
NATURAL HISTORY NOTES

BUFO BUFO (Common toad): FUNGAL OUTBREAK. In species with complex life cycles, like amphibians, early developmental stages like eggs or larvae are the most vulnerable. Predators or pathogens may cause embryo mortalities close to 100% (Petranka & Kennedy, 1999). Episodes of high mortality due to unknown factors have been reported (Carrier & Beebe, 2003) but pathogens are likely causal within these occurrences (Blaustein & Kiesecker, 2002). Fungal infections such as chytridiomycosis (Berger *et al.*, 1998; Welldon *et al.*, 2004) or *Saprolegnia* infections (Blaustein *et al.*, 1994) are important factors causing embryonic mortality in amphibians. Mass mortalities associated with pathogens and chytridiomycosis have been reported in the Iberian Peninsula (Bosch *et al.*, 2001, Bosch & Martinez-Solano, 2006), but episodes of *Saprolegnia* infections have been described only recently (Fernandez-Beneitez *et al.*, 2008).

Bufo bufo reproduction has been monitored over the past decade in the Gándaras de Budiño e Ribeiras do Louro wetland. This wetland is included in the European network of protected places in Natura 2000. It is one of the last remnants of large wetland and gallery forest areas associated with the river Louro basin, which are threatened by industrial development (Ayres & Cordero, 2007). This area consists of a complex of wetlands, gallery forests (*Salix* sp., *Betula* sp., *Alnus* sp.), swamps, bogs, flooded areas, and artificial ponds created by extraction for clay. Reproduction of amphibians has been monitored in more detail during the last five years in these naturalized clay pits.

I monitored a number of clay pits 2-3 times per week, between January and March 2008, walking along the shore in order to detect egg-laying sites, and also to assess the impact of predation by breeding pairs of European otter (*Lutra lutra*). In the third week of January 2008, reproduction of *B. bufo* was detected. I found up to 100 strings in the clay pits during peak breeding. Due to the severe draught that Spain was suffering (the driest autumn-winter in the last 60 years [Xunta de Galicia, unpublished report]) there were few suitable places for egg-laying. Females were forced to spawn in shallow water, and in a variety of unusual places such as among floating pine

branches that drapped over the surface of water.

One week later I detected the first viability problems. Some strings began to desiccate due to a lack of rain and because they were attached to grass in shallow water that was less than 10 cm deep. Other clutches began to develop a fungal coat, with eggs failing to hatch correctly. At least 20% of the clutches failed to hatch, due to desiccation, fungal attack, or both causes. This may be an underestimation because some affected strings could pass unnoticed in places with difficult access; the clay pits have almost vertical banks in some places.

Strings affected by fungal attack presented a "black & white" coloration of eggs in the first stages, changing to a "cotton-like" appearance in the last stages of the pathogen's development. This cotton-like appearance is typical of Oomycete, *Saprolegnia* infection (Fernandez-Beneitez *et al.*, 2008). I detected clutches affected by this pathogen not only in not suitable egg-laying places, but also among suitable underwater vegetation, typical egg-laying areas for *B. bufo*.

This is the first time that I have detected such a large failure in the reproductive success of *B. bufo* in this area. In previous years less than 1% of the strings failed to develop tadpoles (Ayres, unpubl. data). This fact, combined with the impact of predation by Otter on breeding pairs of adults (Ayres & Garcia, 2007), could have a negative impact on the breeding population of *B. bufo* in this wetland.

Emergent pathogens such as *Saprolegnia* are known to be the origin of embryonic die-offs in many amphibians (Blaustein *et al.*, 1994; Kiesecker *et al.*, 2001), including mass die offs of the eggs of European species like Natterjack toad (*Bufo calamita*) and European common frog (*Rana temporaria*) (Banks & Beebe, 1988; Beattie *et al.*, 1991).

However, there are few reports about amphibian die-offs in the Iberian Peninsula from Oomycete attack. Recently an episode affecting *B. calamita* in Sierra de Gredos mountains, Central Spain was described (Fernandez-Beneitez *et al.*, 2008). Observations herein now present a new episode of egg mortality, involving a new species, *B. bufo*, in the NW of Spain at low altitude (30 m a.s.l.).

It is possible that climate-induced reductions in

water depth at oviposition sites may have increased mortality of embryos by increasing their exposure to UV-B radiation, and consequently, their vulnerability to infection (Kiesecker *et al.*, 2001). In Europe, the hatching success of *B. bufo* is generally lower for UV-B exposed eggs than for those shielded from UV-B (Lizana & Pedraza, 1998).

In the area studied it was the driest autumn in the last sixty years, with low water level, and virtually no typical vegetation to lay eggs among such as *Typha* sp. or *Potamogeton* sp. As a result females were forced to lay in 'unsuitable' places like pine branches over the surface of the ponds, that quickly desiccate due to the lack of rain. These unsuitable places likely increased the vulnerability to the eggs to fungal attack. Strings attached in deep water also suffered from fungal attack. Unfortunately no sample of the pathogen was identified to species level.

One species of *Saprolegnia* shown to be involved in natural infections is *Saprolegnia ferax* (Blaustein *et al.*, 1994; Kiesecker *et al.*, 2001). Fernandez-Beneitez *et al.* (2008) states, other species such as *Saprolegnia diclina* could also be responsible for die-offs in Spain. *Saprolegnia diclina-parasitica* complex was also determined as lethal to *R. temporaria* and *B. bufo* eggs under laboratory conditions (Robinson *et al.*, 2003).

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Submitted by: CESAR AYRES. R.O.A.G.A., Centro de Investigación e Información Ambiental -Estrada PO-546 Pontevedra-Marín, km. 4 Apdo. 127- C.P. 36080. Lourizán - Pontevedra, Spain. cesar@herpetologica.org



DIPSAS INDICA (Snail-eating snake): REPRODUCTION. The genus *Dipsas* is a well diversified taxa composed of nocturnal, semi-arboreal and malacophagous species (Greene, 1997) widely distributed in Central and South America (Peters & Orejas-Miranda, 1970). In Brazil eight species occur (SBH, 2008) and published reproductive data are restricted to half of them (Porto & Fernandes, 1996; Martins & Oliveira, 1999; Hartmann *et al.*, 2002; Alves *et al.*, 2005).

Herein, we present unpublished data about oviposition, hatching, clutch size, relative clutch mass (RCM) and size of hatchlings of *Dipsas indica*. This species is distributed in South America and five subspecies are recognized (Peters & Orejas-Miranda, 1970; Hoge & Romano, 1975).

On 10th November 2006, a gravid female *D. indica* with a snout-vent length (SVL) of 600 mm, tail length (TL) of 170 mm, and mass of 60.8 g was collected in Mogi Mirim (22°25'S, 46°57'W, 632 m), São Paulo State. On 17th November 2006 it was housed in 40 x 80 x 50 cm terraria, with a water dish, moistened soil, leaves and branches.

Until oviposition, veronicellid slugs were offered as prey. During this period the snake fed on two slugs with 2.5-3.0 cm. On 27th December 2006 it laid 5 eggs, being 2 weakly adhered and 3 separated. The snake was found under the leaves semi-coiled around the eggs. No behavioral changes of the female were observed during collection of the eggs.



Figure 1. Difference in color pattern of hatchling *Dipsas indica*. Upper: Female. Lower: Male.

Eggs averaged 29.2 ± 1.9 mm in length (range = 27.3-31.7 mm), 12.7 ± 0.4 mm in width (range = 12.2-13.2 mm) and 3.0 ± 0.1 g in mass (range = 2.8-3.1 g). Clutch mass was 14.9 g and after oviposition the female weighed 36.0 g. Relative clutch mass (RCM; total clutch mass/body mass of mother after oviposition + clutch mass; cf. Seigel & Fitch, [1984]) was 0.29. The RCM calculated according to Shine (1980) (total clutch mass/body mass of mother after oviposition) was 0.41. Four out of five eggs were incubated in a plastic container with moistened vermiculite, at room temperature averaging 24 °C (range = 22-26 °C). On 13th April 2007, three eggs hatched after an