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Breeding sites of the Italian Green Toad, *Bufotes balearicus* (Boettger, 1880) in Naples (Italy)

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Abstract

Here we report the results of a longitudinal field study (2002 -2018) aimed at monitoring the presence and the breeding activity of the Italian Green Toad, *Bufotes balearicus,* within the area of the administrative boundaries of Naples. We identified breeding sites in five sub-areas (Bagnoli – ex sito industriale, Mostra d'Oltremare, Parco del Poggio, Scalo Ferroviario-zona orientale, Parco Massimo Troisi), one of which (Parco M. Troisi) since 2018. Only in two subareas (Mostra d'Oltremare and Parco del Poggio) the reproductive activity took place regularly, almost every year, although the development of tadpoles was often compromised by a variety of anthropogenic disturbances. However, the major potential threat to the survival of this amphibian for the entire study area is represented by the isolation of its population mainly due to the scarcity of natural and semi-natural green areas in a highly urbanized territory.

We hope that our research will be helpful to plan management activity for this species in the urban area of Naples.

Keywords: Amphibians, *Bufotes balearicus*, Naples, monitoring, reproductive sites

Riassunto

In questo lavoro sono riportati i risultati di uno studio di campo condotto dal 2002 al 2018 teso ad accertare la presenza e l'attività riproduttiva del rospo smeraldino italiano, *Bufotes balearicus,* nell'area compresa all'interno del territorio comunale della città di Napoli. Abbiamo rilevato siti di riproduzione in 5 sub-aree di Napoli (Bagnoli – ex sito industriale, Mostra d'Oltremare, Parco del Poggio, Scalo Ferroviario – zona orientale, Parco M. Troisi), uno dei quali (Parco M. Troisi) dal 2018. Solo in due sub-aree (Mostra d'Oltremare and Parco del Poggio) abbiamo osservato che l'attività riproduttiva di questo anfibio era avvenuta regolarmente in pressoché tutti gli anni sebbene lo sviluppo larvale spesso risultasse compromesso da diversi tipi di attività antropica. In generale, la principale minaccia per la sopravvivenza del rospo smeraldino italiano nella cinta urbana di Napoli è rappresentata dall'isolamento delle sue metapopolazioni a causa soprattutto della scarsità di ambienti naturali e seminaturali che possano fungere da corridoi ecologici in un ambiente altamente edificato come la città di Napoli. Crediamo che la nostra ricerca possa aiutare a pianificare eventuali azioni di gestione per questa specie nella città di Napoli.

Parole chiave: Anfibi, *Bufotes balearicus*, Napoli, monitoraggio, siti riproduttivi.

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Introduction

The green toads of the Western Palaearctic and Central Asia, formerly ascribed to *Bufo viridis* (*sensu latu*), are currently considered a complex of different evolutionary lineages whose taxonomy and phylogenetic relationships are still intensely debated. In recent years, the different lineages of these green toads were first attributed to the genus *Pseudepidalea* and subsequently to the genus *Bufotes*, as the former has been demonstrated to be synonymous of the latter (Novarini & Bonato 2010; Lo Valvo et al., 2016; Speybroeck et al., 2020). *Bufotes* was accepted as a genus-level taxonomic group as time-calibrated phylogenetic analyses showed its contemporary or even older origin with respect to most of the currently recognized bufonids genera (e.g. *Bufo*, *Epidalea*) (e.g. Speybroeck et al., 2020). According to genetic studies (Stock et al., 2008), the following *Bufotes* species are present in Italy: *B. viridis*, limited to north-eastern Italy (provinces of Udine, Gorizia and Trieste); *B. balearicus* (Boettger, 1880), widespread in the rest of the Italian territory (excluding the Aosta Valley), in Sardinia, north-eastern Sicily and in some minor Tyrrhenian islands (Elba and Ischia); *B. siculus*, endemic to Sicily, excluding the north-east of this island, and to some minor

islands (Ustica and Favignana); *B. boulengeri,* only in Lampedusa (Lo Valvo et al., 2016). However, the reproductive isolation between *B. balearicus* and *B. viridis* and between *B. siculus* and *B. boulengeri* needs further confirmation (Lo Valvo et al., 2016).

Besides the green frogs of the genus *Pelophylax*, *B. balearicus* is the only amphibian currently present within the administrative boundaries of Naples (Guarino et al., 2002, 2012) where, other amphibian species were reported up to the early 1980s (*Rana italica*, *Rana dalmatina*, *Lissotriton italicus*) (Dinardo 1990; Guarino et al., 2012). Unfortunately, unlike other Italian cities (e.g. Rome and Milan), the scarcity and the strong fragmentation of ecologically suitable environments for the survival of amphibians in the urban area of Naples make its populations of *B. balearicus* extremely vulnerable and potentially at risk of extinction.

Figure 1: *Bufotes balearicus* from Mostra d'Oltremare in Naples (photo by FM Guarino, June 2016).

In this study, we report the results of a longitudinal field research aimed at monitoring the presence and the breeding activity of the Italian Green Toad, *B. balearicus,* (Fig. 1) in the urban area of Naples. We believe that our research will be helpful to plan any management activity for this species in the Neapolitan urban area.

Materials and Methods

The study area coincides with that defined by the administrative boundaries of the municipality of Naples (Fig 2). The Cratere degli Astroni Natural Reserve located in the municipalities of Pozzuoli and Naples, of which the herpetofauna is well known (Caputo 1989; Guarino et al., 2002, 2012), was not taken into consideration for this study.

We selected the following four sub-areas where the presence of *B. balearicus* had previously been reported (see Guarino et al., 2012): Bagnoli – ex sito industriale, Mostra d'Oltremare, Parco del Poggio and Scalo Ferroviario -zona orientale (Fig. 2).

The Bagnoli – ex sito industriale (localization of the centre of the area: lat. 14 10 39 E, long. 40 48 35 N) is sited southwest of the city and extends over a surface of about 1.8 km² (0 - 4 m a.s.l.) within the larger area of the Campi Flegrei, in the Bagnoli-Fuorigrotta depression. It hosted the Italsider industrial complex until the beginning of the 1990s and now is abandoned (Fig 3). In this part of the city the breeding sites of the Italian green toads were represented by some artificial water bodies of different size

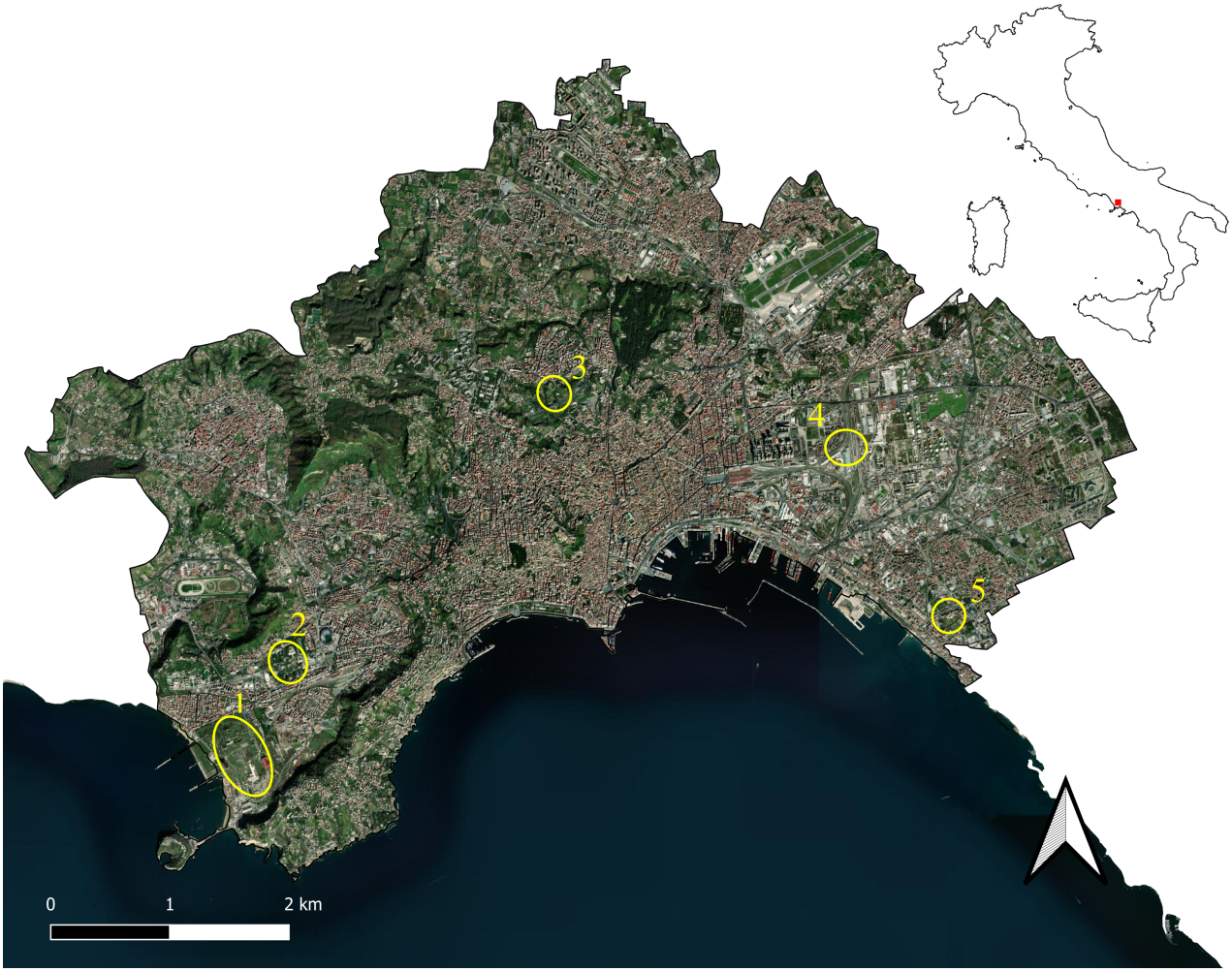

Figure 2: Study area. In the top right insert, location of Naples (red square). In the middle, the five subareas including reproductive sites: 1) Bagnoli- ex sito industriale, 2) Mostra d'Oltremare, 3) Parco del Poggio, 4) Scalo Ferroviario-zona orientale, 5) Parco M. Troisi. (yellow circles) (Picture by ESRI satellite, free edition, 2018, modified by S. Viglietti).

partially surrounded by vegetation. The habitat is characterized by the presence of sub-nitrophilous vegetation, linked to the state of abandonment following the dismantling of the industrial complex, where areas occupied by *Rubus ulmifolius* or *Dittrichia viscosa* are recognizable (pers. comm.). The artificial basins close to the coast-line are surrounded predominantly by *Phragmites australis*. According to the CORINE Biotopes manual, the predominant habitats are: 31.8a Sub-Mediterranean vegetation with *Rubus ulmifolius*, 34.81 Mediterranean sub-nitrophilous grass communities (including Mediterranean and post-cultural sub-Mediterranean vegetation), 53.1 Vegetation of reed beds and similar species (Commission of the European Community, 1991; Angelini et al., 2009). Unfortunately, since 2011 this site could be not investigated because it was subjected to seizure by the Tribunal of Naples (Invitalia 2018).

The Mostra d'Oltremare (localization of the centre of the area: lat. 14 11 12 E, long. 40 49 33 N) is the largest space of the city for trade shows and congresses, located in the western district of the city, a little further

Figure 3: A) Bagnoli area, formerly Italsider industrial complex, in a photo taken in 2019. At the top, in the middle, it is possible to see one of the bodies of water used by the green toad for breeding in this area. Under, on the left, there is the center of Città della Scienza (photo by Guarino, June 2019). B) Educational pond of Città della Scienza with *Carassius carassius* and *Trachemys scripta* (photo by FM Guarino, May 2016).

north of Bagnoli. It covers an area of about 0.7 km² (21 – 31 m a.s.l.) largely occupied by many green spaces, ornamental basins and fountains (Fig. 4). The arboreal species here present are mostly ornamental, while native species are mostly represented in the herbaceous layer. According to the Carta della Natura della Regione Campania (Bagnaia & Viglietti, 2018), this site is categorized as habitat 85 Parks, gardens and green areas.

The Parco del Poggio (localization of the centre of the area: lat. 40° 52' 0.468'' E, long. 14° 14' 22.05'' N) is an urban park located in the North of the city with a surface of approximatively 0.04 km2. It is within an urban area with very varied vegetation, where cultivated areas and small gardens with ornamental species prevail. However, isolated specimens and small nuclei of *Quercus ilex* and *Pinus pinaster* are still found. The spaces no longer cultivated are often occupied by brambles and sub-nitrophilous vegetation ascribable to the category 34.81 Mediterranean sub-nitrophilous grass communities according to the CORINE Biotopes manual (Commission of the European Community, 1991; Angelini et al., 2009). The Parco del Poggio was built on a tuff quarry used in the 1960s for the construction of some surrounding areas. Once the tuff extraction was stopped, in this area an urban park was designed in the 1990s and inaugurated in 2001 (Comune di Napoli 2019). A small lake was built on the bottom of the quarry (167 m a.s.l.) and partially surrounded by ornamental plants such as *Strelizia* sp. and Bambusae gen. sp. (Fig. 5). According to the Carta della Natura della Regione Campania (Bagnaia & Viglietti, 2018), the Parco del Poggio is categorized as habitat 85 Parks, gardens and green areas.

The Scalo Ferroviario- zona orientale (localization of the centre of the area: lat. 14 17 28 E, long. 40 51 53 N) includes several temporary natural ponds and canals extending over a urbanized area (about 0.05 km², about 18 m a.s.l), near the Railway

Figure 4: Reproductive sites of *Bufotes balearicus* in the Mostra d'Oltremare: A) Small artificial lake called Laghetto Fasilides (photo by FM Guarino, May 2015). B) One of the 28 fountains placed at the entrance of the Mostra d'Oltremare (photo by FM Guarino, May 2016) C) Freshly deposited eggs of *B. balearicus*, typically arranged in strings (photo by FM Guarino, April 2016).

Station in the eastern district of the city. The habitat is ascribable to the category 34.81 Mediterranean sub-nitrophilous grass communities according to the CORINE Biotopes manual (Commission of the European Community, 1991; Angelini et al., 2009). It includes sub-anthropic formations with Mediterranean therophytes forming

pioneer stages often very extensive on soils rich in nutrients that are affected by past cultivation practices or more recent weeding practices for the management of the railway network. In this subarea, *B. balearicus* constantly reproduced since the end of the last century (Dinardo 1990; pers. comm.).

The data on the presence and reproductive activity of *B. balearicus* were collected by visual encounter survey (VES) from 2002 to

Figure 5: Parco del Poggio. A), B). Temporary pool formed after the emptying of an ornamental basin (photo by Guarino, May 2016). C) Tadpoles of *Bufotes balearicus* (photo by Guarino, April 2016).

2018, especially from March to June, a period corresponding to the breeding season of the species. Our VES was conducted using multiple transects along the suitable water bodies for breeding of this anuran (Heyer et al., 1994) during daytime hours.

We also recorded the presence of the species based on its typical vocalizations.

Results

The breeding activity of *B. balearicus* in the four subareas identified from 2002 is shown in table 1. Unfortunately, for some years it was not possible to record information on all the different reproductive sites, as reported above.

Table 1: Breeding activity of *B. balearicus* in the municipality of Naples. **C**: Presence of adults using calls; **ND**: not investigated; **P**: Presence of several adults/pairs using VES. **PN**: Presence of isolated individuals in the neighbouring areas using VES; **R**: Reproduction successful: presence of numerous tadpoles and toadlets; **TR**: Fruitless attempt of reproduction: presence of few tadpoles and toadlets and many new metamorphosed individuals dead due to exsiccation of the pool; **PE** Probably extinct due to the destruction of the reproductive site.

Study year	Bagnoli ex sito industriale	Mostra d'Oltremare	Parco del Poggio	Scalo ferroviario zona orientale
2002	ND	R/P	ND	R/P
2003	TR	R/P	TR/C	R/P
2004	TR/C	R/P	R/P	R/P
2005	C	R/P	R/P	ND
2006	ND	R/P	R	ND
2007	ND	ND	ND	ND
2008	ND	R/P	ND	ND
2009	ND	R/P	ND	ND
2010	ND	ND	ND	ND
2011	ND	R/P	R/P	ND
2012	ND	R/P	R/P	ND
2013	ND-PN	R/P	R	ND
2014	ND-PN	R/P	R	ND
2015	ND-PN	R/P	R	PE
2016	ND-PN	R/P	R	PE
2017	ND-PN	R/P	TR	PE
2018	ND	R/P	R/P	PE

Concerning Bagnoli- ex sito industriale (Fig. 3A) we collected data until to 2011. Afterwards, we investigated the neighbouring districts, including the Science Centre called Città della Scienza, recording the presence of different erratic individuals and dead specimens. Anyway, we never registered reproductive events of the Italian green toad in the neighbouring districts, including the educational small pond of Città della Scienza, where freshwater fish *Carassius auratus* and turtles *Trachemys scripta* were abundantly introduced (Fig. 3B).
In the Mostra d'oltremare we observed the reproduction of *B. balearicus* throughout the entire study period. Several sites were used by this species for breeding, such as a small artificial lake called Laghetto Fasilides (Fig. 4A) and the fountains at the entrance walkway (Fig. 4B, C).
In the Parco del Poggio we observed spawning, tadpoles and neometamorphosed of *B. balearicus* in temporary pools that were formed when the artificial lake of the Park was almost completely emptied for annual maintenance (Fig. 5A, B). Reproductive events of this species were generally recorded between April-early June in every year surveyed (see Table 1).
Concerning the Scalo Ferroviario- zona orientale the works for the construction of a new railway line (Fig. 6A and B) since the beginning of 2000 resulted in the gradual disappearance of the breeding sites of the Italian green toad. Since the breeding season of 2005 we were no longer able to find spawning or tadpoles in this area.
Interestingly, since 2018 we also recorded a new reproductive site of *B. balearicus*, in the Parco M. Troisi (4 m a.s.l.) (Fig. 2; 6C), located in the San Giovanni district, east of the study area.

Discussion

Our longitudinal field study (2002-2018) led to the identification of breeding sites of *B. balearicus* in five subareas of Naples. In two of these (Parco del Poggio and Mostra d'Oltremare) the oviposition occurred regularly, although the development of tadpoles was often compromised by a variety of anthropogenic factors, including the alteration of the aquatic habitat owing to water withdrawals and water pollution. However, a major potential threat to the survival of this amphibian for the entire study area is represented by the isolation of its metapopulations mainly due to the scarcity of natural and semi-natural green areas in a highly urbanized territory.
Interestingly, the recent discovery of the reproductive site in the Parco M. Troisi as well as the occasional reproductive events in other areas adjacent to those of the municipality of Naples (e.g. at waterfront of Portici, in June 2018), indicate the need of further field surveys to have a more accurate picture of the reproductive sites of the species in the urban area of Naples. On the other hand, the species is ecologically versatile and can colonize a wide range of anthropic environments (Guarino et al., 2012; Maio et al., 2000, 2001). In this regard, the occasional reports of erratic individuals

Figure 6: Scalo Ferroviario- zona orientale subarea. A) Work in progress for the construction of a new railway. B) Temporary pool with tadpoles of *Bufotes balearicus* (photo by N. Maio, April 2004). C) Parco Massimo Troisi.

of *B. balearicus* for other areas of the city, such as Moiarello, in the northern area of the city (pers. comm.), Soccavo district (pers. comm),); Agnano, and Coroglio (the citizen-science platform iNaturalist: https://www.inaturalist.org) also deserve to be mentioned.

Furthermore, in an urban reality such as Naples where the environments suitable for the reproduction of this amphibian are fragmented and isolated by numerous

anthropogenic barriers (Guarino et al., 2016), it would be very important to check for possible communication routes between the different metapopulations of the species. It is also worth mentioning that *B. balearicus* is included in the Annex IV of the Directive 92/43/CEE on the conservation of natural habitats and of fauna and flora (Habitats Directive) (Lo Valvo et al., 2016) and that its populations in Campania are considered Vulnerable (VU) in the Red List of the Amphibians and Reptiles of the Region (Guarino & Maio 2013) mainly due to the disappearance, alteration, and fragmentation of habitats. Concerning the Mostra d'Oltremare and the Parco del Poggio we proposed to the respective Managing Bodies the following actions aimed at the conservations of *B. balericus:* to ensure the permanence of the bodies of water used by the species for breeding and carrying out the larval development; to ban the use of biocides in the water bodies and neighbouring areas; to monitor and contrast the possible introduction of alien species such as *Trachemys scripta* and *Carassius auratus*; to install information panels on the importance of protecting this species.

To conclude, this study represents an update on the reproduction sites of *B. balearicus* in Naples and a first step for the planning of future management activities for this amphibian in the study area.

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Author contributions

Conceptualisation: F.M.G., M.M., N.M. Data Curation: F.M.G., M.M., N.M. Formal Analysis: F.M.G., M.M., N.M. Investigation: F.M.G., M.M., N.M. Methodology: F.M.G., M.M., N.M. Writing – Original and Final Draft Preparation and Creation and/or presentation of the published work: F.M.G., M.M., G.O., L.O.A.P., A.P., S.V., N.M.

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