

EGG MORTALITY AND EARLY EMBRYO HATCHING CAUSED BY FUNGAL INFECTION OF IBERIAN ROCK LIZARD (*LACERTA MONTICOLA*) CLUTCHES

PEDRO LOPES MOREIRA¹ AND MARGARIDA BARATA²

¹Centro de Biologia Ambiental and ²Centro de Micologia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, Portugal

Infertile and non-viable fertile eggs within a reptile clutch may decrease the incubation success of the remaining eggs, as (1) opportunistic pathogens may use the nutrient resources provided by dead eggs to colonize the clutch and spread to and kill viable eggs; and (2) odours released by spoilt eggs may attract predators to the clutch. These hypotheses were tested on the Iberian rock lizard (*Lacerta monticola*) by comparing the incubation success of fertile eggs between clutches composed solely of fertile eggs and clutches containing a small number of dead eggs. In a laboratory experiment, fungi (*Fusarium* sp. and *Gliocladium* sp.) colonized both infertile eggs and fertile eggs that died during incubation and thereafter spread to and killed adjacent eggs. In addition, offspring hatched earlier from fungal infected eggs than from non-infected eggs. The former were smaller and lighter than the latter, as they hatched before using the full egg yolk content. Results from a field experiment did not corroborate the fungal pathogenic effects observed in the laboratory nor did they confirm that clutches containing dead eggs suffer higher predation. Despite the inconclusive results regarding the role of fungal pathogens in nature, the present study indicates that this subject deserves further investigation in reptiles.

Key words: egg infertility, egg viability, fungal pathogen, early hatching

INTRODUCTION

Many oviparous female reptiles deposit a clutch comprised of several eggs in a nest, with eggs in close proximity or even adhered to each other, without providing care to eggs or hatchlings (Greene, 1997; Pianka & Vitt, 2003). They may provide good model systems to investigate whether infertile and non-viable fertile eggs within a clutch decrease the hatching success of the remaining eggs or the offspring quality. These effects are little studied in reptiles, but they may exert important selective pressures on reproductive traits. Females that lay a single (or only a few) infertile or non-viable fertile eggs within a clutch may jeopardize their entire reproductive output if dead eggs promote clutch colonization by pathogens that kill eggs (Smith *et al.*, 1985; Green, 1999; Robinson *et al.*, 2003), trigger early hatching of embryos (Warkentin *et al.*, 2001; Wedekind, 2002), or infect the tissues of hatchlings (J. Wyneken, unpubl. obs., cited in Eckert & Eckert, 1990). In addition, dead eggs may increase the risk of clutch predation by species attracted by the odor of spoilt eggs (Groves, 1982; Somma, 1989).

Saprolegniaceae water moulds are frequent opportunistic pathogens of fish and amphibian eggs since zoospores readily colonize dead eggs and hyphae thereafter spread to and kill adjacent viable eggs (Smith *et al.*, 1985; Green, 1999; Robinson *et al.*, 2003). Consequently, amphibian communal spawners may suffer from higher egg mortality caused by fungi than species that

space their eggs out (Blaustein *et al.*, 1994; Kiesecker & Blaustein, 1997). Low temperature and pH (Beattie *et al.*, 1991; Bellemakers & Van Dam, 1992), as well as high levels of ultraviolet-B radiation (Kiesecker & Blaustein, 1995), can contribute to egg mortality as they decrease embryo resistance to fungal pathogens. In terrestrial environments, fungal infection of amphibian clutches can also start on dead eggs (Tilley, 1972; Forester, 1979). Females that brood the clutch can reduce egg mortality, as oophagy of dead/infected eggs prevents fungi from spreading to viable eggs and egg mechanical agitation retards fungal growth by disrupting the mycelia (Tilley, 1972; Forester, 1979). As such, egg-laying behaviour and parental care in amphibians have been suggested to have evolved as a defence against egg pathogens (reviewed by Green, 1999). Moreover, embryos themselves may have evolved strategies to escape from pathogens, as both fish (Wedekind, 2002) and amphibian (Warkentin *et al.*, 2001) embryos may hatch earlier from pathogen-infected than from non-infected eggs.

Fungi have also been recognized to contribute to the mortality of lizard, snake and sea turtle eggs (Fitch & Fitch, 1968; Tracy, 1980; Phillott & Parmeter, 2001b). The first appearance of fungi (*Fusarium solani* and *Pseudallescheria boydii*) in sea turtle nests was always on a non-viable egg, and fungal hyphae from this failed egg then spread to and killed adjacent eggs (Phillott & Parmeter, 2001b). Indirect evidence that fungi can be pathogenic to reptilian eggs is provided by the observation that oophagy and removal of dead/infected eggs from the clutch occurs in several lizards with parental behaviour (Mitchell & Groves, 1993; Somma, 2003). These behaviours were hypothesized to prevent both

Correspondence: P. L. Moreira, Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Edifício C2, Campo Grande, 1749-016 Lisboa, Portugal. *E-mail:* plmoreira@netcabo.pt

the spread of pathogens to viable eggs and the detection of the female and/or clutch by predators attracted by the odor of spoilt eggs (Groves, 1982; Somma, 1989).

The present study used the Iberian rock lizard (*Lacerta monticola*) to address the hypotheses that (1) opportunistic pathogens use the nutrient resources provided by dead eggs to colonize the clutch and spread to and kill viable eggs, and that (2) odours released by spoilt eggs attract predators to the clutch. The incubation success of fertile eggs was compared between clutches composed solely of fertile eggs and clutches containing a small number of dead eggs (infertile eggs or fertile eggs killed by freezing), both in the laboratory and in the field. The experimental study was complemented with the identification of the fungi that infected clutches in the laboratory experiment and with the evaluation of the frequencies of egg infertility, egg fungal infection, and egg predation in nature.

MATERIAL AND METHODS

STUDY POPULATION

The Iberian rock lizard is a small insectivorous lacertid endemic to the Iberian Peninsula. In Portugal, it is restricted to a single population at the Serra da Estrela mountain. Lizards are active from March-May to October-November. Adult males emerge from winter hibernation 1-2 weeks prior to adult females, with the copulation season starting soon after female emergence and lasting for 2-4 weeks. Females copulate more than 4-8 times, frequently with several different males, and produce a single clutch per year with 2-11 eggs. Females deposit the entire clutch in shallow burrows that they excavate under rocks (pers. observation), 1-2 months after copulations (Moreira, 2002). They do not appear to provide any form of parental care to eggs or hatchlings (Somma, 2003; and pers. observation).

LABORATORY EXPERIMENT

In order to obtain both fertile and infertile eggs, Iberian rock lizards were bred in captivity and females were allowed to copulate a small number of times. Sixteen males and 44 females were caught on 11-15 October 1998, before entering winter hibernation, near Torre, the top of Serra da Estrela at 1993 m of altitude. Ten other females, caught on 26-30 March 1998 in the same area, were also used. The two groups of lizards were hibernated in captivity on 17 and 22 October 1998, respectively. Males were removed from hibernation on 8 February and females on 15 February 1999 (detailed rearing conditions in Moreira & Birkhead, 2003). Eighty-four copulations occurred between 22 February and 12 March 1999, involving 13 males and 43 females. Females were transferred to terraria without males once they had copulated the required number of times: 24 females copulated once, 11 copulated twice and eight copulated four or more times. When gravid, females were transferred to egg-laying chambers provided with

humid vermiculite as substrate. Eggs were collected soon after being laid. They were weighed (to the nearest 0.01 g) and candled to determine whether they were fertile or not (Olsson & Shine, 1997). Fertile eggs were numbered with a soft graphite pencil on the opposite side of the blastodisc. Forty females oviposited between 23 March and 14 April 1999 and provided 35 infertile and 184 fertile eggs.

Seventeen clutches consisting of six fertile eggs (hereafter termed fertile clutches) and 13 clutches consisting of four fertile plus two infertile eggs (hereafter termed mixed clutches) were assembled from eggs obtained from different females. A maximum of two fertile eggs per female was used per clutch in order to minimize family effects and each clutch was completed within a maximum of three days. Clutches were incubated separately in 600 ml plastic boxes (12 × 9 × 6 cm³) containing 400 ml of incubation medium (1 ml of demineralized water for 10 ml of vermiculite size '2'; Olsson & Shine, 1997) mixed with 20 ml of soil, collected on 21 March 1999 at the site selected for the field experiment (see below). Clutches were placed in the centre of the incubation boxes, and the eggs were arranged in two parallel rows of three eggs each, with eggs touching adjacent ones and their numbers facing up. The two infertile eggs in mixed clutches were placed in the outer positions of each row and both on the same end side of the clutch, either on the right- (six cases) or the left-hand side (seven cases; Fig. 1). Clutches were covered with about 5 mm of incubation medium. Incubation boxes were airtight closed, maintained in the incubator at 26°-28°C and opened regularly for air renewal.

Clutches were inspected at about the middle of the incubation period (16-24 days after the fertile eggs were laid; previous incubations under the same humidity and temperature conditions lasted 36-39 days) and fungal abundance was recorded according to three levels: (1) mycelia not visible to the naked eye; (2) mycelia covering part (less than half) of the clutch; (3) mycelia covering most (more than half) of the clutch. The eggs were then carefully uncovered, so that they were not displaced nor the mycelia disrupted, and categorized as being dead (collapsed), alive (turgid and with white eggshell), and infected by fungi (mycelia closely associated with their surface). Eggs were covered again after examination. Towards the end of incubation, clutches were inspected daily for hatched lizards, and fungal abundance and egg condition were recorded as described above. Forty-seven offspring hatched between 2-15 May 1999, and were measured (snout-vent length and tail length to the nearest millimeter, and head size to the nearest 0.05 mm) and weighed (to the nearest 0.01 g). Eggshells were examined for noticeable yolk residues. Adult lizards and hatchlings were released near Torre.

Eggs that died during incubation and eggshells from hatched eggs belonging to each of the clutches were preserved in 7 ml tubes filled to the top with 70% ethanol. Fungal spores and hyphae were abundant in the alcohol

solution, which allowed the microscopic identification (to the genus) of most fungi that infected the clutches. The fungi characteristic spores were counted on 30 field scopes (400× magnification) in each of two slide preparations. Clutches were considered to have been infected by a particular fungus when its characteristic spores were detected in the counting procedure.

FIELD EXPERIMENT

Eggs were obtained from 39 gravid females captured near Torre between 25 June and 6 July 1999. These females laid in egg-laying chambers (as above) and altogether produced one infertile and 235 fertile eggs. Seventeen other infertile eggs were obtained from three females that laid in captivity without copulating, which had been previously captured between 24 May and 16 June 1999 in the same area. In order to build the mixed clutches, and because infertile eggs were insufficient in number (only 18 available), 20 fertile eggs killed by freezing were also used. Eggs were sorted into 19 fertile clutches (six fertile eggs) and 19 mixed clutches (four fertile plus two dead eggs) in the same manner as for the laboratory experiment.

Fertile and mixed clutches were placed to incubate under flat rocks on 7-10 July 1999 near the Lagoa Comprida lagoon (1580 m of altitude), at a site where a large number of females oviposited every year. Each clutch was placed in the field within a maximum of eight days (mean±SD = 4±1.5 days) after its live eggs were laid. Flat rocks (c. 20-60 cm diameter × c. 5-10 cm thick) were prepared in advance (April 1999) and distributed in clearings over an area of about 50 × 30 m². The soil underneath the rocks was slightly ploughed so that the rocks were well sited on the ground and the soil humidity was preserved. Clutches were placed within a PVC plastic ring (11 cm diameter × 6 cm high; inserted in the ground beneath the centre of each rock) and were covered with about 10 mm of soil. Plastic rings prevented clutches from being crushed when rocks were laid over them and were perforated to allow for water exchange and access of potential predators to the clutch. Eggs in each clutch were spatially arranged as for the laboratory experiment (Fig. 1). Fertile and mixed clutches were equally distributed over the study area.

Clutches were first inspected on 20-22 July 1999 (12-13 days after the beginning of incubation in the field and up to 21 days after its live eggs were laid). Rocks were lifted and eggs uncovered by sweeping the soil with a brush. It was next recorded whether eggs were predated (eggs missing, being destroyed by ants, or with perforations similar to the latter) and also the condition of the remainder eggs (as for the laboratory experiment). After the inspection, the soil and rock were put back in place. Clutches were inspected again on 13-14 August 1999 (35-37 days after the beginning of incubation in the field). None of the eggs had hatched. The condition of the eggs was recorded (as above) and they were brought to the laboratory for the remainder of the incubation period. Females and offspring were released near Torre.

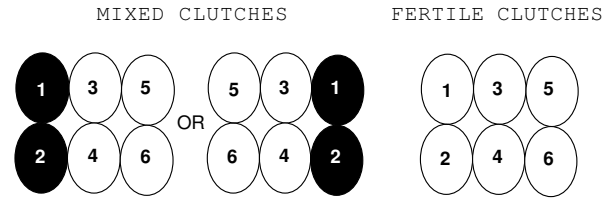


FIG. 1. Egg arrangement of infertile (black) and fertile (white) eggs in the mixed (4 fertile + 2 infertile eggs) and fertile (6 fertile eggs) clutches incubated in the laboratory. For the purpose of analyses, infertile eggs were in positions 1 and 2, fertile eggs that touched them were in positions 3 and 4, and fertile eggs that did not touch infertile eggs were in positions 5 and 6. For the field experiment, both infertile eggs and fertile eggs killed by freezing were used in positions 1 and 2 of the mixed clutches.

EGG INFERTILITY, FUNGAL INFECTION AND PREDATION IN NATURE

The frequency of egg infertility in the population was estimated from 103 clutches laid in captivity by females that were captured when gravid near Lagoa Comprida and near Torre between 1995 and 1999 (Moreira, 2002). The frequencies of egg fungal infection and egg predation in nature were estimated from unhatched eggs and eggshells that were dug out from lizard nests at a site near Torre where a large number of females oviposited every year. These nests were dug out on 28 August and 5 September 1998 (28 and 35 days, respectively, after the beginning of the egg-laying season in that year). A total of 132 unhatched eggs were collected and it was recorded whether they were predated and the condition of the remainder eggs (as for the field experiment). In addition, 348 eggshells from previous years were collected by sieving the soil removed to expose nests. Eggshells were classified as belonging to (1) hatched eggs (eggshells with a slit similar to that observed in eggs that hatched in captivity), (2) unhatched eggs (eggshells not opened), and (3) eggs predated by ants (eggshells with perforations similar to those observed in eggs being predated by ants). Sixty-two much degraded eggshells could not be ascertained to any of the above categories and were not considered for analyses. The unhatched eggs were taken to the laboratory for the remainder of incubation and offspring were released near Torre.

RESULTS

LABORATORY EXPERIMENT

Fungi readily colonized infertile eggs and grew to cover the majority of the mixed clutches. At the middle of the incubation period, all infertile eggs were closely surrounded by mycelia. At this stage, fungal abundance was higher in the mixed clutches (8% clutches with mycelium over part of the clutch; 92% clutches with mycelium over most of the clutch; $n=13$) than in the fertile clutches (35% clutches without visible mycelium, 35% clutches with mycelium over part of the clutch, 30% clutches with mycelium over most of the clutch; $n=17$; $\chi^2=2.1$, $df=2$, $P<0.01$). At the end of incubation, fungal abundance was higher in the mixed clutches

(100% clutches with mycelium over most of the clutch) than in the fertile clutches (18% clutches without visible mycelium, 12% clutches with mycelium over part of the clutch, 70% clutches with mycelium over most of the clutch), but differences were no longer statistically significant ($\chi^2=4.6$, $df=2$, $P>0.10$). This was because fungi also infected fertile clutches by colonizing the fertile eggs that died during incubation.

Fungal spores or hyphae were not detected in the three clutches that were not surrounded by visible mycelia. Spores of *Fusarium* sp. and *Gliocladium* sp. were the most prevalent and abundant among the 27 clutches that were surrounded by visible mycelia. Nineteen of these clutches (70.4%) were infected by *Fusarium* sp., one (3.7%) was infected by *Gliocladium* sp. and six (22.2%) were infected by both *Fusarium* sp. and *Gliocladium* sp. One clutch (3.7%) was infected by a fungus that could not be identified.

The hypothesis that opportunistic pathogens use the nutrient resources provided by dead eggs (infertile or non-viable fertile eggs) to colonize the clutch and spread to and kill viable eggs was supported by results of the laboratory experiment. Both at the middle and the end of incubation, the average proportion of fertile eggs that died per clutch was significantly higher in the mixed than in the fertile clutches (Fig. 2a). Two lines of evidence suggest that fungi were pathogenic rather than simply saprotrophic. Firstly, mycelia were observed in close association with the surface of eggs that appeared to be alive (turgid and with white eggshell), and even with eggs that eventually hatched. Secondly, the spatial

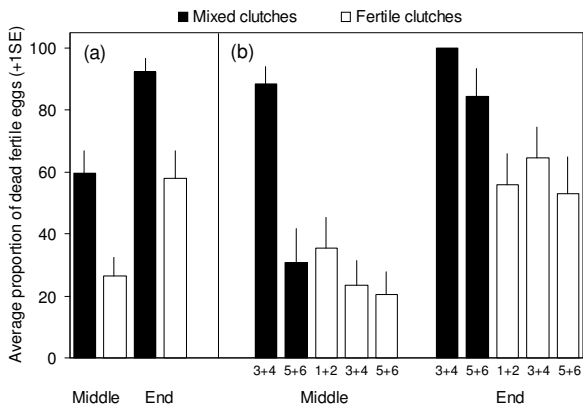


FIG. 2. (a) The average proportion of fertile eggs that died per clutch at the middle and the end of the laboratory experiment was higher in the mixed clutches (4 fertile + 2 infertile eggs; $n=13$) than in the fertile clutches (6 fertile eggs; $n=17$) (Mann-Whitney U -test: Middle: $z=3.33$, $P<0.001$. End: $z=2.97$, $P=0.003$). (b) The average proportion of fertile eggs that died per clutch in the mixed clutches was higher among the eggs (positions 3 and 4) that contacted with infertile eggs (positions 1 and 2) than among the eggs that did not (positions 5 and 6) (results were not statistically significant at the end of the incubation period) (Middle: $z=3.21$, $P=0.001$. End: $z=1.23$, $P=0.32$). In the fertile clutches, the average proportion of fertile eggs that died per clutch at the middle and the end of incubation did not differ significantly according to their position in the clutch (Middle: $z=0.64$, $P=0.52$. End: $z=1.00$, $P=0.22$).

pattern of egg mortality was consistent with a pathogenic basis. In the mixed clutches, the average proportion of fertile eggs that died per clutch was higher among the eggs that were in contact with infertile eggs than among the eggs that were not (results were not statistically significant at the end of incubation; Fig. 2b). Therefore, fungi that colonized infertile eggs (positions 1 and 2) first got in contact and killed adjacent fertile eggs (positions 3 and 4) and then spread from the latter to fertile eggs further away (positions 5 and 6). This pattern of egg mortality was not observed in the fertile clutches (Fig. 2b).

Non-viable fertile eggs also promoted fungal infection of fertile clutches. The pathogenic basis for egg mortality in fertile clutches was supported by the observation that the proportion of eggs that died between the middle and the end of incubation was higher among eggs that were in contact (laterally or diagonally) with eggs that were already dead at the middle of incubation (67%) than among the eggs that were in contact with eggs that were alive at that stage (33%; $\chi^2=4.2$, $df=1$, $P=0.04$). Nonetheless, non-viable fertile eggs were less costly to the remainder of the clutch than infertile eggs, as the proportion of fertile eggs that died in the 14 fertile clutches that were covered by mycelia (70%) was lower than in the 13 (all covered by mycelia) mixed clutches (92%; $\chi^2=9.3$, $df=1$, $P<0.01$).

Among the fertile and mixed clutches, 14 offspring hatched from eggs belonging to 12 different females that were closely surrounded by mycelia and 33 hatched from non-infected eggs belonging to 16 different females. The former hatched significantly earlier and were smaller in every respect and lighter than the latter (Table 1). Large yolk residues were only observed inside the shells of infected eggs, indicating that offspring hatched from these eggs before using the full egg yolk content. The ratio between the average offspring mass and the average original egg mass at oviposition was lower for the offspring that hatched from infected (0.82) than from non-infected eggs (1.00), despite the fact that the average original egg mass was higher for the offspring that hatched from infected eggs (Table 1).

FIELD EXPERIMENT

The hypotheses that dead eggs increase the risks of clutch predation or egg mortality by fungal pathogens were not supported by results of the field experiment. The incubation success of fertile eggs was not lower in the mixed clutches than in the fertile clutches, both when clutch predation and egg mortality not related to predation were analyzed separately and combined. Contrary to predictions, egg mortality tended to be lower among the mixed clutches.

When clutches were first inspected, one fertile clutch contained a broken dried egg (possibly damaged while putting the rock in place) and was therefore excluded from the analyses. At this stage, none of the eggs of the mixed clutches had been predated (0% predated

TABLE 1. Offspring that hatched from fungal infected eggs and from non-infected eggs during the laboratory incubation of mixed clutches (4 fertile + 2 infertile eggs) and fertile clutches (6 fertile eggs), and corresponding duration of incubation, snout-vent length (SVL), tail length, head size and body mass (mean \pm SD). The original egg mass at oviposition for both samples is also shown. Differences were statistically significant between the two groups in every respect (ANOVA). * one missing value for the tail length of a offspring that hatched from a non-infected egg.

	Infected	Non-infected	<i>F</i>	df	<i>P</i>
Offspring (number)	14	33			
Incubation (days)	34.1 \pm 0.86	37.2 \pm 1.04	91	1,45	<0.01
SVL (mm)	28.9 \pm 1.07	29.6 \pm 0.83	5.5	1,45	0.02
Tail length (mm)	36.8 \pm 4.64	41.0 \pm 2.44	17	1,44*	<0.01
Head size (mm)	7.05 \pm 0.23	7.25 \pm 0.21	8.1	1,45	0.01
Mass (g)	0.371 \pm 0.450	0.423 \pm 0.035	18	1,45	<0.01
Original egg mass (g)	0.455 \pm 0.033	0.424 \pm 0.043	5	1,45	0.04

clutches). Ants were destroying four eggs in one fertile clutch and two eggs were missing from another (11% predated clutches). At the second inspection, four fertile eggs were perforated in two mixed clutches (11% predated clutches) and 11 were perforated or missing in four fertile clutches (22% predated clutches). The proportion of predated clutches did not differ significantly between the mixed and the fertile clutches, either at the middle or the end of the experiment (Middle: $\chi^2=2.2$, $df=1$, $P=0.14$. End: $\chi^2=0.9$, $df=1$, $P=0.33$).

Fungi colonized dead eggs in the field experiment (seven of the 19 mixed clutches (37%) had mycelia in the two dead eggs at the middle of the experiment), but did not appear to spread to and kill fertile eggs. At the first inspection, among the 19 mixed clutches (none was predated), only one (5%) contained one (1%) fertile egg that died; among the 16 fertile clutches that were not predated, seven (44%) contained a total of 11 (11%) fertile eggs that died. At the second inspection, among the 17 non-predated mixed clutches, 11 (65%) contained 26 (38%) fertile eggs that died; among the 14 non-predated fertile clutches, all (100%) contained fertile eggs that died, totaling 35 (42%). The proportion of non-predated clutches containing fertile eggs that died was lower in the mixed than in the fertile clutches, both at the middle and the end of the experiment (Middle: $\chi^2=7.3$, $df=1$, $P=0.01$. End: $\chi^2=6.1$, $df=1$, $P=0.01$). The average proportion of fertile eggs that died per clutch did not differ significantly between non-predated mixed clutches and non-predated fertile clutches, both at the middle and the end of the experiment (Mann-Whitney *U*-test: Middle: $z=1.9$, $P=0.06$. End: $z=0.7$, $P=0.50$).

There was no negative overall effect of the presence of two dead eggs within a clutch on the incubation success of the remainder fertile eggs. The proportion of clutches containing fertile eggs that were predated or died from other (undetermined) causes was in fact lower among the mixed than among the fertile clutches (Middle: $\chi^2=9.4$, $df=1$, $P=0.002$. End: $\chi^2=6.8$, $df=1$, $P=0.009$). The average proportion of fertile eggs that were predated or died per clutch was lower in the mixed clutches at the first inspection, but did not differ signifi-

cantly between the two treatments at the end of the experiment (Mann-Whitney *U*-test: Middle: $z=2.3$, $P=0.02$. End: $z=1.0$, $P=0.29$).

EGG INFERTILITY, FUNGAL INFECTION, AND PREDATION IN NATURE

Field estimates of egg infertility and of eggs infected by fungi or predated by ants were all low. Only one of 36 (2.8%) females from Lagoa Comprida and two of 67 (3.0%) females from Torre laid clutches containing infertile eggs, corresponding to one infertile egg out of 248 (0.4%) eggs and two infertile eggs out of 380 (0.5%) eggs, respectively. From a total of 132 unhatched eggs that were dug out from natural nests, 110 (83%) were alive, 17 (13%) were dead, and 5 (4%) were destroyed by ants. Only one of the 17 (6%) dead eggs was surrounded by mycelia. Regarding the 286 eggshells collected from lizard nests, 222 (78%) were from eggs that had hatched, 46 (16%) from eggs that had not hatched and 18 (6%) from eggs that were predated by ants.

DISCUSSION

The present study reinforces previous evidence that fungi can kill reptilian eggs (Fitch & Fitch, 1968; Tracy, 1980) and that dead eggs in a clutch promote clutch colonization by pathogenic soil mycobiota (Phillott & Parmenter, 2001b). In the laboratory experiment, fungi (*Fusarium* sp. and *Gliocladium* sp.) colonized both infertile eggs and fertile eggs that died during incubation and thereafter spread to and killed adjacent eggs. Infertile eggs were more costly to the clutch than non-viable fertile ones, corroborating the idea that the timing at which dead eggs become available for fungal colonization of the clutch determines the proportion of eggs destroyed (Phillott & Parmenter, 2001b). These results also support the hypothesis that oophagy and removal of dead/infected eggs from the nest among lizards that provide care to the clutch (Mitchell & Groves, 1993; Somma, 2003) is an anti-pathogenic mechanism (Groves, 1982; Somma, 1989). Although fungi have been reported to grow under the conditions of lizard

nests (e.g. Hecnar, 1994), the spread of pathogenic fungi from dead to live eggs has not earlier been established in lizards.

Iberian rock lizards hatched earlier (before using the full egg yolk content) from fungal infected eggs than from non-infected ones. In fish and amphibians, early embryo hatching may be a behavioural strategy for escaping from eggs under the risk of pathogen infection (Warkentin *et al.*, 2001; Wedekind, 2002). This hypothesis, which remains to be tested in reptiles, implies that embryos respond to cues that stem from the pathogen or eggs, including embryos' alarm substances (Wedekind, 2002). Alternatively, fungal pathogens may induce early hatching by interfering with the natural mechanisms that trigger hatching (Warkentin *et al.*, 2001). As such, the present observations may be attributed to several causes, including fungal digestion of the eggshell (Ferguson, 1981), fungal exhaustion of the egg water content necessary for the incorporation of yolk into the embryo mass (Packard & Packard, 1988), and the arrest of yolk (precluding its use by the embryo) by hyphae that penetrate through the eggshell (Solomon & Baird, 1980). However, oxygen stress may provide the most parsimonious explanation for the observed results, as it triggers embryo hatching in several taxa (reviewed by Warkentin *et al.*, 2001) and possibly also in reptiles (Losos *et al.*, 2003). Moreover, fungi have been suggested to limit the oxygen available for sea turtle embryos by reducing the egg surface available for respiratory gas exchange (Phyllott & Parmenter, 2001a). Iberian rock lizards that hatched earlier from infected eggs were possibly less fit, as amphibian embryos that hatched earlier (under predation or pathogen infection; Warkentin, 1995, 2000; Warkentin *et al.*, 2001) showed reduced survivorship (Warkentin, 1999). Side-blotched lizards (*Uta stansburiana*) that hatched from eggs that had some of the yolk removed were also smaller and suffered lower survivorship (Sinervo *et al.*, 1992).

The results from the field experiment did not corroborate the fungal pathogenic effects observed under laboratory conditions. The discrepancy between the two experiments may be attributed to two major causes: (1) different incubation conditions and (2) different quality of the eggs. The laboratory incubation conditions may have been optimal for fungal growth, allowing fungi to attain a degree of virulence not common in nature. The eggs used for the laboratory experiment were likely to be of lower genetic quality, as they were obtained from females that copulated fewer times and with fewer males than observed in nature and which were randomly allocated to males (preventing female mate choice). In contrast, eggs used for the field experiment were obtained from females that copulated in the field. Iberian rock lizard females are sexually promiscuous and possibly increase the genetic quality of their offspring through sperm competition, as documented for other reptiles (Olsson & Madsen, 2001). Moreover, female mate choice is described for this species and may result

in genetic benefits for the offspring (Martín & López, 2000; López *et al.*, 2002, 2003). Lower embryo genetic quality for the laboratory experiment might explain the large proportion of fertile clutches that contained non-viable fertile eggs and could have also led to high embryo susceptibility to fungal infections. In agreement with this, genetics (family identity) determined offspring resistance to viral pathogens in common lizards (*Lacerta vivipara*; Uller *et al.*, 2003). Prevention of female mate choice was directly implicated in reduced embryo resistance to bacterial pathogens in whitefish (*Coregonus* sp.; Wedekind *et al.*, 2001, 2004).

Taken together, the results from the present study do not conclusively demonstrate that dead eggs within Iberian rock lizard clutches compromise the incubation success of the remaining eggs. Even though clutches containing infertile or non-viable fertile eggs were colonized by fungi that killed eggs and reduced offspring quality in the laboratory experiment, such effects, or increased clutch predation, were not confirmed in the field experiment. Moreover, low field estimates of egg infertility, egg fungal infection and egg predation indicate that the scope for the above effects to operate in nature may be limited in the studied species. Nevertheless, the above hypothesis deserves further investigation in reptiles, as the laboratory results suggest a potential role of fungal pathogens in the selection of reproductive traits (Olsson & Madsen, 1998) that determine the probability of females laying infertile or non-viable fertile eggs and in the evolution of female egg-laying behaviour (e.g. choice of nesting sites that minimize fungal infection; physical separation of eggs within a nest or between nests). Good models for such studies could be provided by species, such as the sea turtles, that show a significant proportion of failed eggs in natural nests and that suffer from frequent nest contamination by fungi (Phyllott & Parmenter, 2001b).

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