full model. Rv - Riparian vegetation; PCA1 - PCA Factor 1; Pr - Predators; NS - not significant.

Tab. 5: Ökologische Gleichungen für jede Amphibienart des Untersuchungsgebietes und Prozentsatz der durch das logistische Regressionsmodell (forward stepwise conditional design) korrekt klassifizierten Fälle. Die Model Log Likelihood Funktion untersucht die Signifikanz des Modells nach Elimination jener unabhängigen Variablen, die im vollständigen Modell enthalten sind. Rv - Ufervegetation; PCA1 - PCA Faktor 1; Pr - Freßfeinde; NS - nicht signifikant.

Species / Taxon	Logistic equation / Regressionsgleichung	Model Log Likelihood (Significance)	Overall percentage correct Insgesamt korrekt zugeordnet (%)
Lissotriton vulgaris	NS	NS	80.0
Triturus carnifex	NS	NS	86.7
Bufo bufo	Y = -1.4 + 8.2*Rv	-7.506 (< 0.0001)	80.0
Bufotes balearicus	Y = 3.4 – 2.6*Rv	-7.509 (0.018)	80.0
Hyla intermedia	Y = 0.134 – 3.9*PCA1	-10.528 (0.002)	53.3
Pelophylax spp.	Y = 0.7 + 2.7*Pr	-9.548 (0.002)	86.7
Rana dalmatina	Y = -1.4 + 60.9*PCA1	-7.506 (0.014)	80.0

whereas, water frogs were observed to spend most of their annual cycle in these permanent water bodies. Predators affected only the model for *Pelophylax* that showed a positive association with habitats characterized by high predator counts which is also shown by other studies which pointed out the positive association between the presence of potential or effective predators and the distribution of *Pelophylax* (e.g., BEEBEE 1997; FICETOLA et al. 2011). According to these studies, the pattern of habitat selection of *Pelophylax* is interpreted in terms of a tradeoff between the reduced competition due to the absence of other amphibians and the low reproductive success determined by high predator pressure (FICETOLA et al. 2011).

The distribution of the toads was influenced by riparian vegetation (positively in *B. bufo* and negatively in *B. balearicus*), as revealed by the regression models. The presence of vegetated and open areas in the same site allows *B. bufo* to maintain a favorable body temperature over a wider range of air temperatures and hence prolong the daily and seasonal activity. Moreover, the vegetated habitat may provide suitable microclimatic conditions for toad prey, thus enhancing toad feeding activity (SZTATECSNY & SCHABETSBERGER 2005). On the contrary, *Bufotes balearicus* is a species of open fields and scarce surrounding vegetation cover, both these features characterizing disturbed or pioneer habitats (ENSABELLA et al. 2003). Moreover, from all toad sites, extensive livestock farming was recorded. Cattle presence is associated with high levels of nitrogen (HACKTEN BROEKE et al. 1996) in the soil, its effluents and adjacent water bodies as ammonium nitrate can leach from beneath urine and faecal spots (STOUT et al. 1997). It is well known that toads have a higher tolerance to ammonium nitrate than other amphibians (ORTIZ et al. 2004). Thus, reduced competition with other amphibian species may promote the frequent use of such sites by toads for breeding. Other studies in ecologically similar sampling sites demonstrate that the presence of *B. bufo* is negatively related to the presence of *B. balearicus* (ENSABELLA et al. 2003), due to differences in breeding habitat selection. *Bufo bufo* is indeed more tolerant of low temperatures (BABIK RAFINSKI 2001), and can colonize water basins populated by a larger number of predators (GRIFFITHS 1997) because of the unpalatability of its tadpoles (GLANDT 1984; ILDOS & ANCONA 1994; MANTEIFEL & RESHETNIKOV 2002). However, the present study did not find evidence of negative association between the distributions of the two toad species.

Contrarily to the toads, *Rana dalmatina* selected water bodies in deciduous forested habitats, where a huge amount of vegetal material is deposited as aquatic leaf litter. Indeed, the distribution of *R. dalmatina* is well explained by the positive correlation with broad canopy cover and low disturbance of temporary water bodies. All the observations of this species were recorded in four astatic ponds located within the plain forest.

Critical factors for species conservation.– The knowledge of amphibian distribution and habitat use is essential to identify key biotopes appropriate to sustain and develop amphibian populations. These biotopes deserve to be given priority in the Circeo National Park, since they are crucial for the conservation of the batrachofauna and the prevention of further amphibian extinctions in the Circeo National Park. The exploration of the amphibian distribution in the Circeo National Park dates back to the 1960s – 1970s (Bruno 1981) and was largely neglected thereafter until the present study which explored the suitable water bodies within the protected area and provided an updated assessment of the batrachofauna and the basic tools for managing the key biotopes. In particular, the plain forest habitat hosts several seasonal astatic wetlands, locally called 'piscine' (Mediterranean temporary ponds; Annex 1 EC Habitat Directive, 3170* NATURA 2000 code), that are the elective habitat of at least three species listed in the EC Habitat Directive: *T. carnifex*, (Annexes II and IV), *L. vulgaris* (Annex IV), and *R. dalmatina* (Annex IV). This type of water basin is severely threatened by water pollution (Morgana et al. 2005) and groundwater overexploitation for agricultural use that has been greatly reducing the presence and the extension of astatic pools for decades. In order to perpetuate the population viability of amphibian species which were identified as deserving conservation priority, such as the aforementioned species, the protection of the astatic forest pools of the Circeo National Park and the monitoring of ground water levels are urgently necessary.

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