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Olim palus, where once upon a time the marsh: distribution, demography, ecology and threats of amphibians in the Circeo National Park (Central Italy)

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Abstract. The Circeo National Park lies in a territory that was deeply shaped by human activity, and represents one of the few remaining patches of plain wetland habitat in Central Italy. In this study distribution and few demographic information of the amphibians in the Park were provided. Seven species and 25 bibliographic and 84 original breeding sites were recorded, and population size estimations were carried out for a population of these three species: *Pelophylax* sinkl *esculentus*, *Bufo balearicus* and *Rana dalmatina*. For the studied populations of pool frog and green toad the operational sex ratio and the demographic effective population size was also estimated. For *Rana dalmatina*, which is strictly associated to forest environment, a positive and significant correlation between the number of egg clutches and maximum depth of the swamps was found. The State plain forest is the most important habitat for amphibians' conservation in the park. The occurrence of dangerous alien species was investigated and they are evaluated as the major threat for amphibians in the park, especially the crayfish *Procambarus clarkii* in the State plain forest. Index of Calling Survey were performed for anurans and the medians did not differ among species. The potential distribution of amphibians in the Park was evaluated by building a species distribution model. Finally, the absence of three species reported in literature in the 60's of the last century (*Bombina pachypus, Salamandrina perspicillata, Rana italica*) is also discussed.

Keywords. Alien species, Capture-Marking-Recapture, effective population size, Index of Calling Survey, land reclamation, Species Distribution Models, swamps.

INTRODUCTION

Until the early twenties of the past century, marshlands dominated the landscapes along the coast about 45 km southeast of Rome, from Anzio to Terracina towns between the Tyrrhenian sea and the Volsci Chain, at inland distances from 15 to 25 km. The territory, which now belongs to the province of Latina (Latium region), was an extensive marsh at about sea level originated in an alluvial plain (e.g., Linoli, 2005). Forested swamps dominated above sea level while areas below the sea level were covered by mud flats and pools. These so called "Paludi Pontine" (Pontine Marshes) were the subject of land reclamation works, performed periodically since the pre-Roman period, initially by the Italic tribe of Latins, but with scarce success. Land reclamation was conducted extensively with considerable success by Fascist regime, starting in the 1920s and radically changing the landscape of the area, which was converted into an extensive agricultural plain and new towns were founded and built (Littoria, renamed Latina, Pontinia, Sabaudia, Aprila and Pomezia are the most important ones). Out of the origi-

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nal 20,700 hectares of forest and swampland, about 3,200 were put under protection with the creation in 1934 of the Circeo National Park (CNP), which includes also other not forested areas. Among terrestrial vertebrates, amphibians are the class most strictly associated to wetlands. They are an important component of biodiversity and suffer a recent worldwide decline (Stuart et al., 2004; Wake and Vredenburg, 2008). In Italy protected areas play a key role in conservation of amphibians, and act as stepping stones in the face of climate change (D'Amen et al., 2011). The area where CNP falls is classified in the highest irreplaceability naturalistic values (Maiorano et al., 2006). However data on the fauna before the land reclamation was available only for mammals and birds (Lepri, 1935). Consequently, information on which herpetological species occurred in this area before land reclamation could be only deducted by current species distribution on wider areas (Bruno, 1973; Bruno 1981; Bologna et al., 2000), by specific works on the fauna of the CNP (Carpaneto, 1986; Ravenna, 2013; Cinquegranelli et al., 2015), and by herpetological census of surrounding areas which were also subjected to land reclamation in the 30's (Novaga et al., 2013). In particular, the recent paper of Cinquegranelli et al. (2015) provided few updated data on distribution of amphibians in the Park. However Cinquegranelli an co-authors surveyed only 15 sites, and information on habitat use, occupancy level and species detection probability were the main goals of their work.

The aim of our study was fourfold. First, by performing an extensive survey on aquatic habitats, we provide an updated species distribution of amphibians. Second, we estimated some demographic and abundance parameters. Third, we evaluated habitat preferences of amphibians and we provided potential distribution information. Finally, we detected relevant threats for species, population or habitats and we provided information on conservation measures.

MATERIALS AND METHODS

Study area

The Circeo National Park covers 8,484 hectares of a coastal area of the Central Italy, and it consists of five main environments: the plain forest, four coastal lakes, the coastal dune area, the limestone massif of Mount Circeo (541 m asl, a promontory that marks the southwestern limit of the former Pontine Marshes) and the island of Zannone. The plain State Forest covers about 3,190 hectares and mainly consists of deciduous woods. With many areas few meters below the sea level, most of the Park does not exceed the 30 meters a.s.l. The Park ranges from latitude 41°13'N to 41°24'N, and from longitude 12°50'E to 13°07'E. CNP is covered by wooded areas and semi-natural habitats for the 56%, agricultural fields cover the 18%, water bodies the 13%, artificial territories the 11% and, finally, a small portion is covered by wetlands (2%; Giagnacovo et al., 2003). The climate falls in the Lower Mesomediterranean Thermotype, Upper Subhumid Ombrotype (Blasi, 1994). Mean temperature of this area is from 9.5 °C to 17.1 °C, and temperatures below zero are uncommon. Precipitation is mainly concentrated in autumn and early winter (October-December); relative humidity is high all over the year, wind is frequent with a South-Western dominance (Padula , 1985).

Distribution and species occurrence

The data reported in literature, when they were provided with enough accuracy, were georeferenced. Field surveys (March-September 2015) were preceded by a careful analysis of the maps produced by the Istituto Geografico Militare (I.G.M, 1:25000) and by a analysis of satellite images to detect water bodies not reported in the maps (see Romano et al., 2012). Information on methods used to detect the occurrence of amphibians are reported in detail by Romano et al. (2010; 2012).

To describe species rarity and their diffusion we used a method that weights both diffusion (W: wide; M: medium; L: limited) and density (C: common; F: frequent; R: rare) and it consists of a graph of the relationship between the coverage (%) of the UTM grid (we used a mesh of 2x2 km) and the mean number of observations for a square occupied by each species, according to the method proposed by Doria and Salvidio (1994) and used in other herpetological studies (Turrisi and Vaccaro, 2004; Romano et al., 2012). In the computation of the score to build the graph of rarity and diffusion we decided to use only a subset of meshes. We included only the meshes where at least one species record occurred (i.e., 35 meshes), excluding, for example, meshes in highly urbanised areas with no data.

Ecology

Breeding aquatic sites were assigned to the following typologies: (i) ponds and marshes; (ii) slow running waters: ditches, streams and artificial channels (iii) rheocrenic springs (which were checked s up to 50 meters from they source); (iv) forest swamps (including those whose filling is partially due to limnocrenic springs); (v) artificial tanks; (vi) brackish coastal lakes. Correspondence Analysis (CoA) was used to identify associations among amphibian species and aquatic habitats. Considering that the variance of the data was homogenous (Levene's test for homogeneity, based on means, P = 0.758), the hypothesis that habitat categories may host different syntopic number of species was tested using one-way ANOVA. Correlation between the habitat availability and the number of species for each habitat type was tested using non-parametric Spearman's rank correlation. Analyses concerning habitat typologies were performed both on original and bibliographic data, when the latter could be certainly assigned to a given habitat typology. Ecological analyses were performed in the statistical package PAST (Hammer et al., 2001).

Population abundance estimation

Populations estimates were performed using four different methods: Index of calling survey (ICS), capture-mark-recapture (CMR), removal sampling (RES), and egg mass counts (EMC).

For the ICS, which is the relative measure of calling density (Mossman et al., 1998; Weir and Mossman, 2005), surveys were performed as reported in Dorcas et al. (2009) and Romano et al. (2012). ICS may vary among 0 and 3. Considering that ICS provides scores as ordinal measures and with many ties, the scores were analysed using the median test as performed in Statistica® ver. 5.0 (Statistica package, Statsoft Inc., USA). Differences in calling survey scores among anuran species were compared using the non parametric Kruskall-Wallis test and relative post hoc comparisons.

Using photo-identification, CMR analysis was applied on a population of *Bufo balearicus* breeding in an artificial tank in the inner of Sabaudia town; we used the software CAPTURE (Otis et al. 1978) to estimate adult population size (N) analysing data from the four sampling sessions performed in a short time range (23 September – 2 October; light rain, between 9 and 11 p.m.).

RES was applied on a population of *Pelophylax* sinkl. *esculentus* breeding in the artificial pond of the headquarter of the Park (Sabaudia). We used the jackknife estimator of Pollock and Otto (1983) as performed in the program CAPTURE to estimate N on the basis of three removal sessions performed in about three hours.

Both for *Bufo balearicus* and *Pelophylax* sinkl. *esculentus* populations, the operational sex ratio (calculated just on the number of males and females captured and not on population estimates), in accordance with Wilson and Hardy (2002), was expressed as the proportion of mature males, i.e. males/(males + females). The demographic effective population size (N_e) was estimated as

$$N_e = (4*N_m*N_f)/(N_m+N_f),$$

where N is the number of mature males (m) or females (f) individuals (Wright, 1938), which is a widely used equation to obtain demographic estimates of N_e (e.g. Jehle et al., 2001; Schmeller and Merila, 2007).

EMC was used in March to estimate breeding populations of *Rana dalmatina* in seven natural ponds in forest environment; egg counts were performed in a unique sampling session per pond; as *R. dalmatina* is an explosive breeder (Sofianidou and Kyriakopoulou-Sklavounou, 1983; Guarino and Bellini, 1993) and each female lays a single egg mass per season (Nollert and Nollert, 1992), consequently we considered the counts of egg masses as a good proxy of the minimum female population size (Griffiths and Raper, 1994; Grossenbacher et al., 2002). Surface areas of breeding ponds and swamps were estimated by walking the perimeter of each site with a GPS, automatically calculating the area inside the resulting shape. Where egg masses of *R. dalmatina* were counted, Spearman rank correlation was used to test the association between density of egg masses and size or maximum depth of the swamps. Correlation between water surface and their maximum depth was also tested for all 15 swamps where *R. dalmatina* breed (correlations were performed in PAST; Hammer et al., 2001).

Potential distribution

The potential distribution of amphibians in the CNP was evaluated by building a species distribution model (SDM), using the algorithm of maximum entropy (Maxent, Phillips et al., 2006). Selection of environmental data layers, to be employed for these analysis, was based on availability and *a priori* expectation of influences on amphibian population. Considering that the Park area runs along the coast and it is a small area, precipitations and other climatic variables were considered homogenous in that area and were not included among variables. We used the Digital Elevation Model (DEM) with a spatial resolution of 90m to obtain other topographic variables. All variables were resampled in order to match the 90m resolution of the DEM. The environmental variables used were: Corine land cover data 2006; Landsat tree cover, representing the percentage of canopy cover of trees higher than 5 m; distance from forest swamps; distance from running waters; distance from lakes; topographic variables calculated from DEM were elevation, aspect (Northness or Eastness), valley depth, Topographic Wetness Index (TWI; Sörensen et al., 2006), Topographic Ruggedness Index (TRI; Riley et al., 1999), wind exposure, direct insolation (kw/h per square meter). To build the SDM, both original and bibliographic data were pooled together. The whole data set was split in two subgroups by random selection: 70% of points were used for building the model (training), while the remaining 30% of point-data were employed to evaluate its predictive power. This procedure was repeated 10 times for each species, generating an averaged prediction of amphibians' distribution. Finally, the predictive power of the model was evaluated by calculating the area under the receiver operating characteristic curve (AUC). Analysis were conducted in software MaxEnt 3.3.3k and default software settings were used, with the exception of the employment of bootstrapping procedure and number of iterations (1000). All GIS processing to obtain the above mentioned layers was performed using software SAGA Gis.

Threats

Considering that in the Park there is a high-density road network and that road mortality may be considered as an additional factor contributing to the amphibian decline (Puky, 2006; Glista et al., 2008), roads were checked systematically. In particular we controlled the two parallel coastal road and waterfront roads (both about 25 km) after rains in spring and summer.

The causes for amphibian declines are many (e.g., Collins, 2010) but habitat loss and alteration, predator alien species and emerging diseases are among the leading. Habitat loss and habitat alterations in progress was recorded and we searched for exotic animal species for which is well established that they are a threats for amphibians (i.e., predator fishes, crayfishes). Alien species were searched in every site where amphibians surveys were performed, both using visual surveys (using a binocular too) and blind dip netting (20-30 dip netting per site). Information on fish was also obtained by fishermen.

RESULTS

Distribution and species occurrence

Distribution was the result of both bibliographic (when they could be georeferenced with a good approximation, i.e. 500 m; n = 25) and original data (n =84). All available literature (Bruno, 1973; Bruno 1981; Bologna et al., 2000; Carpaneto, 1986; Ravenna, 2013; Cinquegranelli et al., 2015) is consistent in reporting the following species in the park: *Triturus carnifex* (Laurenti, 1768), *Lissotriton vulgaris* (Linnaeus, 1758), *Bufo balearicus* Boettger, 1880, *Bufo bufo* (Linnaeus, 1758), *Hyla intermedia* Boulenger, 1882, *Pelophylax* sinkl. *esculentus* (Linnaeus, 1758) and *Rana dalmatina* Fitzinger in Bonaparte, 1838. The pool frog synklepton is formed by two entities: the parental species, *P. lessonae* (Camerano, 1882) and its hemiclonal hybrid, the klepton *P.* kl. *easculentus* (Linnaeus, 1758). Our research confirmed the occurrence of all these species. Conversely, the occurrence of *Salamandrina perspicillata* (Savi, 1821), *Bombina pachypus* (Bonaparte, 1838) and *Rana italica* Dubois, 1987 reported by Bruno (1981) in many localities of the park was not confirmed by our samplings, consistently with other researches (Carpaneto, 1986; Bologna et al., 2000). Species distributions are reported in Figs. 1 and 2

The graph related to diffusion and density (Fig. 3) showed that *Triturus carnifex* is the species having the most critical situation in the Park, with very limited diffusion and occurrence (i.e., low number of sites). No species are positioned in the upper right corner of the graph, however *Rana dalmatina* and *Pelophylax* sinkl. *esculentus* are of little concern. All the other species, considering their distribution and rarity, do not appear to be particularly threatened. For the particular situation and position in the Fig. 3 of *Bufo balearicus*, at the boundary between 4 quadrants, see Discussion.

Ecology

Fig. 4 shows aquatic habitat preferences of each species. Both newts occurred in a limited number of habitat typologies while the more ecologically plastic species was the tree frog that breeds in all the habitat typologies. Variation in amphibian species composition among habitats is highly explained (more than 80%) by the first two axes of the CoA scatter plot (Fig. 5). Associations were found between the amphibian species and the various aquatic habitat typologies. The results show that all the species are quite different from each other for habitat preference. Few species are strictly associated to only one aquatic habitat typology: *Rana dalamatina, Hyla intermedia* and *Pelophylax* sinkl. *esculentus* are closely linked to forest swamps, ponds and slow running waters respectively. *Bufo balearicus* is associated with ponds and lakes while *B. bufo* seems to be associated with springs and artificial tanks. The newts display a moderate and comparable association with forest swamps, ponds and slow running waters.

The number of syntopic species did not differ significantly among the six habitat categories (one-way ANOVA, $F_{1,5}$ = 0.32, P = 0.90). Number of species in the different aquatic habitat typologies ranges between 3 (lakes) and 7 (ponds and slow running waters). Spearman's rank correlation did not detect a significant association between the habitat availability and the number of species for each habitat type (r = 0.806; P = 0.083).

Population abundance estimation

Index of calling survey (ICS) was performed to several populations of *B. balearicus* (16 populations), *H. intermedia* (16), *P.* sinkl. *esculentus* (52) and *B. bufo* (16). For the three first species all the four ICS scores were recorded (0-3), but for the latter the highest score lacked (Fig. 6). The score 1 for both toads clearly exceeded other scores proportionally. Medians of ICS did not differ among species (χ^2 = 5.678, df = 3, P = 0.128).

Capture-Marking-Recapture (CMR) was applied on a population of *Bufo balearicus* living in a urban meadow (5800 m^2) surrounding the breeding site (a concrete tank). During the four sampling sessions, we performed a total of 56 captures in which 26 different individuals were marked. Closure test confirmed that the population was closed (z = 1.89; p = 0.97). The recapture rate was high and the estimated population was 27 ± 1.32 adult toads (estimate ± SE; CI 95% = 27-33). Considering that the operational sex ratio was extremely balanced (0.46), the estimate of effective population size (N_e=25.8) was similar to that of the adult population census size (N).

The N of *P.* sinkl. *esculentus* breeding in an artificial ponds (88 m^2), was 60 ± 6 individuals (estimate ± SE; CI 95% = 53-78). The operational sex ratio was strongly male biased (0.94) and, as a result, effective population size was much lower than N (N_e=11.25).

The seven swamps where egg mass counting (EMC) of *R. dalmatina* was performed had greatly variable water surface area (mean ± SD = 4637.43 ± 5691.36 m^2; range = 124-15860 m^2) and maximum depth (mean

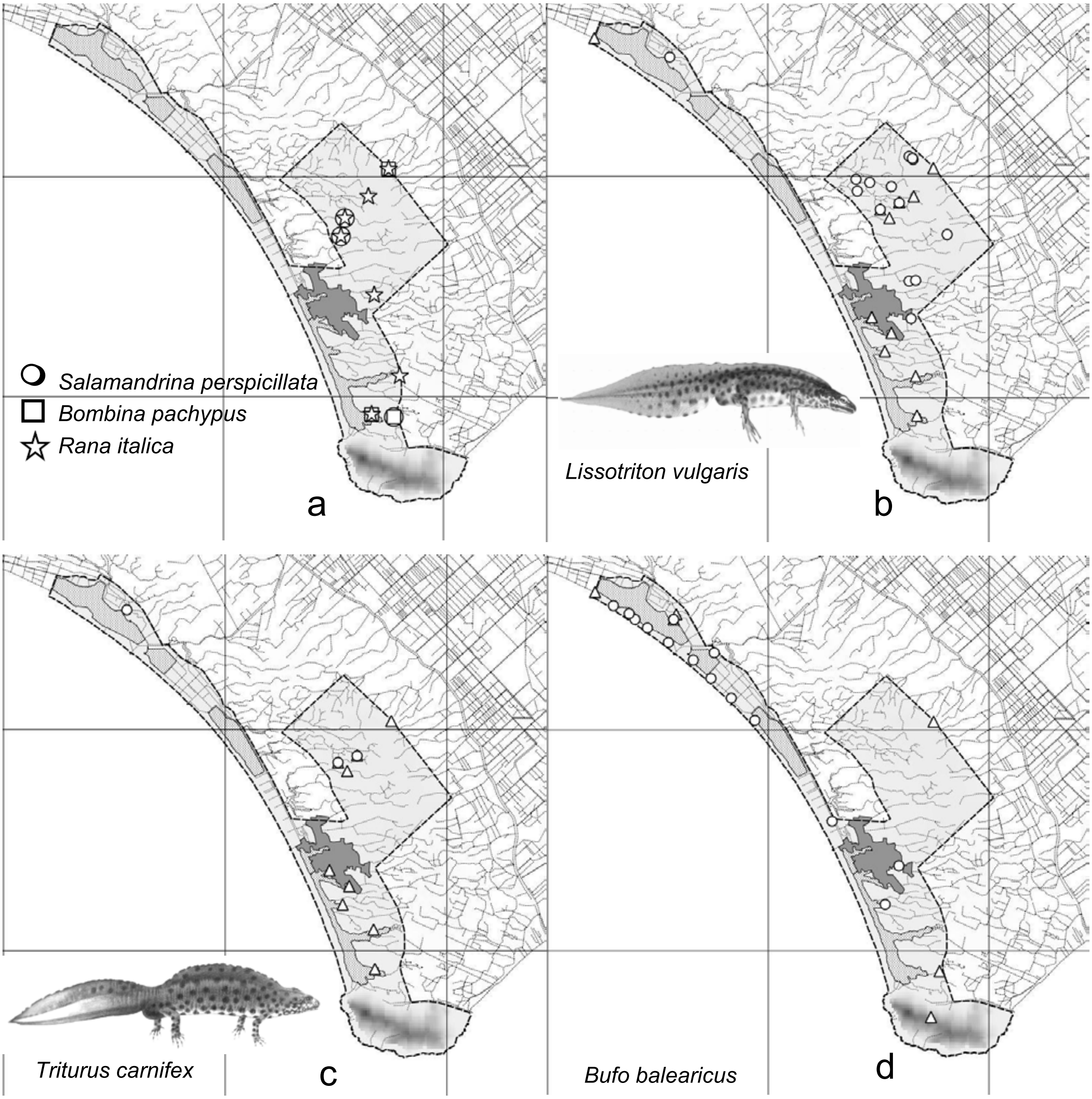

Fig. 1. Distribution of amphibians in the Circeo National Park (Central Italy). a = distribution of three species reported just once in the literature but not recorded during further researches. For figs. b, c and d, circles = original data; triangles = bibliographic data. Grid reports 10x10 km UTM squares. Dashed line: Park boundary. Dotted lines: surface hydrography. Dotted areas: lakes. The urban area of Sabaudia is showed in grey.

± SD =64.28 ± 26.37 cm; range = 40-110 cm). Surface area and maximum depth were not significantly correlated ($r=0.654$; $p=0.128$). Correlation remained above the significance threshold even considering all the swamps ($N = 15$) where reproductive activity was recorded (i.e., pooling sites where egg counts was performed and these where it was not performed) ($r = 0.506$; $P = 0.053$). Number and density of egg masses greatly varied among sites (Fig. S7). A total of 1419 egg masses were recorded (mean ± SD = 202.71 ± 199.09 SD; range 13-604) and their density ranged from 0.01 to 0.47 egg clutches per square meter (mean ± SD = $0.14/m^2$ ± 0.18). A signifi-

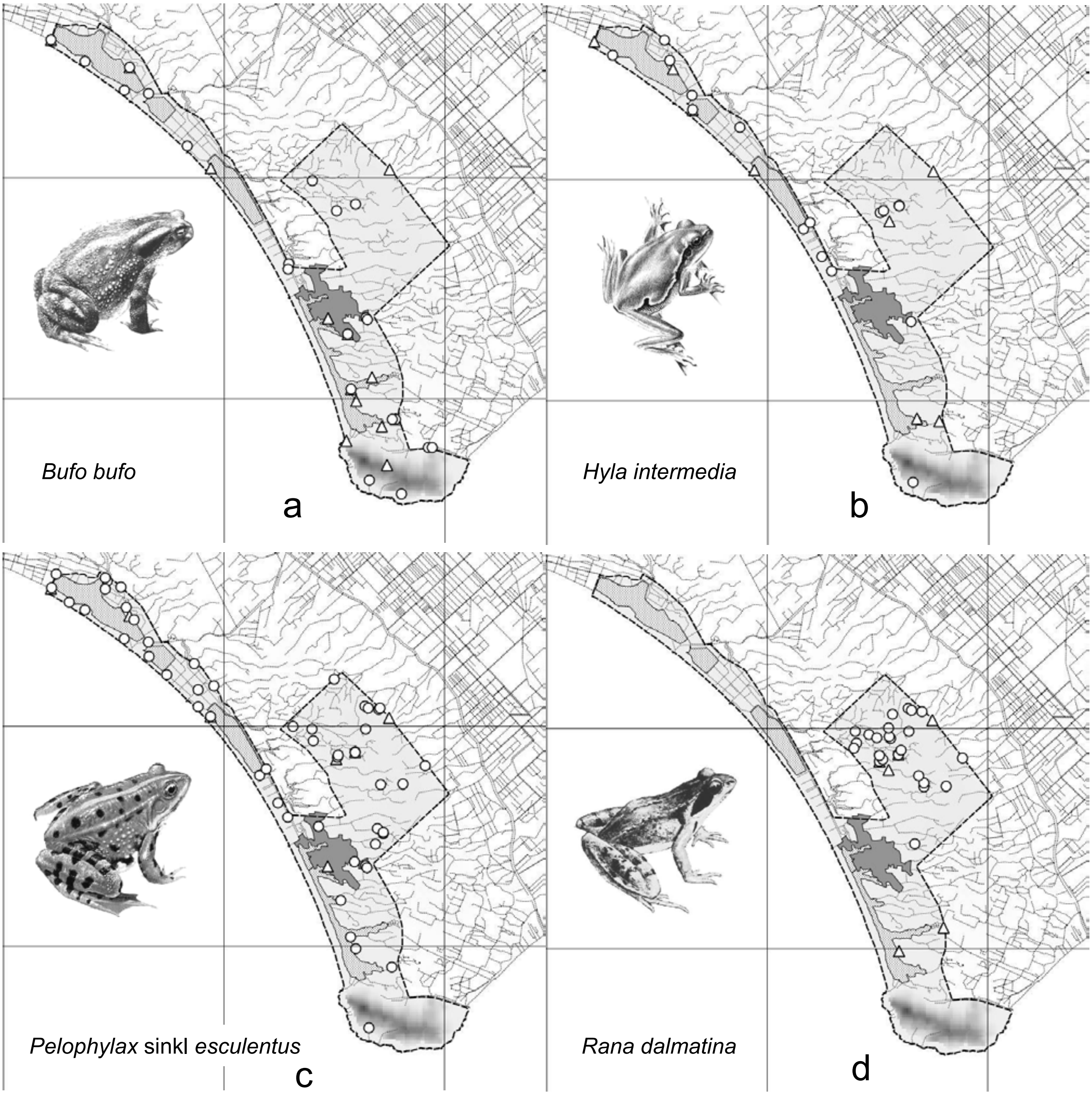

Fig. 2. Distribution of amphibians in the Circeo National Park (Central Italy). Circles = original data; triangles = bibliographic data. Grid reports 10x10 km UTM squares. Dashed line: Park boundary. Dotted lines: surface hydrography. Dotted areas: lakes. The urban area of Sabaudia is showed in grey.

cant correlation among eggs parameters and swamps features was detected only between the number of egg masses and maximum depth of the swamps ($r = -0.509$, $P = 0.249$; $r = -0.055$, $P = 0.919$, for egg density vs. swamp size or depth respectively; $r = 0.643$, $P = 0.109$; $r = 0.836$, $P = 0.025$ for egg number vs. swamp size or depth respectively. See Fig. 7).

Potential distribution

Species distribution models (SDM) were built for all seven amphibian species occurring in the Park. The number of available data to be employed (both as training and test data) for model building was dependent on the species (see Tab. 1 for details) and ranged between

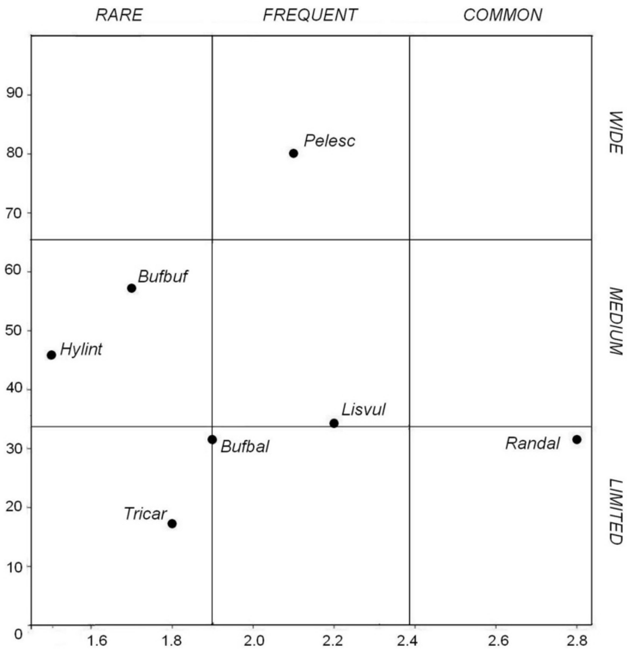

Fig. 3. Relationship between percentage of amphibian species occurrence in UTM square grids (2x2 km) and mean number of observations per UTM square. Tricar = *Triturus carnifex*; Lisvul = *Lissotriton vulgaris*; Bufbuf = *Bufo bufo*; Bufbal = *Bufo balearicus*; Hylint = *Hyla intermedia*; Randal = *Rana dalmatina*; Pelesc = *Pelophylax* synkl. *esculentus.*

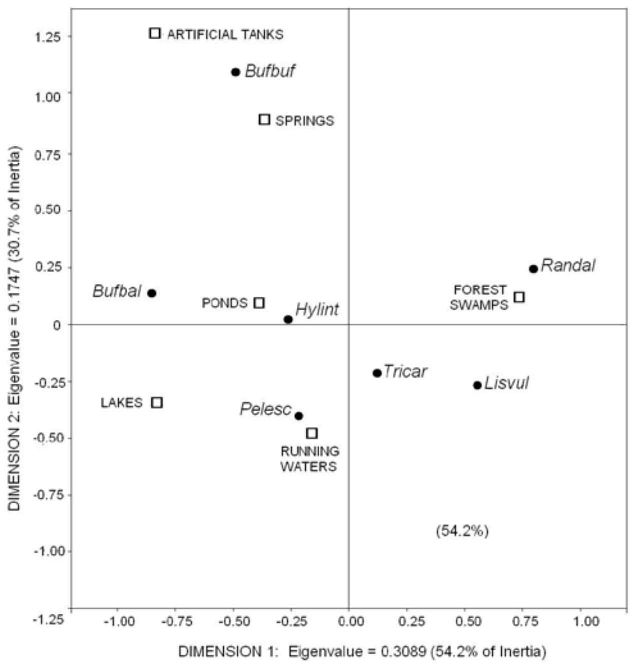

Fig. 5. Correspondence Analysis (CoA) scatter plot illustrating variations of amphibians species (black dots) distribution with aquatic habitat typologies (white squares). The percentages of variation explained by each axis are given in round brackets (codes of species are as reported in Fig.3).

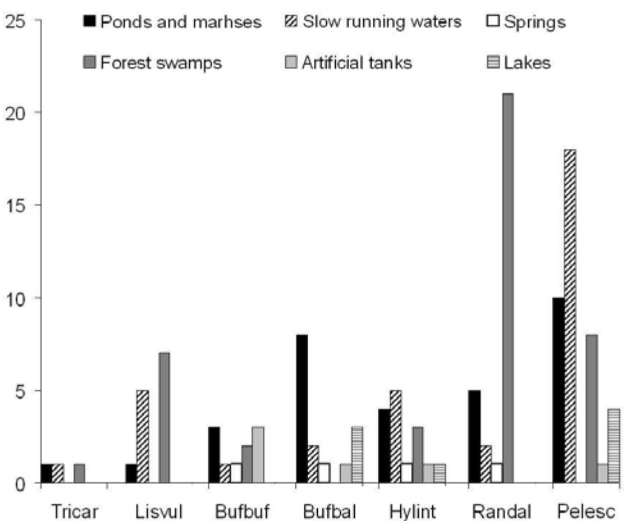

Fig. 4. Habitat partitioning (number of sites on the left vertical axe) of amphibians in the Circeo National Park (Central Italy). Codes of species are as reported in Fig. 3.

11 (*Triturus carnifex)* and 57 (*Pelophylax* sinkl. *esculentus*). All models shown a high predictive power, as revealed by AUC: indeed all averaged models received an AUC value > 0.970 (ranging from 0.974 for *Bufo balearicus* to 0.997 for *Rana dalmatina*) with the exception of the models regarding *Triturus carnifex,* which shown a slightly lower predictive power (AUC = 0.947). As a rule, among all variables included in the analyses, the most important ones for the major part of the species are the distance from water bodies (both lakes and swamps), tree cover and insolation, altogether with many Corine categories. The detailed results, regarding the list of variable effect, together with variable percentage contribution, for each species, is reported in Tab. 1. Potential distribution maps are presented as supplementary materials (Figs. S1-S4)

Threats

In the Circeo National Park we found three ponds and a swamps which are breeding sites of *Lissotriton vulgaris, Pelophylax* sinkl. *esculentus* and *Rana dalmatina* that suffer progressive filling with soil, reduction of the depth and earlier dry up. In April 2015, remains of several *R. dalmatina* eggs desiccated before hatching were recorded in these ponds. The water body with highest danger of disappearing was the one in locality "Cerreto

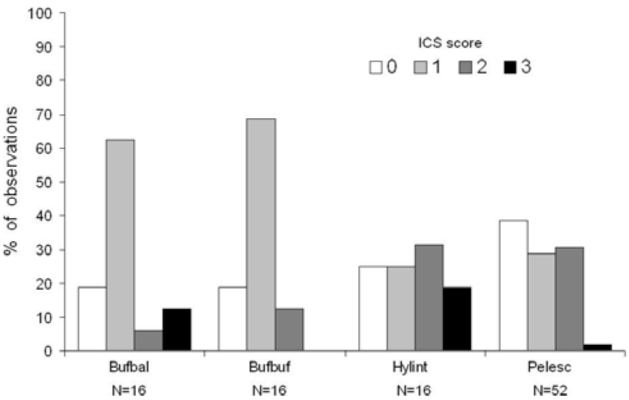

Fig. 6. Percentage of Index of Calling Survey (ICS) scores for four anuran species in the Circeo National Park (code of species as in Fig. 3). N = number of sites for each species.

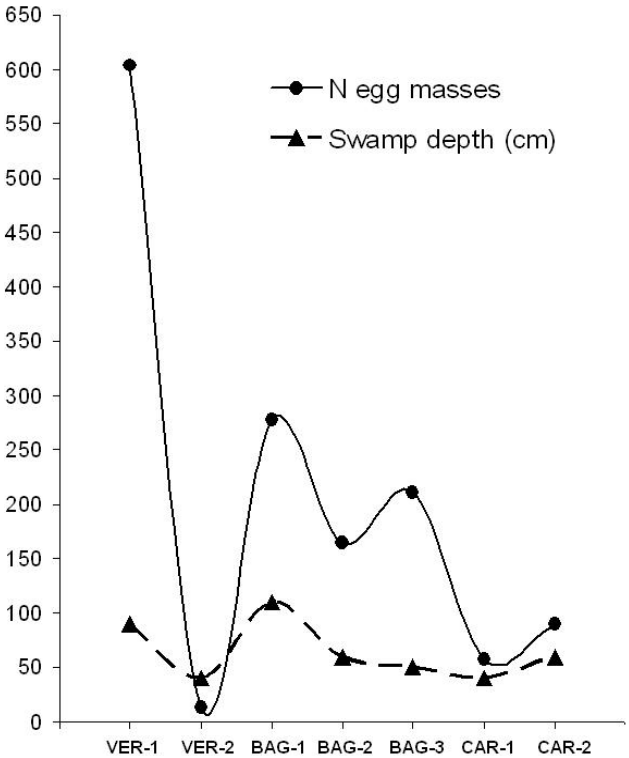

Fig. 7. Trend of the relationship between the number of *Rana dalmatina* egg masses and maximum depth of seven swamps. Alphanumerical codes refer to swamps: VER (Piscina della Verdesca), BAG (Piscina delle Bagnature), CAR (Piscina del Carpino). Correlation was statistically significant (Spearman correlation, r = 0.836, P = 0.025).

Fontana", a pond with surface area of 240 m^2 and maximum depth of about 20 cm.

No relevant evidence of road killing on amphibians were recorded and, during about 20 surveys, just few individuals of *B. balearicus* were found crushed in summer, after a rainy day, in a small stretch of the seafront road (loc. Bufalara, Sabaudia).

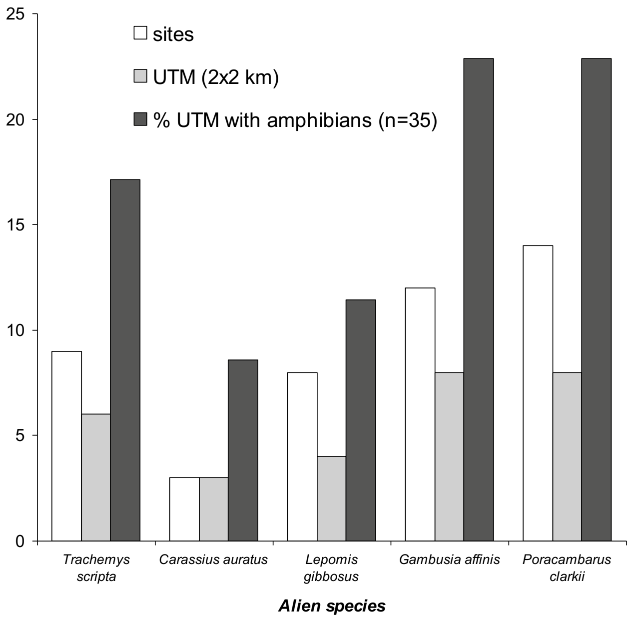

Fig. 8. Number of sites and UTM mashes (2x2 km) where alien species were recorded in the Circeo National Park.

Reproductive populations of five alien species, identified by the literature as threat to amphibians (see Discussion), were detected. In ascending order of threat to amphibians they were one reptile, three fishes and a crustacean: *Trachmeys scripta*; *Carassius auratus, Lepomis gibbosus, Gambusia sp., Procambarus clarkii*. On the whole they occurred at least in 14 UTM mashes (2x2 km), that is 32% of the total meshes occupied by the Park for at least 10% of their surface (n= 44; Fig. 8). Amphibian occurred in all 2x2 km UTM meshes where alien species were recorded. Original data concerning the distributions of these alien species are shown in supplementary materials (Figs S5-S6).

DISCUSSION

Species occurrence and their actual and potential distribution

In the 84 sites with amphibians, we found seven species in the Circeo National Park as reported in literature (Carpaneto, 1986; Cinquegranelli et al., 2015). However the occurrence ratios among species we found (*L. vulgaris*: 17%; *T. carnifex* 4%; *B. balearicus*: 18%; *B. bufo*: 25%, *H. intermedia*: 19%; *P.* synkl. *esculentus*: 62%;

Table 1. Contribution to species distribution models of amphibians in of the Circeo National Park (central Italy). AUC, that may range from 0 to 1 (null-maximum predictive power of the model) is also shown.

Lissotriton vulgaris	**AUC = 0.995**	**Occurrence locations = 24**
Variable	*% Contribution*	*Effect / Corine categories*
Distance from swamps	54.1	Negative
Tree Cover	13.1	Positive for values > 50%
Corine	12.1	Agricultural areas with significant portions of natural vegetation, Forested areas – Scrubs and herbaceous – Sclerophyllous vegetation, Inland water-bodies – Water courses
Triturus carnifex	**AUC = 0.947**	**Occurrence locations = 11**
Variable	*% Contribution*	*Effect / Corine categories*
Corine	50.3	Agricultural areas with significant portions of natural vegetation, Agro-Forestry areas, Forested areas – Scrub and herbaceous – Sclerophyllous vegetation
Distance from Swamps	24.3	Negative
Distance from Lakes	14.2	Negative
Bufo balearicus	**AUC = 0.974**	**Occurrence locations = 20**
Variable	*% Contribution*	*Effect / Corine categories*
Corine	42.6	Agricultural areas with significant portions of natural vegetation, Forested areas, Open spaces with little vegetation
Distance from Lakes	36.1	Negative
Insolation	11.2	Negative
Bufo bufo	**AUC = 0.993**	**Occurrence locations = 24**
Variable	*% Contribution*	*Effect / Corine categories*
Corine	25.7	Forested areas
Distance from Lakes	21.9	Negative
TRI	20.3	Positive
Hyla intermedia	**AUC = 0.989**	**Occurrence locations = 23**
Variable	*% Contribution*	*Effect / Corine categories*
Tree Cover	19.2	Positive
Distance from Lakes	18.7	Negative
Corine	18	Inland water-bodies - Wetlands, Forested areas
Distance from Swamps	17.5	Negative
Pelophylax* sinkl. *esculentus	**AUC = 0.981**	**Occurrence locations = 57**
Variable	*% Contribution*	*Effect / Corine categories*
Distance from Swamps	44.4	Negative
Tree Cover	15.6	Positive
Distance from Lakes	14.4	Negative
Rana dalmatina	**AUC = 0.997**	**Occurrence locations = 29**
Variable	% Contribution	Effect / Corine categories
Distance from Swamps	75.1	Negative
Tree Cover	10.7	Positive for intermediate values

R. dalmatina: 30%) differed, in some cases, from those reported by Cinquegranelli et al., 2015 (20%, 13%, 20%, 20%, 53%, 67%, 20%, respectively). These authors, performing 14 visit in each site, provide an interesting contribution testing the species detection probabilities (p), misdetection rates (Mr) and minimum number of visits (Nm maximum=10.3) necessary to be 95 % certain that an unrecorded species is in fact absent from a given site. We performed 1-5 samplings in each site and the species presence we recorded is, obviously, affected by species detection probabilities. However in the 40% of the sites sampled by Cinquegranelli et al. (2015) we found at least one additional species (1-4). We think that this discrepancy among the species' ratios could be probably due to the high difference in the number of sampling sites, to different sampling protocols, and to different years (difference in annual precipitation may affect, intuitively, amphibian species detectability)

Our original data showed that urodelans have a more limited diffusion and occurrence than anurans. In particular, *T. carnifex* has been detected only in three sites during our survey, resulting as the rarest species in the Park (Fig. 3). Its distribution appeared associated with the deep swamps and slow running waters of the plain forest (Fig. 4; Tab. 1), and this datum agrees with the habitat preferences known for this species (Andreone and Marconi, 2006). However, data for the surrounding areas (Novaga et al., 2013) indicate that vernal ponds and marshes are often colonized by both *T. carnifex* and *L. vulgaris*, suggesting that the limited distribution of newts outside the State forest might also be affected by the occurrence of alien species (Fig. S5), especially *P. clarkii* (Fig. S6). *Bufo balearicus* was recorded in open habitat with sandy and clay soils, as typically showed by this species in other Italian areas (Balletto et al., 2007), around the coastal lakes (Fig. 1) where retrodunal ponds and marshes in grazing lands are the most preferred breeding sites (Fig 4; Tab. 1). The concentration of its elective habitats along the coastal areas explains the overall limited diffusion of this toad in the Park area. On the contrary, *B. bufo* appears widespread in different habitat typologies (Fig. 2), and uses a greater variety of breeding sites (Fig. 4). *Hyla intermedia* has been detected both in open and forest habitats but the ICS scores showed higher concentrations in the wooded habitats, as could be predicted for a semi-arboreal species. *Rana dalmatina* can be considered to be the most representative species of the hygrophilous plain forest and no breeding sites were found outside the forest boundary. Finally, *P.* sinkl. *esculentus* is the commonest species in the park (Fig. 2 and 3), as expected for a species which is ecologically plastic and tolerant to anthropic disturbance in a highly urbanised context.

During our surveys we did not find *S. perspicillata, R. italica and B.pachypus*. The question is whether these species actually occurred in the 60s' as reported by Bruno (1981) and became extinct in the last decades, or their records reported by this author have to be considered as erroneous. These three species are characterised by different ecological requirements, with *Salamandrina* and *R. italica* strictly associated to clean running waters, and mainly shady, cool and damp areas (Utzeri et al., 2004, Angelini et al., 2007). Conversely, *Bombina pachypus* has a realtively wide ecological niche, but it is an heliophilous and thermophilus species tipically linked to open, lentic and shallow waters (Guarino et al., 2007). While for the latter species suitbale habitat were actually identified in the park and its occurrence in past decades cannot be excluded, for the first two amphibians we did not find terrestrial and aquatic habitats matching their ecological requirements. Suitable breeding sites could presumably be available before land reclamation (20s' of the XX century) in a restricted piedmont area on the northern slope of the Circeo massif (loc. "sorgente Mezzomonte" and "Rio Torto").

Ecology

The full range of aquatic habitats available in the Park is largely exploited by toads, tree frog and pool frog, while the two newts and *Rana dalmatina* exhibited a narrow habitat niche (Fig. 4). Larger pools are generally deeper in environments similar to that of the CNP (Brooks, 2005) but in the forest we studied this correlation was not significant. Wet phase duration is generally correlated with both pond surface area and maximum depth (Schneider and Frost, 1996; Brooks and Hayashi, 2002) but, if these two features are considered independently, maximum water depth is generally the best predictor of hydroperiod (Calhoun et al., 2003; Skidds and Golet, 2005). The Habitat Suitability Index for *R. dalmatina* elaborated by Radiguet (2012) showed that the date of drying of the pond is one of the key components to make habitats highly suitable for this frog. The positive and significant correlation we found between the number of egg clutches of *R. dalmatina* and maximum depth of the swamps (Fig. 7) is probably related to wet phase duration. Furthermore, as in other Italian areas (e.g., Bernini et al., 2004), also in the CNP *Rana dalmatina* was strictly associated with swamps and eggs were preferentially spawned in water bodies with intermediate values of tree cover (Tab. 1; Fig. 4 and 5). Distance from swamps also negatively affected the occurrence of both newt species (Tab. 1; Fig. 5). Toads are associated with the environment surrounding the lakes (Tab. 1), but the two species differed in their canopy requirements, as the green toad was associated with open spaces with little vegetation, while the common toad was associated with forested areas.

Population abundance estimation

Abundance of *B. bufo*, *B. balearicus*, *H. intermedia* and *P.* sinkl. *esculentus* greatly varied among sites, as emerged from the ICS (Fig. 6); the maximum score (3) was recorded for almost all species (2-19% of the surveyed sites, see Fig. 6), except the common toad. The question of whether Index of Calling Survey (ICS) can be considered a good proxy of actual population size is controversial (see for instance Jansen, 2009 and Corn et al., 2011 for articulated discussions on this topic). The reliability of ICS depends on species and is higher for species with loud calls (i.e., higher detectability), as that we studied, and for tree frogs and pool frogs in particular (Pellet and Schmidt, 2005; Tanadini and Schimdt, 2011). For *B. bufo*, *B. balearicus*, *H. intermedia* and *P.* sinkl. *esculentus* in the Circeo National Park, ICS could be easily used in extensive monitoring programs with relatively low effort. Furthermore, monitoring data collected for ICS may be elaborated using a recent class of statistic models that provide abundance estimations and that consider detectability (N-mixture models; Royle, 2004; Royle and Link, 2005).

Fort three species we elaborated demographic estimates. Capture-Marking-Recapture (CMR) method estimated about 27 green toads in a population, displaying an even sex ratio; a population of pool frogs, using removal method, was estimated to consist of about 60 adults, with strongly male biased sex ratio.

In several Italian populations of *B. balearicus* sex ratio is typically male biased and their N_e are highly variable (see table 1 in Giacoma, 1999). In 26 Italian populations demographic parameters are (range, mean ± s.d., 25th-75th percentile): N= 3-292; 89.46 ± 79.66, 28-144.5; sex ratio = 0.53-1, 0.82 ± 0.13, 0.73-0.94, N_e = 0-254.94, 50.27 ± 61.85, 12.84-64.05; (data elaboration from synoptic table 1 in Giacoma, 1999). These two anurans populations showed a contrasting situation if N and N_e are considered independently. One season of data collection provides the size of effectively breeding individuals (N_b) which is directly connected and derived by N_e because N_b times the generation time approximates N_e (Waples, 1990). Thus both N_b and N_e are connected to population's persistence probability, and may be used as indicators of a population's viability (e.g., Frankham et al., 2002). Considering both the high accessibility of the two sites and the ease of the sampling, these two populations of *B. balearicus* and *P.* sinkl. *esculentus* could monitored in be long term to corroborate our data and to assess populations trends.

Rana dalmatina may be considered the most representative species of the Park, because this frog is strictly associated with the swamps in the State Forest which represents the residual environment of the pre-reclamation (Tab. 1). Although this frog has a limited distribution it is common (Fig. 3) and abundant (Figs 2d and 7) in its elective environment. The sex ratio in *Rana dalmatina* is male biased in 90% of the breeding ponds (Lodé et al., 2004; Lodé et al., 2005; Lodé 2009) ranging from about 0.8 to 3. Considering this range of sex ratio, in the seven swamps we studied in the state forest, the whole population size might approximately range between 2550 and 5700 adults.

Threats and conservation strategies

Habitat loss and alteration. The CNP is among the most urbanized protected areas at national level. As a consequence, aquatic habitat loss and alteration (which are the main causes of amphibian decline at global level; see Collins, 2010) associated with land development, present the greatest challenge to the persistence of these habitats and their animals. However we did not find significant evidence of aquatic habitat loss due to current anthropic pressure. The aquatic habitat characterizing the PNC are swamps and ponds with a typical semi-perennial or seasonal hydroperiod. They are lentic shallow water bodies that are deep, on average, about 60 cm (see results). The main conservation problem of swamps and ponds was an habitat evolution toward dryer situations in shorter time, which is largely due to the dramatic change in the hydrological regime resulting from the past land reclamation. An excavation to increase water depth and the hydroperiod duration was planned for the pond in the locality "Cerreto Fontana" where three amphibian species spawn and that is exposed to a fast drying (see results).

Road mortality. Road mortality in the CNP does not seem to be a problem for amphibians and, probably, only one situation (loc. Bufalara) deserves further researches to estimate the actual impact on the green toad population.

Alien species. Invasive alien species are among the key factors threatening biodiversity (EEA, 2012). We found five alien species that, considering the available literature, may be considered as threats to amphibians (Fig. 8, Fig. S5-S6). The red-eared terrapin *Trachemys scripta* may have a large negative impact on amphibian populations (tadpoles; Polo-Cavia et al., 2010) in water bodies with high numbers of alien turtles. *Trachemys scripta* seems to be more diffused in the northern part of the Park (Fig. S5a). Its occurrence in the swamps of the State Forest was never recorded, even in the past (A. Romano pers. obs.). We did not find massive aggregations of *Trachemys*, although some sites (e.g., the surroundings of the Fogliano lake) support higher population density than southernmost areas. As a consequence of the apparently low density of *Trachemys*, we think that its management, for the conservation of amphibians, is less urgent than that of other aliens species.

Three alien fishes were detected in small water basin too. The gold fish, *Carassius auratus* can strongly affect amphibian populations either by predation at different life stages (e.g., Monello and Wright, 2001) and influencing reproductive behavior (Winandy and Denoël, 2013). However when gold fish does not reach high demographic density, it seems that its presence is compatible with persistence of native amphibians (Hartel, 2004). Therefore to well understand the threat level to amphibians, the occurrence of gold fish in the Park should be evaluated both in the distribution and in population size. The presence data here reported (Fig. S5b), which shows a scattered and limited distribution, are the only one recorded during this study, but it should be considered that this fish is more widespread in the park (Zerunian, 1984; Zerunian and Leone, 1996). Gold fish did not occur in the water bodies of the State Forest, probably because these aquatic habitats have a seasonal hydroperiod. The pumpkinseed sunfish, *Lepomis gibbosus,* was introduced for the first time in Italy in the Varano Lake in 1900 (Central Italy) and experienced an impressive increase of distribution in these last years (Zerunian, 2002). Due to its relatively small size *L. gibbosus* mainly preys upon amphibian larvae and eggs, but can severely damage also the adults (Hartel et al., 2007). Information about its negative impact on amphibian populations are corroborated from experiments with controlled conditions (Adams, 2000). *L. gibbosus*, that we recorded in several ditches, is probably widespread in this park characterised by a high connectivity among linear water bodies.

It is worth to mention that *L. gibbous* was recorded at high density in a large concrete artificial tank (30x5 m, about 2 m depth) on the southern slope of the Circeo massif, (Fig. S5c), which is the area with lowest water habitat availability in the Park. We found a female of *Bufo bufo* in that tank but no breeding activity was recorded. The artificial tank is a potential habitat for at least three amphibians species, because *B bufo, P.* sinkl. *esculentus,* and *H. intermedia* breed in a similar tank 1.6 km away on the same slope (Fig. 2). The eradication of *Lepomis gibbosus* for these sites is planned and will be carried out in 2016 by the Park. Vredenburg (2004) demonstrated that removing introduced fish can enable amphibian populations to recover to pre-decline levels.

The mosquitofishes of the genus *Gambusia* were introduced into natural or artificial water environments in many parts of the world as a biocontrol to mosquito populations, in particular where there are (or there were) malaria infections. The effectiveness of this fish in combating malaria is still debated and this discussion is outside the scope of this paper. By the way, it was imported for the first time in Italy, in Pontine marshes on 1922 by G.B. Grassi (Sella, 1926; Ronchetti, 1968) where, as the other Italian areas, is widespread. The introduction of *Gambusia* sp. strongly depresses all amphibian populations (Adams, 2000; Katz and Ferrer, 2003; Wells, 2007; Segev et al., 2009). The observations in the Park indicate widespread presence (Fig. S5d), as the mosquitofish occurs in a least in a quarter of aquatic sites that host amphibians (Fig. 8). The mosquitofish seems to be absent from the water bodies in the State Forest, probably because they have of a seasonal wet regime. Eradication of mosquitofish was planned and carried out in 2015, in an artificial ponds where only pool frogs breed. Around this aquatic site, located in a meadow garden, six rock piles were also placed to offer additional refugia to small vertebrates. After fish removal and the placing of the artificial shelters (November 2015), on February 2016, the pond was colonised by *B. bufo*, but it is a potential breeding site also for tree frogs that are in the surroundings. The pond is in the area devoted to tourist and visitor reception; information panels about the performed conservation action were also placed.

The last alien species we recorded, and the most dangerous one, is the red swamp crayfish, *Procambarus clarkii,* which is an efficient predator of amphibian larvae of several European species (Gherardi et al, 2001; Cruz and Rebelo, 2005; Cruz et al., 2006a; Ficetola et al., 2011). The presence of this crayfish is a deterrent for the colonization of potentially suitable aquatic habitats by amphibians (Cruz et al., 2006a, b; Ficetola et al., 2011). Furthermore, *P. clarkii* is the cause of massive local extinction of amphibians, as happened for instance in a Portugal natural reserve where crayfish caused the disappearance of more than 50% of amphibian species (Cruz et al., 2006b), or, in Italy, the extinction of *Rana latastei* from one part of its already small distribution range (Mazzotti et al., 2007). It is also a vector of the pathogen *Batrachochytrium dendrobatidis* which is capable to depressing or to extinct of amphibian populations. In the CNP it is the alien species with more records and widest distribution (Figs 8 and S6) and its occurrence is likely underestimated. *Procambarus clarkii* is the only alien species reported within the State Forest (Fig. S6), so all amphibians occurring in the forest are severely threatened. Eradication of *P. clarkii* from the site in the State Forest, is an aim of the Park. To achieve this goal, an integrate strategy is under consideration, using both intensive removal (Hein et al., 2007) and biological control (Aquiloni et al., 2010). An annual monitoring to detect further invasions into other areas of the forest was also planned. A pilot study to test the effectiveness of active removal (i.e., the less expensive method) on the State Forest populations is currently in progress.

An updated, large, and geo-referenced database of species distribution is the essential prerequisite for any protected area to effectively manage its resources and to plan appropriate conservation strategies. In the CNP seven species were recorded, few of them strictly associated with particular aquatic habitats and thus with limited distribution, as *Rana dalamatina* is. For this species the maintenance of swamps is an essential prerequisite for conservation. The debated issue about the presumed presence of *Salamandrina perspicillata*, *Bombina pachypus* and *Rana italica* can reasonably be regarded as solved: these species are absent in the Park and their closest sites are at least 10 km away, on the Volsci chain. No reliable information are available to assess if they occurred before land reclamation or not. Despite the highly urbanised territory, habitat loss and alteration seem to be limited, and few practical and rather simple actions can be made to improve the current situation. The main threats to amphibians in the park, in our opinion, are the spread of alien species. Particular concern deserves the invasion of the red swamp crayfish in the State plain forest which is the area with highest level of species richness.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at < http://www.unipv.it/webshi/appendix >.

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