

PREDATION ON AMPHIBIAN EGGS AND LARVAE IN TEMPORARY PONDS:
THE CASE OF *BUFO CALAMITA* IN SOUTHWESTERN SPAINAlexandre PORTHEAULT¹*, Carmen DÍAZ-PANIAGUA¹ & Carola GÓMEZ-RODRÍGUEZ¹

RÉSUMÉ. — *Prédation des œufs et des larves d'amphibiens dans les mares temporaires: le cas de Bufo calamita dans le sud-ouest de l'Espagne.* — Les œufs et les larves des bufonidés sont souvent désagréables ou toxiques. Nous avons évalué la vulnérabilité des œufs et des larves de *Bufo calamita* face à neuf prédateurs potentiels. L'ingestion n'a pas exercé d'effets toxiques ou désagréables sur la plupart des prédateurs, en revanche les petits prédateurs ont eu des difficultés pour extraire les œufs de la gélatine. Au laboratoire, les prédateurs les plus importantes ont été exercées par les larves de Pleurodèles de Waltl *Pleurodeles waltl* (Amphibiens, Salamandridés), les Triops *Triops cancriformis* (Crustacés Notostracés) et les Ecrevisses de Louisiane *Procambarus clarkii* (Décapodes, Cambaridés), espèce introduite. Le taux de prédation est lié à la taille des prédateurs. La prédation dans les mares de reproduction a affecté jusqu'à 95-100% des pontes, et le succès reproducteur n'a été constaté que dans les mares où ces trois prédateurs principaux sont absents. Tandis que d'autres espèces de *Bufo*, qui contiennent des substances toxiques, peuvent coloniser les habitats aquatiques permanents, chez *Bufo calamita* seule la gélatine compacte des œufs sert à décourager les petits prédateurs.

SUMMARY. — Eggs and larvae of bufonids are often unpalatable or toxic. We assessed the vulnerability of *Bufo calamita* eggs and larvae to nine potential predators. Ingestion did not exert toxic or distasteful effects on most predators, but small predators had difficulties extracting eggs from the jelly. In laboratory, the most intensive predation was by larvae of Sharp-ribbed Salamander *Pleurodeles waltl* (Amphibia, Salamandridae), the common crustacean *Triops cancriformis* (Crustacea Notostraca) and the introduced Red Swamp Crayfish *Procambarus clarkii* (Decapodes, Cambaridae). Predation rate was related to the body size of predators. Predation in breeding ponds reached up to 95-100% eggs, and breeding success was only recorded in ponds without the three major predators above. While other species of *Bufo* that contain toxic substances are able to colonize permanent aquatic habitats, for *Bufo calamita* only the compact jelly of eggs serves to deter small predators.

Eggs and larvae of most amphibians are vulnerable to a wide variety of predators. As a consequence of this predation pressure, amphibian species have developed defensive mechanisms, ranging from secretion of toxic substances which make eggs or larvae unpalatable or toxic to predators (Licht, 1968; Kats *et al.*, 1988; Gunzburger & Travis, 2005) to selection of specific breeding habitats such as temporary ponds in which key-predator species are absent (Snodgrass *et al.*, 2000; Semlitsch, 2002, 2003; Jakob *et al.*, 2003). In temporary aquatic habitats fishes are absent, while amphibians and some invertebrate species may become important predators. Identity, density and size distribution of predators are related to pond characteristics such as hydroperiod, and only species which exhibit plasticity in behaviour, life history or morphology may deal with the spatial or temporal variation in predator density within specific

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types of temporary ponds (Wellborn *et al.*, 1996). The complex life cycle of amphibians is completed in most species by breeding in temporary ponds, where eggs and larvae may develop in a productive medium in which predation is not as intense as in permanent ponds. In most species, their adaptation to the use of temporary ponds is evidenced by the wide flexibility in reproductive timing (Díaz-Paniagua, 1992), a wide variation of the length of the larval phase, and of the metamorphic size (Wilbur & Collins, 1973), as well as by the high fecundity and capability of sustaining occasional high annual offspring mortality.

In this study we analyse the predation pressure on amphibian eggs in temporary ponds. Predation is studied on the Natterjack Toad, *Bufo calamita*, a common anuran in Spain that mainly breeds in shallow, ephemeral pools during winter and spring (Díaz-Paniagua, 1988; Díaz-Paniagua *et al.*, 2005). Females lay long strings of about 2500 eggs at the pond edges, where vegetation is scarce or lacking and where eggs are widely exposed to the sun. Some authors have considered that eggs and larvae of this species, as in other bufonids, are unpalatable to certain predators (Banks & Beebee, 1988; Semlitsch & Gavasso, 1992), although predation by several species of aquatic predators has been documented (Beebee, 1979; Banks & Beebee, 1988; Denton & Beebee, 1991; Semlitsch & Gavasso, 1992). The aim of this study was to evaluate the importance of predation on the reproductive success of an amphibian species and to assess if the use of temporal habitats, where most predators are absent, may be a strategy to increase egg and larval survival. We examine whether *Bufo calamita* eggs and larvae are vulnerable to several species of potential predators that are frequently or occasionally found at their breeding sites and if the intensity of predation may explain the selection of breeding habitats and their requirements for successful breeding. We also evaluate the possible impact of two exotic species (*Procambarus clarkii* and *Gambusia holbrooki*) that were introduced to this area in past decades.

MATERIALS AND METHODS

STUDY AREA

This study was carried out in Doñana National Park (37°00'N, 6°38'W), southwestern Spain. This is a coastal sandy area with marshes, moving and stabilized dunes, and a wide area of stabilized sandy substrate on which large numbers of temporary ponds are usually filled by autumn or winter rains. These ponds are the breeding habitats of most amphibian species of this area (Díaz-Paniagua, 1988; Díaz-Paniagua *et al.*, 2005).

LABORATORY EXPERIMENT

Spawns of natterjack toads were collected in small temporary ponds in Doñana during the 2003 and 2004 breeding seasons (winter) and transported to our laboratory located within the Park. Individuals of nine species of potential predators commonly inhabiting temporary ponds of this area were also captured and separately transported to the laboratory. The potential predators were: a) insects: adults and larvae of aquatic beetles (*Cybister lateralmarginalis*), larvae of dragonflies (*Libellulidae* spp.); and backswimmers (*Notonecta glauca*); b) crustaceans: a common planktonic crustacean of temporary ponds (*Triops cancriformis*) and a very abundant exotic introduced crayfish (*Procambarus clarkii*); c) an introduced fish species (*Gambusia holbrooki*); d) urodele amphibians: adults of *Triturus pygmaeus* and *Lissotriton boscai* and larvae of *Pleurodeles waltl*. The frequency in which these predator species occur in ponds where *Bufo calamita* was observed breeding (as assessed from their presence in a total of 29 ponds) is presented in table I.

We made observations in laboratory to assess the vulnerability of *Bufo calamita* eggs and larvae to the different predator species. Predator-prey encounters were staged in plastic containers 18 x 18 x 12 cm filled with 2 litres of water. We introduced 30 eggs or larvae of *Bufo calamita* (10 eggs or larvae at the same developmental stage each from three different spawns) into each container. For each predator species, every day of experimentation we used six containers, introducing one individual predator in three experimental containers while the other three containers without predator were used as controls. All predators were starved 48 h before the experiment. We counted the number of eggs or larvae remaining in the tank after 24 h and after 48 h. We repeated the trials in different days for the same or other predator species; the number of tests made for each predator species is shown in table I. The experiments were made using eggs (embryos within their jelly capsule) at stages 3, 5, 10, 13 and 15 (Gosner, 1960) and larvae at stages 22 and 25 (Gosner, 1960). Larvae mostly remained motionless but free on the floor of the tanks. All eggs or larvae of the same trial were at the same stage. Body length of individual predators was recorded after every trial.

TABLE I

Presence of predators in breeding ponds of *Bufo calamita*, averages and standard deviations of body size of the predator species used in experiments and number of trials (number of times \times number of replicates) are shown.

Predators	Presence in ponds (%)	Body size (mm) (Mean \pm standard deviation)	Number of trials	
			Eggs (stage 5-13)	Larvae (stage 17-21)
<i>Gambusia holbrooki</i>	0.0	18.2 \pm 20.2	5 \times 3	7 \times 3
<i>Pleurodeles waltl</i> (larvae)	37.9	29.8 \pm 1.6	1 \times 3	1 \times 3
<i>Triturus pygmaeus</i>	69.0	42.4 \pm 3.1	7 \times 3	6 \times 3
<i>Lissotriton boscai</i>	58.6	29.0 \pm 2.5	6 \times 3	6 \times 3
<i>Procambarus clarkii</i>	20.7	42.6 \pm 15.2	5 \times 3	7 \times 3
<i>Triops cancriformis</i>	31.0	27.9 \pm 1.1	4 \times 3	3 \times 3
<i>Notonecta glauca</i> (larvae)	20.7	15.2 \pm 0.1	2 \times 3	1 \times 3
<i>Libellula</i> sp. (larvae)	41.4	16.3 \pm 1.5	2 \times 3	2 \times 3
<i>Cybister lateralimarginalis</i> (adults)	37.9	30.2 \pm 2.6	5 \times 3	7 \times 3
<i>Cybister lateralimarginalis</i> (larvae)	44.8	35.9 \pm 1.3	1 \times 3	1 \times 3
Any major predator species	68.9			
Four major predator species	13.8			
No major predator species	31.0			
No predators	20.7			

FIELD EXPERIMENT

From January to June 2004, we surveyed 29 ponds in Doñana National Park, 20 of them were monitored monthly, the remaining nine were monitored only once during the breeding season (in March 2004). In every survey, we counted all amphibians or potential predators captured with a dip-net at 12 points or more within the pond. We considered that *Bufo calamita* had bred successfully in a pond if we observed metamorphics or tadpoles in the last stages of development.

To monitor predation on *Bufo calamita* eggs, we selected four additional ponds where we detected eggs just after oviposition. In every pond we monitored three to five groups of three strings of eggs: a) one with total protection from predators with a rigid plastic wall; b) one spawn enclosed with a mesh of 1 cm-grid which did not allow access to *Procambarus clarkii*, *Triops cancriformis* and adult *Cybister lateralimarginalis*, but could be crossed by other predators; c) one unprotected spawn, which allowed access to all predators. We estimated the number of eggs consumed after 8-11 days.

STATISTICAL ANALYSES

For each predator species we used χ^2 tests to compare the total number of eggs or larvae remaining in tanks with predators versus the total number of eggs counted in control tanks after 24 or 48h. P values less than 0.05 were considered indicators of significant predation. Also the number of eggs and the number of larvae consumed per predator species were compared using χ^2 tests.

After checking normality and homogeneity of variances of body length of predators, we tested differences among two groups of predators (with predation rate larger or shorter than 50%) with ANOVA. Pearson correlation coefficients were calculated to analyse the relationship among predator size and their ingestion rate of eggs or larvae.

RESULTS

PREDATION ON EGGS AND LARVAE IN LABORATORY

Of the nine potential predators, only *Notonecta glauca* did not prey on any egg of *Bufo calamita*. Adults and larvae of *Cybister lateralimarginalis* and *Libellula* spp. ate a small number of eggs. *Gambusia holbrooki* and *Lissotriton boscai* did not prey many eggs after 24h, but their predation rate increased remarkably after 48h. *Pleurodeles waltl*, *Triturus pygmaeus*, *Procambarus clarkii* and *Triops cancriformis* were the most important predators, ingesting more than 60% eggs after 48h. *Pleurodeles waltl* ingested all the eggs after 48h (Fig. 1).

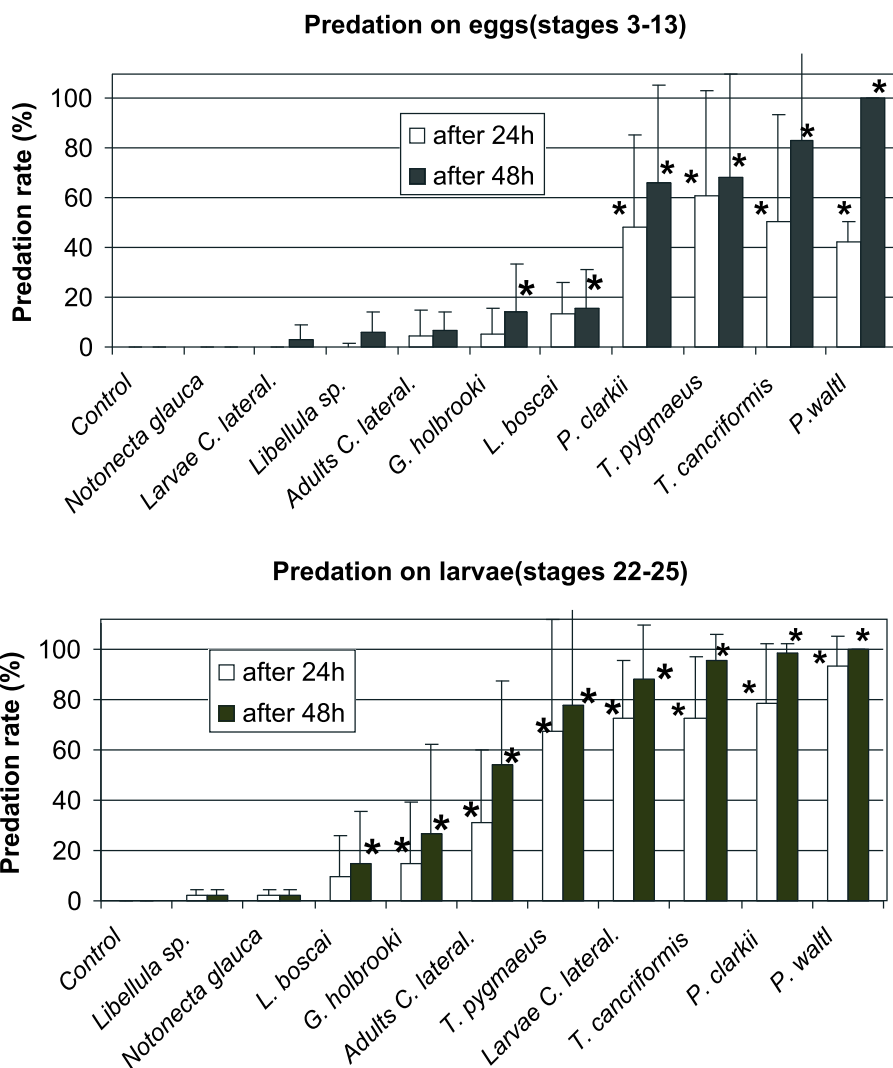


Figure 1. — Predation rates on eggs and larvae of *Bufo calamita* after 24h and 48h of exposure to nine potential predator species. *: χ^2 values with $p < 0.005$

All potential predators ingested some *Bufo calamita* larvae, although the predation rates of *Notonecta glauca* and *Libellula* sp. were non significant. The number of larvae consumed by *Lissotriton boscai* did not significantly differ from controls in the first 24h, but it was significant after 48h. *Lissotriton boscai*, *Gambusia holbrooki* and adults of *Cybister lateralimarginalis* consumed lower proportions of larvae than the other predators; while *Triturus pygmaeus*, larval *Cybister lateralimarginalis*, *Triops cancriformis*, *Procambarus clarkii* and *Pleurodeles waltil* ingested more than 60% larvae. *Pleurodeles waltil* ate 100% larvae after 48h.

Comparing the proportion of eggs and larvae consumed by each predator, *Pleurodeles waltil* fed all items offered although this species ingested faster larvae (93.3% in 24 h) than eggs (42.2% after 24 h). The remaining species consumed more larvae than eggs, although differences were significant only in *Procambarus clarkii*, *Cybister lateralimarginalis* (adults and larvae), *Gambusia holbrooki* and *Triturus pygmaeus* (χ^2 (in all cases) $p < 0.005$; Fig. 1).

Adults and larvae of the aquatic beetle *Cybister lateralmarginalis* were rare predators on eggs, but they significantly shifted to frequent predators of larvae (χ^2 (in both cases) $p < 0.005$).

PREDATION RATE IN RELATION WITH PREDATOR SIZE

Body size of predators significantly differed among the species tested (trials with eggs: $F_{9,125} = 7.907$, $p < 0.001$; trials with larvae: $F_{9,77} = 29.034$, $p < 0.001$). In relation to their predation rate, predator species ingesting more than 50% of the available eggs were significantly larger than those ingesting less than that ($F_{1,133} = 39.338$, $p < 0.001$). Species eating more than 50% of the available larvae had significantly larger body size than species which ingested less than that ($F_{1,85} = 24.109$, $p < 0.001$).

There was a significant correlation among predator size and their predation rate, both on egg and larval predation ($r_{\text{eggs}} = 0.477$, $p < 0.0005$, $n = 45$; $r_{\text{larvae}} = 0.531$, $p < 0.001$, $n = 87$).

BREEDING SUCCESS VERSUS PRESENCE OF PREDATORS IN THE PONDS

We recorded metamorphic individuals of *Bufo calamita* in only 25% of the 20 breeding ponds that were monitored monthly. In all of them we did not detect any of the major predators (those with larval or egg predation over 50% in experimental trials). Nevertheless, no breeding success was detected in 75% of the ponds, of which 40% were due to early desiccation, while in the others predation could be the cause of mortality (20% ponds had important predators) (Fig. 2).

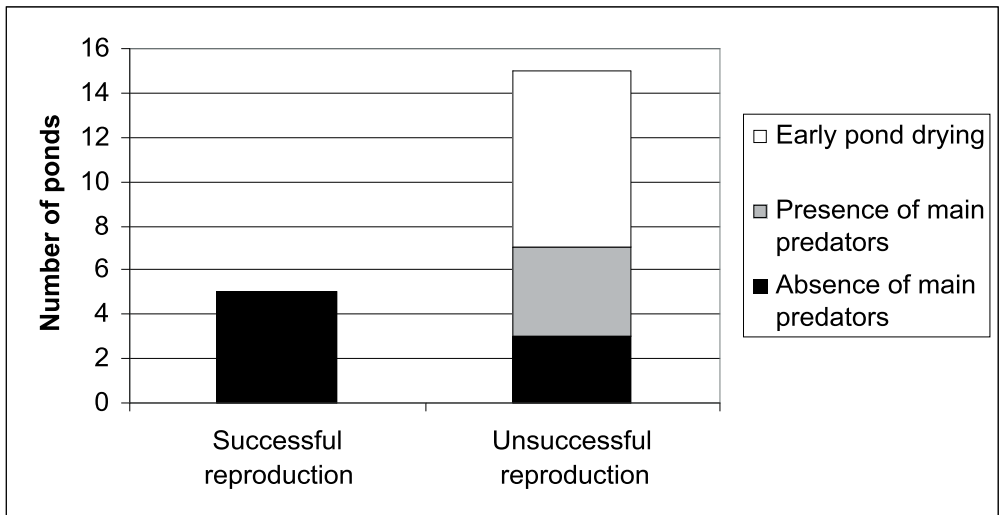


Figure 2. — Number of ponds with successful or unsuccessful reproduction of *Bufo calamita*, classified as a function of the presence or absence of major predators (*Pleurodeles waltl*, *Triops cancriformis*, *Procambarus clarkii* and *Triturus pygmaeus*) and by pond desiccation before metamorphosis of tadpoles.

In the field predation experiment, in three ponds containing major predators, predation reached 95-100% eggs when eggs were unprotected but small predation occurred when there was some protection. No predation was recorded in the pond in which no major predators were detected (Table II).

TABLE II

Percentage of eggs predated (mean \pm standard deviation) in spawns in predator exclusion enclosures in four breeding ponds of *Bufo calamita*: A= No access by predators, B= protection from big predators, C= no protection from predators. Presence (+) or absence (-) of major predators (egg predation rate >50%) or minor predators (egg predation rate <50%) is indicated

Pond	Pond hydroperiod	Percentage of eggs predated			Number of groups	Presence of predators	
		A	B	C		Predation >50%	Predation < 50%
1	5 months	3.33 (± 5.78)	3.33 (± 5.78)	100	3	+	+
2	5 months	0	20.0 (± 40.0)	95.0 (± 10.0)	4	+	+
3	2-3 months	0	0	100	3	+	+
4	2-3 months	0	0	0	5	-	+

DISCUSSION

In this study we observed differences in intensity of predation among predator species. Some species exerted a very intensive predation, consuming all or a high proportion of eggs and larvae. We suggest that their presence in breeding ponds would condition the reproductive success of *Bufo calamita*. The most intensive predation was due to *Pleurodeles waltl* larvae, which may reach large body sizes at the time of oviposition and larval period of *Bufo calamita*. However, this urodele breeds frequently in more permanent ponds than those typically used by *Bufo calamita* in our study area (Díaz-Paniagua, 1988). Thus, it is rare to find the co-occurrence of these two species into the same breeding pond. Therefore, although *Pleurodeles waltl* larvae may be important predators, they probably do not exert a high influence on *Bufo calamita* population. In contrast, another major predator, *Triops cancriformis*, an omnivorous crustacean (Longhurst, 1955) reaching great population densities in the ephemeral ponds where it is observed, may be more dangerous for toad eggs and larvae. Although we have found this species in 31% of the *Bufo calamita* breeding ponds, it is likely that the frequency of co-occurrence between these two species was underestimated, because the high numbers of individuals of such efficient predators in relative small ponds could rapidly have consumed eggs of *Bufo calamita*, decreasing their probability of detection. The possibility of high impact of predation by this crustacean on regulation of amphibians populations was reported by Knoepffler (1978), after observations of predation on *Pelobates cultripes* tadpoles.

It was also relevant the predation exerted in laboratory experiments by *Procambarus clarkii*, an exotic introduced crayfish which is present in many aquatic habitats of the study area. Its capability of predation on *Bufo calamita*, *Hyla meridionalis* and *Pelobates cultripes* eggs and larvae has been previously demonstrated by Cruz & Rebelo (2005). This species is omnivorous (Gutiérrez-Yurrita *et al.*, 1998) and may include amphibian eggs in its diet whilst consuming the vegetation supporting them (Gamradt & Kats, 1996; Gamradt *et al.*, 1997; Pérez-Santigosa *et al.*, 2003). However, crayfish are not frequent in the typical breeding ponds of *Bufo calamita*.

Triturus pygmaeus is the only major predator which has been widely found in ponds where *Bufo calamita* breeds. The high number of adult individuals at the study area could be expected to exert a high predation pressure. However, when only newts (and no other major predators) had access to the eggs, predation was considerably reduced, suggesting that they cannot be considered responsible of the absence of *Bufo calamita* breeding success from temporary ponds where eggs had been previously observed. Moreover, adult newts enter the ponds immediately after their flooding in autumn, but they migrate to land in February-March (Díaz-

Paniagua, 1990, 1998). Thus, only the earliest clutches of *Bufo calamita*, laid in late January or in February, are exposed to predation by newts.

The other predators tested did not exert high predation on toad eggs in laboratory, and thence are likely not very important for the regulation of toad populations, although a considerable predation on larvae was exerted by dytiscids.

Since almost all predator species tested in this study fed on eggs or larvae of *Bufo calamita*, it is likely that these are not toxic or distasteful for their predators. Higher consumption was related to predator body size, and this is not surprising as the smallest predators may have trouble in extracting eggs from the jelly and capturing larvae, thus being satiated with fewer items than larger predators. In fact, adult *Triturus pygmaeus* and *Lissotriton boscai* consumed eggs of *Bufo calamita*, but only the largest species exerted a considerable predation rate. Although *Lissotriton boscai* frequently ingests eggs of other amphibian species (Lizana & Martín-Sánchez, 1994; Pérez-Santigosa *et al.*, 2003), the relatively low predation rate found in our experiments could be better explained by the difficulty to extract eggs from the jelly or to capture larvae. Similar differences were also observed in British newts, of which only the largest species (*Triturus cristatus*) consumed eggs and larvae of *Bufo calamita*, while the small-sized newts (*Triturus vulgaris*) did not (Banks & Beebee, 1988; Denton & Beebee, 1991).

We observed difficulties in other predators to pierce the jelly and extract the eggs, as occurred with dytiscid larvae which were glued to the jelly in their failed attempts of egg predation. Adult dytiscids did not eat eggs in early developmental stages (3-5 Gosner stages), while embryo consumption increased when jelly became less compact in later stages, as well as in post-hatching embryos (correlation between ingestion rate and developmental stage of the eggs: $r=0.701$, $p<0.005$). Although dragonfly larvae were toad eggs predators in British populations (Banks & Beebee, 1988), in our experiments 4 out of 6 *Libellulidae* spp. larvae were observed to die after being caught within the jelly in their predation attempts. The scarce or nil predation observed may have been influenced by the small size of these predators, or also by the conditions of our experimental trials, as Banks & Beebee (1988) commented that these predators only caught tadpoles when submerged vegetation was provided in the tanks.

Eggs and tadpoles of *Bufo calamita* are considered unpalatable to fishes (Banks & Beebee, 1988; Semlitsch & Gavasso, 1992). Here we only used *Gambusia holbrooki*, a small sized introduced exotic fish that may occasionally occur in temporary ponds in very wet years. Their low but significant frequency of larval and egg consumption suggests that these items are not unpalatable to this species, but probably these fishes were too small to exert a high impact on *Bufo calamita*. In any case, *Bufo calamita* commonly breeds in temporary ponds (Andrén & Nilsen, 1985; Díaz-Paniagua, 1990; Semlitsch & Gavasso, 1992; Lardner, 2000) where they are not exposed to predation by fishes.

The preference of small temporary ponds as breeding sites and the avoidance of permanent water-bodies may be related to predator avoidance reasons. This is confirmed by the results of our field experiments, which revealed reproductive success only in small pools where major predators were absent.

The high vulnerability of eggs and larvae of *Bufo calamita* to a wide number of aquatic predators constrains their reproductive success to ephemeral ponds where they can complete metamorphosis. The stability of their populations is therefore conditioned by the availability of areas including ponds of short hydroperiod, where eggs and larvae do not face high predation pressure.

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