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## ECOLOGICAL NOTES ON THE THREATENED AMPHIBIAN *DISCOGLOSSUS SARDUS* IN THE TUSCAN ARCHIPELAGO

### SUMMARY

*Discoglossus sardus* is an amphibian endemic to the Tyrrhenian area, with a predominantly island distribution. Less is known about the ecology of the Italian populations of the species, even if it is classified as Vulnerable in the national IUCN assessment. In this study, we present data on population density along with notes on the characterization of the breeding sites in the Tuscan Archipelago.

*Key words:* breeding sites, density, herpetofauna, Tyrrhenian painted frog

### RIASSUNTO

*Note sull'ecologia di Discoglossus sardus, anfibio a rischio di estinzione, nell'Arcipelago Toscano.* *Discoglossus sardus* è un endemismo tirrenico che presenta un areale prevalentemente insulare. Sono poche le informazioni ecologiche disponibili sulle popolazioni italiane di questo anfibio, nonostante la specie sia classificata come Vulnerabile nella valutazione nazionale svolta dalla IUCN. In questo studio vengono presentati dati e osservazioni riguardanti prevalentemente densità di popolazione e caratterizzazione dei siti riproduttivi di *D. sardus* nell'Arcipelago Toscano.

*Parole chiave:* densità, Discoglossos sardo, erpetofauna, siti di riproduzione

### INTRODUCTION

The Tyrrhenian painted frog *Discoglossus sardus* Tschudi in Otth, 1837 is an amphibian endemic to the Tyrrhenian area, occurring on the Îles Hyères, Corsica, Sardinia (including Asinara, Caprera, La Maddalena, Spargi and San

Pietro islands), the Tuscan Archipelago and the Monte Argentario, a fossil island in southern Tuscany (CORTI *et al.*, 1991; CAPULA *et al.*, 2007). The Archipelago, made up by seven main islands (Gorgona, Capraia, Elba, Pianosa, Montecristo, Giglio and Giannutri) and some minor islets, is most likely an emerged portion of the Paleo-Apennine (ARINGOLI *et al.*, 2009) and its faunal composition is influenced by its position between the Sardinian-Corsican microplate and the Italian Peninsula, and by the sea-level changes which, especially during the Pleistocene, got some of these lands in contact. The presence of *D. sardus* in the Archipelago is still to be clarified. Probably the ancestor of *D. sardus* was present on the Sardinian-Corsican microplate before its detachment from the continent, then it could have differentiated in *D. sardus* during insular isolation; the species range could have enlarged during the Messinian period and later, in the Quaternary period, it could have restricted to some islands, disappearing from the continental areas (LANZA, 1980; ZANGARI *et al.*, 2006). The species occurs on Montecristo and Giglio and it is one of the five amphibians present on the Archipelago, along with *Bufo bufo*, *Bufo viridis balearicus*, *Pelophylax* sp. (Elba), and *Hyla sarda* (Capraia, Elba). *D. sardus* is included in Annexes II-IV of 92/43/CEE Directive and it is classified as Vulnerable, among the Threatened categories, in the IUCN Red List of the Italian Vertebrates (RONDININI *et al.*, 2013).

The distribution of *Discoglossus sardus* in the Tuscan Archipelago was recently updated (BIAGGINI *et al.*, 2015), but less is known about the ecology of these species in the Archipelago for which only dated papers are available (i.e., BRUNO, 1968, 1975). A more recent paper by LEBBORONI *et al.* (2014) gives notes on the reproduction of *D. sardus* on the Monte Argentario. In this study, we present data on population density along with notes on the characterization of the breeding sites in the Tuscan Archipelago.

## MATERIALS AND METHODS

We performed larval counts to estimate density values (SHAFFER *et al.*, 1994). For each water body, we repeated counts during three consecutive sampling sessions and the mean number of larvae was used to calculate density. We approximated the shape of ponds to semi-ellipsoids to estimate their volume. Samplings were performed in spring 2014 (early April) and 2019 (early April on Giglio and early June on Montecristo) on a smaller number of sites (Table 1).

We used Generalized Linear Models (GLM) to test the possible influence of water supply (present/absent, categorical predictor) and volume of the breeding sites (continuous predictor) on the density of larvae in the two

islands. For Giglio island, we pooled density values from 2014 and 2019 after verifying the absence of significant differences between the two years, through a Mann-Whitney U Test. On Montecristo, the surveys in 2019 were performed later in the season compared to 2014 and for this reason, we analysed data from the two years separately. We applied GLM, as above described, on data from 2014. In 2019 the only pools hosting larvae were the ones with water supply (while the isolated ones were already dried), thus we only tested the influence of pool volume on density, using a Spearman correlation.

In 2014, on both islands, we also measured some physical features (salinity, pH and dissolved oxygen) on a subsample of breeding sites and the snout-vent length (SVL) and body mass of some individuals.

**Table 1**

List of the study sites (the numbers correspond to the ones reported in Fig. 1), with indication of the amount of sampled pools, their volume range and the density of *Discoglossus sardus* larvae recorded in the two years of samplings (LD<sub>2014</sub>, LD<sub>2019</sub>; outlier values are also reported).

**Montecristo (IV 2014, VI 2019)**

1) Along the stream *Fosso del Santo*, at 240-260 m a.s.l. Four pools with slow running water and LD<sub>2014</sub> = 0.036 ± 0.045 n/dm<sup>3</sup> and one isolated pool with lentic water and LD<sub>2014</sub> = 0.80 n/dm<sup>3</sup>. Volume range = 251-879 cm<sup>3</sup>.

2) From *Cala Maestra* to *Grotta del Santo*, along a track (about 1 km long), at 33-180 m a.s.l. 14 small sized pools (vol. range: 1-16 cm<sup>3</sup>) with lentic water and LD<sub>2014</sub> = 3.30 ± 2.08 n/dm<sup>3</sup>.

3) Along the stream *Fosso della Cala, Cala Maestra*, at 25-60 m a.s.l. Pools with a wide size range (2.5 - 1570 cm<sup>3</sup>), mainly characterized by slow running water. LD<sub>2014</sub> = 5.77 ± 1.63 n/dm<sup>3</sup> (3 sites); LD<sub>2019</sub> = 0.92 ± 0.88 n/dm<sup>3</sup> (14 sites).

4) Along the stream *Fosso di Santa Maria*, at 15-65 m a.s.l. Pools with volume ranging from 1 to 2763 cm<sup>3</sup>, mainly characterized by slow running water. LD<sub>2014</sub> = 1.17 ± 1.66 n/dm<sup>3</sup> in 7 sites; 27.30 n/dm<sup>3</sup> of newly hatched larvae in a tiny pool of 1 cm<sup>3</sup>. LD<sub>2019</sub> = 0.15 ± 0.243 n/dm<sup>3</sup> (3 sites).

5) *Cale Gemelle, East*, at 10 m a.s.l. One pool (vol.: 25 cm<sup>3</sup>) with slow running water, near the shoreline with LD<sub>2014</sub> = 5.97 n/dm<sup>3</sup>.

**Giglio (IV 2014, IV 2019)**

1) Along the stream *Fosso della Valle dell'Olivello*, at 30-140 m a.s.l. Series of pools (vol. range = 628 - 785000 cm<sup>3</sup>), distributed along 200 m, with slow current water and some lateral pools with no water supply. LD<sub>2014</sub> = 0.13 ± 0.14 n/dm<sup>3</sup> in 6 sites and an outlier value of 3.07 n/dm<sup>3</sup>; LD<sub>2019</sub> = 1.09 ± 1.49 n/dm<sup>3</sup> in 11 sites and 84.93 n/dm<sup>3</sup> in a very tiny pool.

2) Along the stream *Fosso delle Grotte*, at 80 m a.s.l. A single pool of slow lotic water, in a village, with volume of 30144 cm<sup>3</sup> and LD<sub>2014</sub> = 0.83 n/dm<sup>3</sup>.

3) *Valle dei Nobili*, at 10-130 m a.s.l. Three pools along the stream *Fosso della Valle dei Nobili* and two isolated pools. Volume range = 2198-54430 cm<sup>3</sup>; LD<sub>2014</sub> = 6.42 n/dm<sup>3</sup>.

4) *Fosso di Valle del Pentovaldo and Fosso del Santo*. A small isolated pool (about 419 cm<sup>3</sup>) with a very high LD<sub>2014</sub> 95.54 n/dm<sup>3</sup> and a pool with lentic water (12500 cm<sup>3</sup>) with LD<sub>2014</sub> = 2.39 n/dm<sup>3</sup>.

5) *Fosso di Valle di San Giorgio*. A pool (10990 cm<sup>3</sup>) with slow current and LD<sub>2014</sub> = 0.91 n/dm<sup>3</sup>.

6) Along the stream *Fosso di Valle dei Mulini*, at 260 m a.s.l. (data not included in the analyses).

In 2014, we found three isolated pools (vol. range = 628 - 6300 cm<sup>3</sup>) with eggs and newly

hatched larvae with very high density, and one big pool (about 2 m<sup>3</sup>) with lotic water and  $LD_{2014} = 0.014$  n/dm<sup>3</sup>; in 2019 only one small cavity with newly hatched larvae (1570 cm<sup>3</sup>).

7) *Fosso del Dobbiarello*. A single pond with lentic water (471000 cm<sup>3</sup>), in the final part of the stream with  $LD_{2014} = 3.06$  n/dm<sup>3</sup>. The stream is interrupted by a road and a tourist village built behind the beach.

8) *Fonte del Prete*, at 35 - 53 m a.s.l. Six isolated pools with lentic water (vol. range: 628 - 94000 cm<sup>3</sup>) within an area of about 2 ha. Very variable density,  $LD_{2014} = 8.61 \pm 13.57$  n/dm<sup>3</sup>.

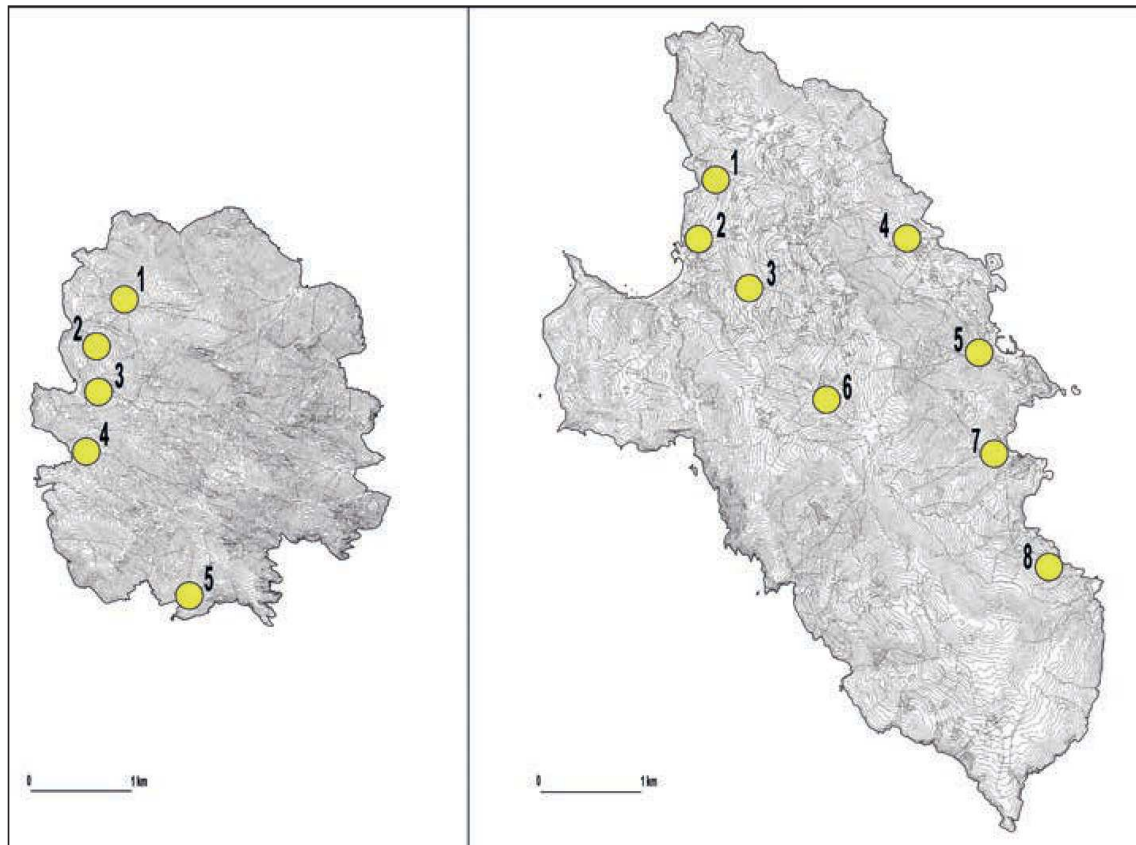


Fig. 1 — Localisation of the study sites for density estimation of *Discoglossus sardus* on Montecristo (left) and Giglio (right) islands (an approximate point is indicated for surveys along tracks or stream). Numbers of sites correspond to the ones listed in Table 1.

## RESULTS

In Table 1, we provide a brief description of the breeding sites surveyed in 2014 and 2019 along with the estimation of *D. sardus* larvae density. On Giglio island, larvae density (not differing between years:  $n_{2014} = 22$ ,  $n_{2019} = 12$ ,  $U = 121$ ,  $p = 0.705$ ) increased with decreasing pool size ( $n = 34$ , Wald stat. = 7.249,  $p = 0.007$ ), but it did not vary among isolated pools ( $n = 16$ ) and pools with water supply ( $n = 18$ ; Wald stat. = 1.276,  $p = 0.259$ ). On Montecristo, in 2014, we got analogous results, with a significant (negative) influ-

ence of pool volume on larvae density ( $n = 31$ , Wald stat. = 5342,  $p = 0.0208$ ), and no significant differences among pools with ( $n = 15$ ) and without ( $n = 16$ ) water supply (Wald stat. = 5.342,  $p = 0.120$ ); in 2019, no correlation between larvae density and pool volume was found ( $n = 17$ ,  $r = -0.328$ ,  $p = 0.198$ ).

On Montecristo, breeding sites ( $n = 8$ ) were characterized by salinity = 0.01 – 0.02 ‰;  $\text{pH} = 7.49 \pm 1.19$ ; dissolved oxygen =  $8.8 \pm 1.81$  mg/L (recorded at a mean water temperature of  $15.55 \pm 2.08$  °C). On Giglio island ( $n = 6$ ), we measured comparable values for the three variables: salinity = 0.02 - 0.05 ‰;  $\text{pH} = 7.48 \pm 0.75$ ; dissolved oxygen =  $9.66 \pm 1.91$  mg/L (at a mean water temperature of  $14.23 \pm 1.26$  °C).

The size (SVL) and body mass recorded for *D. sardus* on Montecristo were  $49.54 \pm 7.84$  mm and  $13.18 \pm 7.27$  g in males ( $n = 23$ ),  $43.02 \pm 7.43$  mm and  $7.30 \pm 3.01$  g in females ( $n = 10$ ),  $34.22 \pm 5.12$  mm and  $3.64 \pm 1.33$  g in five sub-adults. On Giglio, we measured slightly bigger males with SVL of  $60.3 \pm 9.51$  mm and body mass of  $26.27 \pm 12.56$  g ( $n = 11$ ), and one female 36 mm long and 4.69 g heavy.

## DISCUSSION

On Montecristo and Giglio, *Discoglossus sardus* spawns in pools formed in the granitic rocks, isolated or connected with each other along streams or small creeks, the first ones being characterized by no water supply, and the second ones by very slow running water. The water bodies where *D. sardus* larvae occurred were mostly characterized by small size, shallow clear water and scarce or absent vegetation, which makes it possible to count larvae with good accuracy (BOSCH *et al.*, 2018).

Larvae density did not significantly vary when comparing pools characterised by lentic and lotic (slow) water, while it increased with decreasing volume of water bodies (larvae were found even in tiny cavities in the rock, Table 1), except for data gathered on Montecristo in 2019 when, however, the scarce availability of breeding sites, mostly dried up, resulted in a smaller small sample size. On this island, during both the years of sampling, we noticed that the spawning activity across sites was slightly differentiated in time according to the different persistence of water. An earlier spawning activity occurred at Cala Santa Maria where most pools are subject to early desiccation while at Cala Maestra, where water persists late in the breeding season, *D. sardus* spawning activity seems to be delayed (and prolonged).

On Giglio, *D. sardus* was observed also in artificial sites (not included in the analyses), such as small wells and water tanks inside cultivated plots or vegetable gardens. The abandonment of traditional cultivation, made up of

tiny land ownings and related structures for the collection of water, is one of the possible menaces for the species on the island, along with the increase of intensive cultivation (e.g., vineyards). Further causes of habitat loss and degradation on Giglio may be associated to the increase of buildings and infrastructures, often related to tourism and to water abstraction (related to agriculture but also to private gardens management) and pollution, as addressed also by LANZA *et al.* (2009) and GIOVACCHINI *et al.* (2015). On Montecristo, due to its protection status, no human activities are performed except for a low impact tourism. On both islands, we repeatedly observed dead adult females in water with no detectable signs of predation attempts or cutaneous abnormalities, which may suggest that deaths could be associated with mating (lumbar amplexus) as observed in other amphibians (e.g., KAGARISE SHERMAN & MORTON, 1993).

*Acknowledgements* — We thank M. Raffa and E. Paggetti for helping during field work. Research activity was allowed by *Parco Nazionale dell'Arcipelago Toscano* and *Ministero dell'Ambiente e della Tutela del Territorio e del Mare* (Prot. 0044068–04/12/2012–PNM II; 67681-27/11/2018; 0011888-24.05.2019).

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