The reproduction of the Apennine yellow-bellied toad (*Bombina pachypus*) in central Italy

Marzia Mirabile¹, Mario Melletti², Alberto Venchi¹, Marco A. Bologna¹,∗

Abstract. Amphibians are declining worldwide and many species are threatened for unknown reasons. In fact, information on ecology of several species are not available due to the lack of studies. The Apennine yellow-bellied toad (*Bombina pachypus*) is an Italian endemic species in progressive decline in many areas, often for unclear reasons. A two-year study of 116 temporary ponds in a grazing area of the Majella National Park (central Italy) from 2001 to 2002 revealed that: (a) Apennine yellow-bellied toads reproduced in small ponds characterized by high desiccation risk; (b) breeding activity occurred for a prolonged period (at least from May to the beginning of August), during which females spawned clutches of very few eggs in several, successive events; (c) the reproductive success was very low especially because of high egg mortality; and (d) the main cause of mortality was the desiccation of ponds. Consequently, the characteristics of breeding areas (i.e., small, ephemeral ponds) are the most important feature influencing the reproduction. The safeguard of temporary ponds is crucial for the conservation of this amphibian and could preserve also the other species using these pools.

Keywords: clutch size, desiccation, prolonged breeder, reproductive success, temporary ponds, yellow-bellied toad.

Introduction

Worldwide populations of amphibians suffer declines and extinctions and although the main causes of decline are linked to habitat loss and fragmentation (Stuart et al., 2004), they could be related to more complex and not always completely understood factors, such as climate change (both global and local; Corn, 2005), ultraviolet radiation and fungal diseases (Kiesecker, Blaustein and Belden, 2001; Rachowicz et al., 2005), pollution as well as predation and competition by introduced species (Beebee and Griffiths, 2005). Thus, detailed information on the ecology of species is fundamental to understand the causes of population declines (Green, 2003).

In Italy, a region very rich in endemic species, major factors influencing survival of amphibians are habitat fragmentation, life-history specialization and a distribution limited to low altitudes, generally subjected to anthropogenic disturbance (Andreone and Luiselli, 2000; Bologna and La Posta, 2004). Moreover, mycotic infections caused by *Batrachochytrium dendrobatidis* (as the chytridiomycosis) seem to represent an additional factor affecting the survival of some Italian amphibians, as shown for the Apennine yellow-bellied toad, *Bombina pachypus* (Bonaparte, 1838) (Stagni et al., 2004).

The analysis of reproductive strategies is crucial for the conservation of pond-breeding amphibians: in fact, the status of the population of many species is commonly assessed studying breeding assemblages (Beebee and Griffiths, 2005) and, moreover, embryos are particularly susceptible to several risks (e.g., UV irradiation and fungal infections; Kiesecker, Blaustein and Belden, 2001). Especially amphibians that breed in temporary ponds experience a highly uncertain environment for eggs and larvae, which are more susceptible to mortality because of the drying of ponds than those developing in permanent water bodies, that, nevertheless, can face other risks such as predation (Duellman and Trueb, 1986; Wells, 2007).

The Apennine yellow-bellied toad occurs mainly in temporary ponds (Di Cerbo and Ferri, 1997).
2000; Venchi, Papa and Bologna, 2001; Venchi, 2002) and its breeding season ranges from March-April to September-October (Venchi, 2002). Knowledge on reproduction of this species is still very poor, especially ecological aspects (Meloni, Bonifazi and Gibertini, 1994; Guarino et al., 1998).

The aim of this work is to contribute to the knowledge of the ecology of the Apennine yellow-bellied toad by studying its reproduction, also to provide useful information for its conservation, which in general could support the safeguarding of the other species using temporary ponds. To do this, we analysed the: (1) phenology of spawning; (2) characteristics of clutches; (3) features of reproductive performance; (4) causes of mortality; and (5) embryonic and larval development as a function of duration and water temperature.

Materials and methods

Study area

The study area is located in the Majella National Park (central Italy) on the north-east side of the Mt. Morrone, near Roccacaramanico (42°06′N – 14°01′E; 1000-1280 m a.s.l.; fig. 1). We surveyed 73 ponds in 2001 and 43 in 2002 (fig. 1; table 1). All ponds were temporary, sunny and fed by rainfall and in few cases by ground water springs. Several puddles were created by horse footprints. In fact, this area is used for grazing of both horses and sheep. The landscape is characterized by a secondary mountain pasture, surrounded by...
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### Table 1. Surveyed ponds in the Majella National Park (central Italy) in 2001 and 2002.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total number of ponds</th>
<th>Number of ponds used by <em>B. pachypus</em></th>
<th>Number of used ponds desiccated</th>
<th>Number of ponds with successful metamorphosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>73</td>
<td>69</td>
<td>63</td>
<td>6</td>
</tr>
<tr>
<td>2002</td>
<td>43</td>
<td>32</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td>Tot</td>
<td>116</td>
<td>101</td>
<td>89</td>
<td>12</td>
</tr>
</tbody>
</table>

*31 ponds were present in both years.

*Ponds with successful metamorphosis could be underestimated because the sampling frequency did not ensure that all metamorphs were detected.*

woods of beech (*Fagus sylvatica*) and downy oak (*Quercus pubescens*; Tammaro, 1998). Although the massif is primarily calcareous, the soil of the eastern slope is rich in quite impermeable substrates like clay and sandstone, which support the forming of temporary ponds. Moreover, this area is subjected to slight landslides which alter its morphology. The local climate is continental and the temperature can go below 0°C for long periods between November and March and sometimes later (such as in April 2001). Rainfall is distributed throughout the year, with a maximum in November and a minimum in summer. In the study area the mean annual rainfall is 1418.51 mm, and the mean annual temperature is 11.06°C (Tammaro, 1998). Summer is the most critical period for the reproduction of amphibians, due to the desiccation risk of water bodies. During our study, the period May-September 2002 was more rainy (monthly rainfall more than 50 mm) than in 2001 (monthly rainfall always less than 50 mm).

### Study species

The Apennine yellow-bellied toad was previously considered the Italian endemic subspecies *pachypus* (Bonaparte, 1838) of the yellow-bellied toad *Bombina variegata* (Linnaeus, 1758), but it is currently considered a distinct species based on genetic studies (Nascetti et al., 1983; Lanza and Vanni, 1991; Fromhage, Vences and Veith, 2004; Guarino, Picariello and Venchi, 2007). This amphibian is distributed in peninsular Italy, while populations north of river Po are referred to as *B. variegata*. The Apennine yellow-bellied toad is preferably located in hills and low mountains, but ranges from the sea level to about 1600 m a.s.l. (Barbieri et al., 2004; Guarino, Picariello and Pellegrini, 2006). Information on taxonomy, faunistics and biology of this toad has recently been summarized by Guarino, Picariello and Venchi (2007). This species is listed by IUCN (2007) as Least Concern (LC) in view of its relatively wide distribution and presumed large population, but specific studies assess this species as Vulnerable (V), in particular due to its endemism (Andreone and Luiselli, 2000) and to fast decline in different Italian regions (Barbieri et al., 2004).

### Field methods

Data were collected during two years (2001 and 2002) between April and September. During this period all ponds (used and unused by the species) were visited every 4-10 days for a total of 20 visits in 2001 and 20 in 2002. Used ponds were those where adults and/or eggs were detected. Maximum depth was measured (0.1 cm accuracy) and the maximum water surface was estimated with the equation for an ellipse. For those ponds for which precise measures of both depth and area were available (49 in 2001 and 35 in 2002), we calculated the maximum pond volume (*V*\(_{max}\)), which was estimated following the equation:

\[
V_{max} = \frac{A_{max} \times d_{max}}{3}
\]

where *A*\(_{max}\) and *d*\(_{max}\) were the maximum water surface and the maximum depth, respectively (see Brooks and Hayashi, 2002 for more details).

Adults were captured, identified and sexed (capture permits DPN/2D/10441 of the Italian Ministry for the Environment, Land and Sea) and each newly captured specimen was recorded by taking a picture of the specific belly pattern (yellow belly with dark spots), that allowed individual identification thanks to its uniqueness. To prevent the possible spread of any infections, we used disposable gloves when handling each toad and the equipment (i.e., metre stick, net, multi-parametric probe) was previously disinfected before the use in each pond. Both juveniles (determined according to Lanza, 1983) and adults were immediately released after the examination. Number of days of permanence in the same pond and possible movements were recorded for individuals captured more than once.

The Apennine yellow-bellied toad lays eggs irregularly, most frequently as small scattered masses (“clutches”), which can also result as isolated eggs. We counted clutches in the ponds, but we may have underestimated their number in few large and densely vegetated ponds, where small clutches were difficult to spot. Clutch locations were marked with numbered flagging, also for distinguishing new clutches from those already found.

The exact number of eggs of each clutch was counted and their developmental stage was assessed using the table for staging Anuran embryos of Gosner (1960), referring to stages 1-25 that represent the embryonic development. Eggs at Gosner stages 1-14 were used to estimate duration of development, because such eggs had been laid recently, at most a few hours earlier (Duellman and Trueb, 1986; Mirabile, personal observation).

Tadpoles and metamorphs of each pond were captured with a net. For the analyses we used the maximum count of every pond. It may be possible that some metamorphs left

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the ponds undetected, thus their number may be underestimated for those ponds that did not have complete mortality due to early pond drying. The snout-vent length (SVL) of metamorphs was measured when they emerged, using digital callipers (0.02 mm accuracy). As for adults, the specific belly pattern of each new metamorph was photographed for individual identification. Moreover, we could estimate the duration of development in days, both embryonic (from first appearance of eggs to their hatching) and complete (until metamorphosis), in ponds where breeding occurred only once and all eggs were first detected at Gosner stages 1-14. Due to the desiccation of most ponds, developmental time could be assessed only for very few clutches.

We recorded the depth at which clutches were stuck to their support, using a metre stick (0.1 cm accuracy), and the water temperature in the exact location of the clutch, using a multi-parametric probe. For each survey, we measured water temperature of all ponds and for the related analyses we used the mean, maximum and minimum values. For ponds where the species occurred (101 in total), we recorded the duration (hydroperiod) from late April until the pond was dried up. Finally, we recorded the presence of potential predators, both vertebrates and invertebrates.

Parametric and nonparametric tests were used according to the distribution of data. For all analysis, means are given ±1 SD, tests were two-tailed and statistical significance was set at $\alpha \leq 0.05$.

Results

Pond characteristics

Ponds present in the study area, included those unused by *B. pachypus*, were generally very small: $\bar{x}_{2001} = 5.25 \pm 13.14$ m², range 0.009-60.78 m², $n = 66$ and $\bar{x}_{2002} = 9.46 \pm 19.44$ m², range 0.027-92.88 m², $n = 40$. In particular one third of all ponds had a water surface less than 0.1 m². Furthermore, in both years, water bodies were on average shallow: $\bar{x}_{2001} = 17.4 \pm 15.9$ cm, range 0.5-64 cm, $n = 49$ and $\bar{x}_{2002} = 16.3 \pm 14.0$ cm, range 1-61 cm, $n = 35$.

The small ponds held scarce water, in fact the maximum estimated volume was: $\bar{x}_{2001} = 0.57 \pm 1.37$ m³, range 0.00005-7.29 m³, $n = 48$ and $\bar{x}_{2002} = 0.68 \pm 1.50$ m³, range 0.0001-7.09 m³, $n = 34$. The only two ponds with a water volume > 10 m³ (12.02 m³ in 2001 and 18.89 m³ in 2002) were excluded from this last analysis.

Adulst permanence and spawning phenology

We captured 137 individuals (82 males, 52 females, 3 juveniles) during 2001 and 91 (52 males, 33 females and 6 juveniles) during 2002. All females ($n = 13$) and most of males re-captured in 2002 ($n = 13$) were found in the same pond or at a distance within 1 meter. Only 4 males recaptured in 2002 moved along a distance greater than 1 meter. Finally, two metamorphs of 2001 were recaptured in 2002 in the same pond.

Generally adults remained in the reproductive site about 2 months; no significant difference between males and females emerged ($\bar{x}_{\text{males}} = 54 \pm 36$ days, range = 8-144 days, $n = 40$; $\bar{x}_{\text{females}} = 49 \pm 30$ days, range = 7-138 days, $n = 33$; $t = 0.66, P = 0.51, n = 73$).

In both years, eggs were found from May to early August (fig. 2). During this period, spawning occurred repeatedly with peaks in May and June (fig. 3). The number of ponds available for reproduction decreased in July, especially in 2001 (fig. 3).

Clutch characteristics

We found a total of 310 clutches (173 in 2001 and 137 in 2002). Generally they were very small (typically <10 eggs, range 1-114 eggs; fig. 4) and the number of eggs per clutch was not significantly different between years (Wilcoxon test, $Z = -0.085, P = 0.93, n = 7$). Forty-six percent of the clutches ($n = 143$) contained five eggs or less and only four percent ($n = 12$) numbered more than 30 eggs. Masses of eggs were stuck to aquatic vegetation, to grasses at the edge of pond or to little branches fallen accidentally in the water. In the smallest ponds eggs were simply sunk at the bottom. Clutches were generally stuck at low depth ($\bar{x} = 4.3 \pm 5.2$ cm, range = 0.1-30 cm, $n = 298$). Forty-five percent of clutches ($n = 134$) were found within 2 cm under the water surface.
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Figure 2. Phenology of the reproduction in populations of Apennine yellow-bellied toad (Bombina pachypus) in a pond by pond basis in 2001 and 2002: horizontal lines represent the presence of eggs in each pond (stars indicate ponds in which reproduction was successful).

Reproductive success and causes of mortality

Although the number of metamorphs may be underestimated, total mortality from spawning to metamorphosis was likely to be very high in both years (table 2). We found only 6 damaged eggs in 2001 and 2 in 2002, suggesting that fungal and other infections were not a primary cause of mortality.

The mortality was due mainly to desiccation of ponds (table 2). In fact, 91.3% (n = 63) of temporary ponds used in 2001 and 81.3% (n = 26) in 2002 dried up during summer (table 1, fig. 2). On average, the pond hydroperiod was shorter in 2001 (\(\bar{x} = 45 \pm 29\) days, range = 8-172 days, \(n = 69\)) than in 2002 (\(\bar{x} = 92 \pm 56\) days, range = 6-171 days, \(n = 32\)) and the difference was highly significant (Mann-Whitney test, \(Z = -3.92, P < 0.001, n = 101\)).

Another cause of mortality could be the predation of both eggs and tadpoles. In the study area potential predators were present only in 27 out of 73 ponds in 2001 and 18 out of 43 ponds in 2002. Identified possible predators were: newts, Lissotriton italicus (Perracca, 1898) and Triturus carnifex (Laurenti, 1768); green frog, Pelophylax synkl. lessonae/esculentus (Linnæus, 1758); grass snake, Natrix natrix (Linnæus, 1758) and several aquatic invertebrates, such as crawling water beetles (Coleoptera: Dytiscidae), dragonfly nymphs (Odonata) and some species of Hemiptera (Gerridae and Notonectidae). Predation was observed twice, only on tadpoles: by grass snake and by crawling water beetle.

Development and water temperature

We estimated that hatching time varied between a week and a month (\(\bar{x} = 16 \pm 7\) days, range = 8-29 days, \(n = 10\)) and the complete development until metamorphosis occurred within three months (\(\bar{x} = 68 \pm 12\) days, range = 56-88 days, \(n = 6\)). Water temperature was negatively correlated to the duration of embryonic development, but not to the duration of complete development (table 3). Mean, minimum and maximum values of water temperature entered the correlation with the duration of embryonic development were 19.1°C, 10.9°C, 28.4°C, respectively. Mean, minimum and maximum values of water temperature used in the correlation with the duration of complete development were 20.6°C, 10.9°C, 29.3°C, respectively. Finally, SVL at metamorphosis showed a wide range (\(\bar{x} = 14.55 \pm 1.57\) mm, range = 11.75-19.61 mm, \(n = 68\); fig. 5).
Figure 3. Number of ponds filled with water (black bars) and number of eggs (grey bars) over the study period in 2001 and 2002.

Figure 4. Distribution of clutches per number of eggs in 2001 (white bars) and 2002 (black bars) in a population of Apennine yellow-bellied toad.

Discussion

Breeding activity of the Apennine yellow-bellied toad occurred for a prolonged period, from May until the beginning of August (fig. 2), during which females spawned small clutches in several events. Similar results were obtained in a population from southern Italy (Guarino et al., 1998) and in *B. variegata* (Barandun and Reyer, 1997). Although it is known that, in many prolonged breeding anurans, males spend longer time than females in the reproductive site in order to increase the probability of multiple mating (Duellman and Trueb, 1986; Wells, 2007), our results show no sex differences in pond permanence. Adults of Apennine yellow-bellied toad stayed at the breeding site for a prolonged period possibly in order to optimize the use of resources as soon as they were available (e.g., ponds filled after heavy rain; Barandun and Reyer, 1997), as also observed in other species (Morey and Reznick, 2004). In fact, where the conditions for reproduction are not continuously suitable (e.g., temporary ponds), species should devote more effort to their opportunities to breed than those living in more stable environments (Giesel, 1976).

We found that clutch size was very small (fig. 4) as recorded in other populations of both yellow-bellied toad species (Barandun and Reyer, 1997; Guarino et al., 1998). Clutches of few eggs, spawned opportunistically in several puddles, could be interpreted as an adaptation to the limited carrying capacity of the temporary breeding ponds: scattering eggs possibly maximises the chance of the offspring to reach meta-
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### Table 2. Eggs and tadpoles mortality (number and percentage) in 2001 and 2002.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total number of eggs</th>
<th>Number of eggs desiccated</th>
<th>Number of tadpoles desiccated</th>
<th>Number of tadpoles disappeared for unknown reasons</th>
<th>Number of metamorphs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>1644</td>
<td>1008 (61%)</td>
<td>349 (21%)</td>
<td>275 (17%)</td>
<td>12</td>
</tr>
<tr>
<td>2002</td>
<td>1527</td>
<td>762 (50%)</td>
<td>319 (21%)</td>
<td>390 (26%)</td>
<td>56</td>
</tr>
</tbody>
</table>

*a* Because of our sampling periodicity (4-10 days), number of metamorphs may be underestimated for those ponds that did not have complete mortality due to pond drying.

### Table 3. Spearman’s correlations between pond water temperature (mean, maximum and minimum values) and the duration of embryonic development and of complete development until metamorphosis.

<table>
<thead>
<tr>
<th></th>
<th>Duration of embryonic development</th>
<th>Duration of development until metamorphosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( R )</td>
<td>( P )</td>
</tr>
<tr>
<td>Mean water temperature (°C)</td>
<td>(-0.75)</td>
<td>0.01</td>
</tr>
<tr>
<td>Maximum water temperature (°C)</td>
<td>(-0.31)</td>
<td>0.37</td>
</tr>
<tr>
<td>Minimum water temperature (°C)</td>
<td>(-0.70)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### Figure 5. SVL at metamorphosis (in mm) in relation to date in 2001 and 2002 (the number of metamorphs is given for dates with more than three captures).

morphosis (risk-spreading strategy; Abbühl and Durrer, 1998; Guarino et al., 1998). Thus, an unpredictable environment affects the reproductive success: for example, in years with optimal environmental conditions *B. variegata* is probably able to produce a higher number of offspring and, moreover, females in a better condition lay more clutches over a breeding season (Buschmann, 2002). Finally, especially within larger ponds, the behaviour of scattering eggs individually or in small packets can be interpreted as an antipredator adaptation that makes eggs inconspicuous and, therefore, difficult for predators to locate (Marshall, Doyle and Kaplan, 1990).

Estimated breeding success of the studied population was very low in both years (table 2), as also detected in another population of the Apennine species in central Italy (Mattoccia et al., 2006) and in a Swiss popula-
tion of the yellow-bellied toad (Barandun and Reyer, 1997). High mortality was due mainly to pond desiccation (table 2), as observed also in the yellow-bellied toad (Barandun and Reyer, 1997). In 2001 the hydroperiod was very short and most ponds dried up between May and July (fig. 3), hence most of eggs were lost. In the same period in 2002, thanks to a longer availability of water, more tadpoles achieved metamorphosis than in 2001 and more eggs were laid until the end of July. Paton and Crouch (2002) underlined the importance of hydroperiod in pond-breeding amphibians of Rhode Island (USA), showing that the date of drawdown was critical for successful reproduction. In spite of high risk of desiccation, many amphibians breed in temporary ponds mainly because of the scarcity of predators, in particular fishes (Paton and Crouch, 2002; Ficetola and De Bernardi, 2004; Babbitt, Baber and Brandt, 2006), as in the examined ponds of the Majella National Park. In fact our results show that predation was a negligible cause of mortality compared to desiccation.

In amphibians, water temperature and food availability are the major external factors controlling the duration of development (Duellman and Trueb, 1986; Wells, 2007). Our results show that, in general, the development was shorter at higher temperatures (table 3), in accordance with other studies (Newman, 1989; Barandun and Reyer, 1997; Morand, Joly and Grolet, 1997; Morrison and Hero, 2003). We observed that most clutches were laid just under the water surface (depth < 2 cm), also in the deepest ponds, probably for benefiting from the higher water temperatures. In fact, in a field experiment on waterfrog tadpoles, Thurnheer and Reyer (2001) observed a clear preference of tadpoles for the shallow zones of the pond, significantly warmer with respect to the deepest regions.

Time to metamorphosis showed a wide range of variation in length of larval period as well as body size in accordance with Wilbur and Collins (1973), who observed that in species adapted to temporary ponds, factors other than date of hatching affect the duration of complete development and the size at metamorphosis (e.g., water temperature, Morand, Joly and Grolet, 1997; water volume, Denver, Mirhadi and Phillips, 1998). In our study, metamorphs showed a wide range of SVL (fig. 5) and an average size a quarter of that of adults (usually 6-8 cm; Lanza, 1983). Apennine yellow-bellied toad takes three years to reach the minimum size for reproduction and consequently the age of first breeding is delayed. However it is a long-lived species: the maximum longevity ascertained in nature is 16 years (Guarino, Angelini and Cammarota, 1995).

In conclusion, our results show that the characteristics of breeding sites (i.e., small temporary ponds) are the most important feature influencing the reproduction of the Apennine yellow-bellied toad. In particular the safeguard of water bodies that have suitable breeding hydroperiod in even a minority of years is greatly needed for the conservation of all long-lived amphibians (Paton and Crouch, 2002), like the Apennine yellow-bellied toad. The protection of areas with ponds of this kind is essential for this endemic species, because both sexes show high pond fidelity and adults could not reproduce yearly (Guarino et al., 1998; Mattoccia et al., 2006). Finally, the protection of these water bodies could preserve also the other species using these pools.

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References


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