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# Breeding phenology of Bufo viridis Laurenti, 1768 in Sicily

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**Abstract.** *Bufo viridis* Laurenti, 1768 is a common species that inhabits a wide variety of habitats. The different climates characterising its broad range lead to a high degree of variability in its seasonal activity and reproductive cycle. This paper reports some observations carried out on the breeding phenology of this species over a two year period in Mediterranean temporary ponds in Sicily. The reproductive period of Sicilian green toads extends into the autumn months, making it longer than that of other Italian populations. This behaviour seems due to the impact of xeric environmental conditions on the seasonal activity of the studied populations. The present study confirms that *B. viridis* is an opportunistic breeder with a wide margin of variability in annual reproductive cycle patterns, as would be expected of an ecologically variable species. The duration of the reproductive season varied between populations in the same year and between different years for the same population.

Keywords. Amphibian, *Bufo viridis*, phenology, autumn breeding, Mediterranean environments, Sicily.

# INTRODUCTION

*Bufo viridis* Laurenti, 1768 is a widespread species with a range which extends from eastern France and Italy to central Asia, including northern Africa and numerous Mediterranean islands (Bologna and Giacoma, 2006). Because of the high morphological variability of the green toad, several forms, as species and subspecies, have been described within its extensive range (Stöck et al., 2001). In Italy seems to be present the nominate subspecies (Bologna and Giacoma, 2006); however, the taxonomical status of the green toad has not so far been clearly assessed, and further investigations are needed (Roth, 1986; Balletto et al., 2000; Stöck et al., 2001).

The species inhabits a wide variety of habitats, from mesic to arid, from subtropical to cold temperate, and from below sea level in Israel to more than 4000 m a.s.l. in the Hima-

layas (Dessauer et al., 1975). It is common along coasts, due to its ability to survive and breed in brackish waters (Lanza, 1983). Its typical reproductive sites are temporary and shallow water bodies (Roth, 1997; Bologna and Giacoma, 2006).

The variety of climates covered by its broad range gives rise to high degree of variability in the seasonal activity and reproductive cycle of the green toad (Giacoma et al., 2000). Breeding periods observed for populations from continental Italy and Sardinia are generally comprised between the end of February and the end of August, with a maximum length of about three and a half months. A longer reproductive period, extending into the autumn months, was discovered for a population found near Palermo (Capo Gallo, La Fossa) in a preliminary study carried over three years from 2000 to 2002 (Sicilia, 2006).

Our paper reports further observations on the reproductive activity and the duration of larval development in the La Fossa and Ustica Island (province of Palermo) populations and analyses the differences between these and other Italian populations, in order to verify previous observations and to better understand the breeding phenology of *B. viridis* in Sicily.

## MATERIALS AND METHODS

#### Study area

Sicily has a Mediterranean climate with mild winters and long, dry summers, characterised by unpredictable rains. The plain areas of the Island and some circum-Sicilian islands are arid or semiarid in terms of De Martonne and Gottmann's aridity index. The west coast and central-southern areas are arid according to Lang's Rain Factor (Duro et al., 1997).

Its insularity, geographic position, orography, the nature of its soils and environmental degradation preclude Sicily having a hydrographic system like that of mainland Italy. Sicilian freshwater environments are unstable, with wide seasonal oscillations (Riggio, 1978) and most natural freshwater ecosystems are temporary. The breeding sites of *B. viridis* studied in this research are temporary water bodies located in the NW part of the Island.

La Fossa is located in the Capo Gallo Nature Reserve, on the coast in the outskirts of Palermo (Fig. 1). The only reproductive site of *B. viridis* in La Fossa is a small concrete basin with a surface area of about 40 m<sup>2</sup> and a maximum depth of 80 cm, located about 50 m from the shore-line at sea level ( $38^{\circ}12'45''N$ ,  $13^{\circ}17'30''E$ ). The basin is only filled by rainfall and due to underlying calcareous soil, the climate and the presence of a leak, is subject to frequent drying up. The area has an average rainfall of 680 mm per year and an average temperature of 17 °C (Zampino et al., 1997a). The vegetation surrounding this site consists of degraded Mediterranean scrub. No other amphibian species are present in the area.

Ustica is a volcanic island about 9 km<sup>2</sup> wide located in the southern Tyrrhenian Sea, 60 km north of Palermo (Fig. 1). Precipitation is scarce, with rainfall averaging 320 mm per year. Average temperature is 17.1 °C (Zampino et al., 1997b). Because of the climate and the permeability of the substratum, a permanent surface hydrological network does not exist on the island. Occasional rain water concentrates in some less permeable depressions locally called "gorghi" (Buffa and di Palma, 1990). Our observations concern two of these reproductive sites of *B. viridis*, the only amphibian species present on Ustica Island.

The first, Gorgo Salato  $(38^{\circ}43'07"N, 13^{\circ}10'35"E)$ , is a temporary pond with unpredictable hydrology, about 150 m<sup>2</sup> wide and with a maximum depth of 50 cm. It is about 30 m from the shore-line and about 20 m a.s.l. The other, Gorgo San Bartolicchio  $(38^{\circ}42'00"N, 13^{\circ}10'21"E)$ , located



Fig. 1. Study area.

at 100 m a.s.l., is a concrete basin built in the depression of a natural temporary pond. It fills up partially to reach a maximum surface area of about 100  $m^2$  and a maximum depth of 60 cm. The landscape surrounding the water bodies consists of cultivations and grassland. The two ponds are about 3 km apart.

# Sampling

The data on the breeding phenology of *B. viridis* populations of La Fossa and Ustica Island are based on field observations carried out from September 2002 to August 2004. Samplings were performed twice weekly, once weekly or fortnightly at La Fossa, and fortnightly on Ustica Island. Male calling activity, pairs, eggs, embryos and toadlets were recorded. Male calling activity and pairs were observed from sunset to 12 pm over a surface area of about 1500 m<sup>2</sup> which included the water bodies and their neighbouring area. The presence of eggs and embryos was detected by means of a hand net during the daytime. The presence of toadlets was checked in both diurnal and nocturnal surveys. On some occasions sampling allowed the timing of metamorphosis to be determined.

Air and water temperatures (°C) were measured at 10 pm on every nocturnal visit, and information on the hydrological phases of the water bodies was recorded. Occasional observations were carried out at the reproductive sites of La Fossa and Coda di Volpe (another concrete basin near Palermo, at 310 m a.s.l.) during August, September and October 2005 to detect possible spawning events.

## RESULTS

Variation in maximum depth of the La Fossa basin during the two years of this study can be seen in Figs. 2 and 3, which show the hydroperiods and frequent drying up of the basin. The peak in depth shown for the second half of May 2004 was due to management of the Nature Reserve artificially filling up the basin.

Reproductive period results for La Fossa are shown in Table 1. Eggs and tadpoles often did not complete development because of drying up of the water body. No toadlets were seen during the first year of study, while during the second year they were observed in Octo-

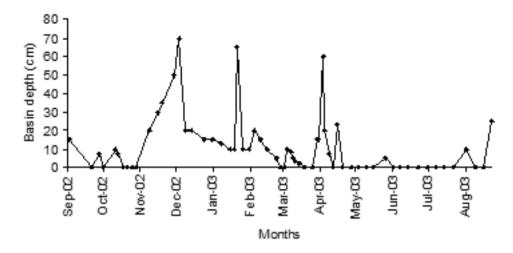


Fig. 2. Variation of maximum depth of La Fossa basin during the first year of survey.

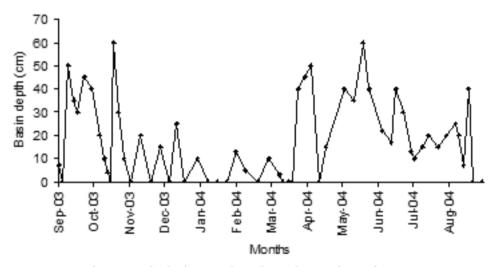


Fig. 3. Variation of maximum depth of La Fossa basin during the second year of survey.

ber 2003 and during June, July and August 2004 only thanks to artificial filling of the water body. In the metamorphosis event observed in October 2003, development took 33 days. A spawning event observed in August 2005 was followed by metamorphosis after 27 days.

Observations from the Ustica sites are shown in Tables 2 and 3. As in La Fossa, reproductive events in Gorgo Salato were often not successful due to frequent drying up of the pond. Sampling on Ustica probably was not frequent enough to detect all spawning events and toadlets, however.

	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
	2002	2002	2002	2002	2003	2003	2003	2003	2003	2003	2003	2003
Calling activity							х	х	х			х
Pairs						х	х	х				х
Eggs/Embryos	х						х	х				х
Toadlets												
	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
	2003	2003	2003	2003	2004	2004	2004	2004	2004	2004	2004	2004
Calling activity	х					х	х	х	х	х		
Pairs	х				х	х	х	х	х	х		
Eggs/Embryos	х						х	x	х			
Toadlets		х								х	х	x

Table 1. Breeding phenology of *Bufo viridis* at La Fossa (Palermo).

Table 2. Breeding phenology of Bufo viridis at Gorgo Salato (Ustica Island, Palermo).

	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
	2002	2002	2002	2002	2003	2003	2003	2003	2003	2003	2003	2003
Calling activity		x		x		x		x				
Pairs		x	x	x	x	x	x					
Eggs/Embryos	x	x	x	x	x	x	x		x			
Toadlets									x			
	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
	2003	2003	2003	2003	2004	2004	2004	2004	2004	2004	2004	2004
Calling activity	х				х	х			х			
Pairs	х	х			x		x	x	x			
Eggs/Embryos		x					x					
Toadlets												

Table 3. Breeding phenology of Bufo viridis at Gorgo San Bartolicchio (Ustica Island, Palermo).

	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
	2002	2002	2002	2002	2003	2003	2003	2003	2003	2003	2003	2003
Calling activity		x						x				
Pairs		x	x	x	x		x					
Eggs/Embryos		x		x	x	x	x		x			х
Toadlets				x			x					
	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
	2003	2003	2003	2003	2004	2004	2004	2004	2004	2004	2004	2004
Calling activity	х						х	х	х			
Pairs	х	x	x				x	x	x			
Eggs/Embryos	х	x	x			x	x	x				х
Toadlets							x					

In all the study sites, although the toads' breeding activity occurred for several consecutive months, it was not continuous and characterized by many irregularly distanced spawning events. In some cases, the presence of pairs and male calling activity represented only an attempt at reproduction and were not followed by spawning.

The minimum air temperatures at which breeding activity was observed around the reproductive site was 7.6 °C at La Fossa and 7.4 °C on Ustica; while the maximum values recorded during breeding activity were 29.4 °C at La Fossa and 23 °C on Ustica. The minimum water temperature at which pairs were observed in the ponds was 6.5 °C on Ustica. *B. viridis* eggs were observed in the Coda di Volpe basin in September and October 2005.

### DISCUSSION

Lanza (1983) reported that the reproductive season of *Bufo viridis* in Italy ranges from March to August. In a more recent review of data from several Italian populations, Castellano et al. (2000) report that the breeding period starts between the end of February and the end of March depending on the latitude, and lasts from two weeks at Amendolea (province of Reggio Calabria) in Calabria to three and a half months at Spotorno (province of Savona) in Liguria. According to these authors, reproductive activity is contingent on a combination of temperature and rainfall. A study carried out on 163 green toad reproductive sites in Calabria (S Italy) by Tripepi et al. (2000) and other information reported for Italian populations confirm this cycle of annual breeding activity (Vandoni, 1914; Delmastro, 1994; Aprea, 1996; Lapini et al., 1999; Bologna, 2000; Farinello and Del Cengio, 2000; Caldonazzi et al., 2002; Falcioni and Poggiani, 2003; Furlani and Fiacchini, 2003; Bologna and Giacoma, 2006). Only in Lombardy reproduction was occasionally observed at the beginning of September: usually it takes place between April and June (Bonini and Bressi, 2004).

The breeding season of the Sicilian populations studied is longer than that observed in other Italian populations, covering a maximum of seven non-consecutive months at La Fossa during the second year of this study, and nine months on Ustica Island during the first year. Reproductive activity generally took place in all months except July, the least rainy month of the year in the areas studied (Zampino et al., 1997a, 1997b); spawning events occurred in all months except June and July. This seems in contrast with Castellano et al. (2000), according to whom the activity cycle of *B. viridis* can be divided into three phases: winter latency, the reproductive period and post-reproductive period. The authors maintain that in Italian populations, winter latency terminates between the end of February and the end of March, when the reproductive period begins (Castellano et al., 2000).

Studies on several amphibian populations have shown that they can modify their reproductive patterns in Mediterranean environmental conditions. In sub-Mediterranean areas, *Triturus carnifex* extends the length of its breeding period (Cvetkovic et al., 1996). Jakob et al. (2003) investigated reproductive strategies in an amphibian community that breeds in hydrologically unpredictable ponds in southern France and found that some species (*Pelobates cultripes, Pelodytes punctatus, Hyla meridionalis, Rana perezi*) exhibit flexibility in timing their breeding periods, a strategy which the authors hypothesize

ensures more reliable breeding success. *P. cultripes* and *P. punctatus* were found to breed in autumn as well as in spring: autumn reproduction is known of only in Mediterranean populations of these species (Salvidio and Quero, 1987; Sindaco and Andreone, 1988; Diaz-Paniagua, 1992; Jakob et al., 2003). *Hyla arborea, Rana ridibunda, R. dalmatina* and *B. viridis* in Greece exhibit differences compared to the same species in northern Europe in several aspects of their reproductive strategies, especially length of breeding season. For example, the spawning season of *B. viridis* starts at the end of February or in the first week of March and ends at the beginning of July, lasting for 4.5 months, while in the middle European countries the reproductive period shows the shorter length of 3 months (Kyriakopoulou-Sklavounou, 2000).

Mediterranean conditions also affect the length of reproductive period in the studied Sicilian populations. Xeric environments, unpredictable rainfalls and the absence of severe winter temperatures are probably responsible for the extension of the period of breeding activity of the green toad. This adaptation may be very important to the survival of the populations, especially at La Fossa and Gorgo Salato where toads try to exploit every opportunity to attain successful reproduction. Indeed, if the reproductive effort were limited to a short period, the risk of failure would be high, as demonstrated by the continual loss of eggs and tadpoles due to the frequent drying up of the water bodies.

It is known that amphibians, particularly toads, can successfully inhabit dry environments characterized by the intermittent availability of water. Williams (1987) reported general adaptations of amphibians to dry areas to be: the use of temporary water bodies for reproduction, an indefinite breeding season, breeding behaviour cued by rainfall and rapid development of eggs and tadpoles; adaptations all shown by the populations of *B. viridis* we studied. Development from eggs to toadlets takes between one and a half to three months in previously observed Italian populations (Lanza, 1983; Aprea, 1996; Lapini et al., 1999; Tripepi et al., 1999; Farinello and Del Cengio, 2000; Falcioni and Poggiani, 2003; Furlani and Fiacchini, 2003; Bonini and Bressi, 2004), whereas at La Fossa we recorded a time of 27 days. Interestingly, a similarly short metamorphosis (21.1 to 31.8 days) was recorded as an adaptation to desert environments in temporary ponds in Egypt (Hussein and Darwish, 2000). However, it is important to note that the rate of larval development can be also affected by other environmental factors, such as temperature, scarce food and high population density (Hussein and Darwish, 2000).

Data reported for green toad populations in other dry environments show a shorter breeding season than in the Sicilian populations reported here; and there are no records of an autumn reproduction period. In Israel, *B. viridis* spawning mainly occurs from early February to May (Blaustein and Margalit, 1995; Degani and Kaplan, 1999). In North Africa mating lasts from the end of the winter to May (Schleich et al., 1996; Hussein and Darwish, 2000). Mertens (1929) concluded that there were two mating seasons (spring and late summer) on Djerba Island (central-southern Tunisia) (Mertens, 1929 in: Schleich et al., 1996). In reality, knowledge about the reproductive behaviour of the green toad in dry environments is probably incomplete and further studies are required: in another investigation, one of the authors of this study found tadpoles of *B. viridis* at stage 25 (according to development stage tables of Gosner, 1960) at the end of December 2004 in a *sebkha* on the extremely arid Kerkennah Islands (Tunisia). Observations carried out from 2004 to 2006 on the hydroperiod of this temporary *sebkha* suggest that the tadpoles observed in

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
2000	х	х	х	х	х					х	х	
2001		х	х	х	х							
2002		х	x	х	х			х	х			

Table 4. Spawning events of Bufo viridis at La Fossa (Palermo) during the triennium 2000-2002 (modified<br/>from Sicilia, 2006).

2004 were born from spawnings occurred in a period comprised between the autumn and the beginning of the winter season (A. Sicilia, pers. obs.).

It seems that not all Sicilian populations of *B. viridis* prolong their breeding season, or at least not every year. In the Gorgo di Santa Rosalia, another *B. viridis* breeding site located near Palermo (at 458 m a.s.l.), the 2000 and 2001 reproductive seasons started at the end of January and lasted 41 and 24 days respectively (Sicilia, 2006). This pond is a temporary water body which dries up only during summer months. In the same years spawning events, at La Fossa (Table 4), were recorded from January to May and during October and November (in 2000); and from February to May (in 2001). It is also worthy of note that the *B. viridis* reproductive period at La Fossa was shorter in 2001 than in other years and there was no autumn reproduction period (Sicilia, 2006).

Evidence of environmental variability also comes from the differences observed in reproductive patterns between Sicilian and southern Italian populations, e.g. those found in Calabria and Apulia (Tripepi et al., 2000; Frisenda, 2002). It is not clear whether this difference is due to lack of data or to different environmental conditions, although the latter is likely, given that in Calabria *B. viridis* prefers medium and large size water bodies such as rivers and lakes (Tripepi et al., 2000).

To conclude, this study confirms that this taxon is an opportunistic breeder with highly variable annual reproductive cycle patterns. In the populations studied in Sicily, the duration of the reproductive season varied between populations in the same year and between different years for the same population.

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