



Reproductive phenology and environmental temperatures in the Smooth Newt *Lissotriton vulgaris meridionalis* (Boulenger, 1882), (*Amphibia*, *Urodela*) in a Mediterranean habitat

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Abstract— The present work aims to contribute to the knowledge of the influence of environmental temperatures on the reproductive dynamics of the population of the Smooth Newt Lissotriton vulgaris meridionalis (Boulenger, 1882) at "Bosco di Palo" Natural Park (north of Rome, Central Italy). The data collection took place for a long – term investigation from the breeding season of 1995 – 1996 until the breeding season 2014 - 2015 (with the exception of the seasons 2003 - 2004, 2004 - 2005 and 2005 - 2006); the breeding season is considered the beginning of the filling of temporary ponds until they are completely drained. Samples were taken every fifteen days. During the breeding seasons investigated, the greatest concentration of individuals in water occurs in the period between January and May. The maximum presence of newts in water occurs with a maximum temperature range that oscillates from $14 \,^{\circ}C$ to $17 \,^{\circ}C$ and a minimum temperature that oscillates from $3 \,^{\circ}C$ to $7 \,^{\circ}C$. From the analysis of the data it would therefore emerge that the minimum atmospheric temperature may constitute one of the most significant environmental parameter for the reproductive phase of the Smooth Newt. This data agrees with the observations reported in the present work and suggests that the identified trend can be extended to populations of Mediterranean environments.



Keywords— Lissotriton vulgaris, long-term study, Mediterranean habitat, reproductive phenology, temperature.

I. INTRODUCTION

The present work aims to contribute to the knowledge of the influence of environmental temperatures on the reproductive dynamics of the population of the Smooth Newt *Lissotriton vulgaris meridionalis* (Boulenger, 1882) at "Bosco di Palo" Natural Park.

The smooth newt *Lissotriton vulgaris meridionalis* is a Urodelian Amphibian distributed in the Italian peninsula, with the exclusion of the southern regions (RAZZETTI & BERNINI, 2006). Its ecology, in the Mediterranean, is strictly influenced by environmental parameters. The vitality of populations is closely linked to the conservation of small wetlands, often temporary, which allow egg deposition and larval development (BELL & LAWTON, 1975; ACCORDI & NOBILI, 1999; PIZZUTI PICCOLI, 2008; PIZZUTI PICCOLI, 2017).

The newt reproduces both in temporary and perennial waters (ponds, lakes, fountains), never in flowing waters (BELL, 1977; RAZZETTI & BERNINI, 2006). Given the absence of fish, temporary ponds have the advantage of significantly reducing the number of predators present. Temporary ponds, however, are extremely unpredictable habitats, and premature drying can often destroy an entire generation of larvae. Metamorphosed individuals spend

about two years in the undergrowth before reaching sexual maturity and returning to breed in ponds. The adults live a terrestrial life outside the breeding season (GRIFFITHS, 1984).

In the "Bosco di Palo" Natural Park, the populations of *Lissotriton vulgaris meridionalis* have been monitored since 1995 and we have seen how their state and reproductive biology are closely related to rainfall, temperatures and the seasonal filling of temporary ponds (PIZZUTI PICCOLI, 2008; PIZZUTI PICCOLI, 2010).

In recent decades, amphibian populations in the world have shown a sharp decline due to global warming and the reduction of rainfall in many sites of the distribution range (METTOURIS *et al.*, 2017; PIZZUTI PICCOLI, 2017).

The study aims to verify, through long-term observations, whether it is possible to identify an optimal temperature range for reproduction of the Smooth Newt in Mediterranean habitat.

II. STUDY AREA

The "Bosco di Palo" Natural Park (Fig. 1) is located 37 km to the north of Rome (Central Italy - IGM Topographic Map Sheet 149 NE IV) and is situated between the sea and the Via Aurelia in locality of Palo Laziale, in the town of Ladispoli (41 $^{\circ}$ 56 'N, 12 $^{\circ}$ 05 'E). The study area is part of a narrow coastal plain that extends from the delta of the Tiber River and that was formed during the Quaternary period (BONO *et al.*, 1993).



Fig.1: The "Bosco di Palo" Natural Park.

The territory was divided into three longitudinal strips parallel to the sea, a band made up of silt deposits and marshy black lands, an intermediate band characterized by ancient fossil dunes and a third more recent band formed by coastal dune and beach (currently in strong erosion). The soil wooded area is characterized by clay. The climate is part of the type mesomediterranean with mild winter, a summer period of about three months of dryness and rainfall regime of maritime type (BLASI 1994; BLASI 2018).

ISSN: 2456-1878 (Int. J. Environ. Agric. Biotech.) https://dx.doi.org/10.22161/ijeab.86.26 The environments that we find in the Park are the Mediterranean scrub, planitial wood and grassland. The planitial wood, characterized by the presence of temporary ponds, consists of a mixed forest of deciduous oaks of about 60 hectares, with the dominance of *Quercus ilex* L., *Quercus cerris* L., *Quercus pubescens* Willd. and *Ulmus minor* Miller (LUCCHESE, 1990; LA MONTAGNA *et al.*, 2023).

The amphibians of the study area are represented by four species: *Bufo bufo* (Linnaeus, 1758), *Hyla intermedia* Boulenger, 1882, *Pelophylax bergeri* (*Günther*, 1986) / *Pelophylax klepton hispanicus* (*Bonaparte*, 1839) and *Lissotriton vulgaris meridionalis* (Boulenger, 1882) (PIZZUTI PICCOLI, 2008).

The temporary ponds are temporary water collections whose depth varies between 20 and 150 cm. These environments are extremely precarious because they are influenced by the seasonal weather patterns. Because of the shallow, thermal stratification is absent; the temperature of the water, from surface to bottom, is under the direct influence of the sun and reflects the seasonal and daily variations in air temperature, even if it remains always few degrees below respect to it. The ponds undergo a drying period, from June to September, and freezing at the surface for few days during negative peaks of temperatures in the months of January and February. The oxygen concentration is subject to daily and annual fluctuations and also varies vertically; it is higher in surface for the presence of photosynthetic organisms and less abundant on the bottom for the presence of organisms decomposers. The water pH decreases with the onset of warm weather (GATTA, 1990; MURA & BRECCIAROLI, 2003). The bottom of the ponds is characterized by a strong decomposing activity; the half-submerged trees growing around the ponds and directly into the water (mainly Fraxinus oxycarpa Bieb.) release a considerable mass of leaves on the bottom of ponds. Within the ponds, the vegetation is very scarce and characterized by terrestrial grasses that withstand periods of immersion.

For the research were chosen three ponds in the wood that have the following characteristics: Pond 1, called "pond of *Emys*", with a maximum diameter of 20 m, a maximum area of 62.8 sq. m. and a maximum depth of 120 cm; Pond 2, called "pond of newts", with a maximum diameter of 4 m, maximum area of 12.56 sq. m. and a maximum depth of 81 cm; Pond 3, called "pond of reeds", with a diameter of 22 m, maximum area of 69 sq. m. with a maximum depth of 83 cm. The Pond 3 is characterized by the coverage of rushes, *Juncus sp.* and *Typha sp.* in about a third of the surface (LUCCHESE, 1990; LA MONTAGNA *et al.*, 2023).

III. MATERIAL AND METHODS

The data collection took place from the breeding season of 1995 - 1996 until the breeding season 2014 - 2015 (with the exception of the seasons 2003 - 2004, 2004 - 2005 and 2005 - 2006, in which the study was temporarily interrupted); the breeding season is considered the beginning of the filling of temporary ponds until they are completely drained. Samples were taken every fifteen days.

The meteorological data (maximum and minimum daily temperature, rainfall) were provided by the Survey Station of the Ministry of Agricultural and Forestry Resources present in the "Bosco di Palo" Natural Park and, subsequently, since 2006, by the Survey Station of the Ministry of Agricultural and Forestry Resources of Massimina (Rome).

The capture of the specimens was performed by dipnetting, according to pre-established transects, by using a net square shape with side of 36 sq. cm, with square mesh of 0.5 cm side.

For each sampling has been established dipnetting mode according to the size of the pond (HEYER, 1988); in the "pond of reeds" the research was carried out with an average of 80 dipnetting for sampling, in the "pond of *Emys*" the research was carried out with an average 80 dipnetting for sampling and in the "pond of newts" the research was carried out with an average of 30 dipnetting for sampling.

In each season the specimens were marked through photos of the ventral pattern and was made an estimation of the population density applying the Lincoln -Petersen Method modified by Bailey, suitable for small populations of temporary ponds (ACCORDI & NOBILI, 1999; PIZZUTI PICCOLI, 2017); Bailey's modification is thought to yield a better estimate when sample size is small (less than circa 20) (BAILEY, 1951; GREENWOOD & ROBINSON, 2006).

The field monitoring was conducted in accordance with applicable laws and authorizations provided for this kind of studies. Handling of individuals was made in compliance with the standards necessary to prevent transmission of pathogens between individuals (RAZZETTI & BONINI, 2001; STOCH & GENOVESI, 2016).

IV. RESULTS

The graphs in Fig. 2, Fig. 3 and Fig. 4 show the data relating to the comparison between the maximum and minimum daily temperatures and the average number of newt captures in the study period for each sampling day. The garph refers to the total number of newts captured and to the male and female specimens of the population. In the breeding seasons monitored, the presence of water in the pools since autumn has led to the presence of the animals for an average long period (with presences also in autumn) even if the capture frequencies have a spring maximum in the months of March and April. In some seasons, the entry into the water occurs concentrated in a short time if the water in the pools forms late (late winter). The exit from the water generally always occurs in a short time at the end of spring.

In TABLE 1 are shown the number of individuals caught per breeding season and the population size estimated for the site. The data produced can be considered an underestimation of the population of "Bosco di Palo" for the presence of other breeding sites besides those investigated, certainly has been identified the range size of the population.



Fig.2: Daily mean of total specimens captured in the study seasons at the minimum temperatures recorded.



Fig.3: Daily mean of total specimens captured in the study seasons at the maximum temperatures recorded.



Fig.4: Daily mean of male and female specimens captured in the study seasons at the minimum temperatures recorded.

Breeding season	Number of caught individuals	Males	Females	Male percentage	Female percentage	Population estimate (numbers of individuals)
1995 – 1996	100	43	57	43%	57%	257
1996 – 1997	172	52	120	30%	70%	246
1997 – 1998	80	29	51	36%	64%	221
1998 – 1999	145	52	92	48%	52%	267
1999 - 2000	131	61	70	46%	54%	329

Table 1. The data of the breeding seasons from 1995 – 1996 to 2014 – 2015.

2000 - 2001	104	53	51	51%	49%	295
2001 - 2002	114	55	59	48%	52%	387
2002 - 2003	134	47	87	35%	65%	256
2003 - 2004	-	-	-	-	-	-
2004 - 2005	-	-	-	-	-	-
2005 - 2006	-	-	-	-	-	-
2006 - 2007	154	51	103	33%	67%	294
2007 - 2008	165	63	102	38%	62%	232
2008 - 2009	136	56	80	41%	59%	267
2009 - 2010	145	63	82	45%	55%	360
2010 - 2011	109	46	63	42%	58%	342
2011 - 2012	130	48	82	37%	63%	259
2012 - 2013	112	52	60	46%	54%	212
2013 - 2014	147	71	76	48%	52%	324
2014 - 2015	138	50	88	36%	64%	287

V. DISCUSSION AND CONCLUSIONS

During the breeding seasons investigated, starting from the 1995-1996 season, the greatest concentration of individuals in water occurs in the period between January and May. The maximum presence of newts in water occurs with a maximum temperature range that oscillates from 14 °C to 17 °C and a minimum temperature that oscillates from 3 °C to 7 °C.



Fig.5: Significant correlations between the total abundance of smooth newts in the ponds and the minimum air temperature in the reproductive seasons.

The aggregated abundance data for the whole reproductive seasons show that the only covariate really negatively influencing the abundance of the smooth newts in the ponds was the minimum temperature (Spearman's rho = -0.307, P = 0.022) (Fig. 5). All the other covariates showed no significant association with the abundance of *ISSN:* 2456-1878 (Int. J. Environ. Agric. Biotech.) https://dx.doi.org/10.22161/ijeab.86.26 newts (P always > 0.05). On the other hand, while a negative correlation was observed between female abundance and both mean (Spearman's rho = -0.279, P = 0.039) and minimum temperatures (Spearman's rho = -0.353, P = 0.008) (Fig. 6 and Fig. 7), abundance of males was not significantly correlated with any covariate.



Fig.6: Significant correlations between the female abundance in the ponds and the minimum air temperature in the reproductive seasons.

From the analysis of the data it would therefore emerge that the minimum atmospheric temperature may constitute one of the most significant environmental parameter for the reproductive phase of the Smooth Newt.

This trend was already highlighted in previous studies on the population of the "Bosco di Palo" Natural

Park (PIZZUTI PICCOLI, 2008); the strong point of the present study consists precisely in the long-term study of the population and its reproductive phenology as a function of the temperatures recorded.



Fig.7: Significant correlations between the female abundance in the ponds and the mean air temperature in the reproductive seasons.

Furthermore in a study carried out in a site about 20 km away from the study area, from 2006 to 2008, it is indicated between 1°C and 11°C the minimum temperature range most suitable for the reproductive phase of the Smoth Newts. The maximum peak of presence of individuals in water occurs with a maximum temperature of 13.2°C and a minimum temperature of 6.9°C (DI GIUSEPPE, 2012).

This data agrees with the observations reported in the present work and suggests that the identified trend can be extended to populations of Mediterranean environments.

In conclusion, it is believed that the present work, based on a long-term study of the population of *Lissotriton vulgaris meridionalis*, can constitute an important contribution in defining the optimal environmental parameters for the reproductive phenology in a Mediterranean environment and can be of support in actions of conservation of the species.

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