ACID TOLERANCE OF NATTERJACK TOAD (*BUFO CALAMITA*) DEVELOPMENT

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ABSTRACT

The tolerances of spawn and tapoles of the natterjack toad to varying degrees of acidity have been investigated. The results show that:

1. Spawn and small tadpoles are more vulnerable than large tadpoles to low pH.
2. Total mortality of spawn occurs below pH 4.0 with the critical range for survival being between pH 4.0-4.5.
3. Growth rates of tadpoles are increasingly inhibited by pHs between 6.0 and 4.0 even in the presence of excess food.
4. It takes more than 24 hours for spawn to be killed by exposure to low pH (3.5).
5. Healthy spawn is less vulnerable to acid damage than spawn containing large numbers of dead eggs at the outset.

INTRODUCTION

Natterjacks toads *Bufo calamita* are endangered and protected in Britain, having undergone major declines during the 20th century (Beebee, 1976;1977). Most of the losses have been from heathland sites in southern and eastern England and much conservation effort is currently orientated towards reversing this trend which has left only two very small populations on this type of habitat in the United Kingdom. One special aspect of heathland ecology relevant to amphibian fauna is the abundance of acid ponds on the podsolised sandy substrates. In extreme cases pools with pHs of less than 3.0 have been found and most usually fall between pH 3.0-5.0 with only a small proportion closer to neutrality. It is already known that natterjacks avoid acid ponds whenever possible and that larval development is impaired at low pH (Beebee and Griffin, 1977; Strijbosch, 1979). This study set out to obtain more detailed information on the effects of acid conditions on natterjack egg and larval development.

MATERIALS AND METHODS

Very small sections of natterjack spawn (generally no more than 100 eggs from the end of a string containing 5-7,000) were taken from a maximum of four strings at one of the surviving heathland populations of the species. Batches of 10-20 eggs were allowed to develop in 2 litres of tapwater at a variety of pHs adjusted and maintained by the addition of H_2SO_4. Control tanks at neutral pH contained equivalent amounts of added Na_2SO_4 and all were supplied with food *ad libitum* (Beebee, 1983). Development was monitored under laboratory temperature (18-25°) or environmental temperature (2-23°) regimes and tadpole growth rates together with survival of eggs and tadpoles recorded. After metamorphosis surviving toadlets were released at the site of capture.

RESULTS

Eggs, partly grown (12mm) and well grown (20mm) natterjack tadpoles were tested initially for their abilities to survive a 10 day period at a variety of pHs. Prior to the experiment, the small and large tadpoles had been reared in tapwater at pH 7.0. It can be seen from Table 1 that large tadpoles survived at pHs as low as 3.5 whereas spawn and young larvae were killed at or below pH 4.0. Evidently early stages of development were more susceptible to acid toxicity than later ones.

Development Stage	pH 3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0
Eggs	0	8	10	10	8	10	10	10
12mm tadpoles	0	10	8	8	10	10	10	10
20mm tadpoles	9	10	10	10	10	10	10	10

TABLE 1: Sensitivity of spawn and tadpoles to low pH. Experiments started with 10 eggs or tadpoles per tank, and figures are numbers surviving after 20 days at laboratory temperatures.

	pH							
	3.5	4.0	4.5	5.0	5.5	6.0	7.0	4.0[1]
Numbers hatching (laboratory temp)	0	16	19	19	20	20	20	
Growth rates (laboratory temp)	–	0 (0.00)	0.58 (0.06)	0.67 (0.18)	0.92 (0.12)	0.81 (0.03)	0.92 (0.06)	0.33 (0.12)
Numbers surviving 10 days (laboratory temp)	–	0	16	16	18	18	20	6
Numbers metamorphosing	–	0	10	14	18	18	19	0
Numbers hatching at low conductivity (laboratory temp)	0	20	20	20	20	20	20	
Numbers hatching (environmental temp)	0	2	18	16	20	20	20	
Hatch time (days) (environmental temp)	–	20	18	15	14	14	14	

TABLE 2: Effects of pH on survival and growth from spawn. Tanks initially contained 20 eggs each in 2 litres of water, and all figures except line 2 (growth rates) and line 7 (hatch times) represent numbers surviving to a particular stage. Growth rates are given as mm day^{-1}, over a 10 day period with standard deviations in parentheses. 'Low conductivity' tanks contained a 1:5 dilution of tapwater: glass distilled water, with a conductivity at 25° of 180μS cm^{-1}. 1 = Fate of larvae transferred from pH 7 to pH 4 after hatch.

A closer study of pH effects on spawn development is reported in Table 2. Acid-induced mortality was similar at two different water conductivities, indicating that at least over the range tested (which was similar to that seen in the wild) nutrient status of the water did not act in any cooperative way with pH to affect the viability of spawn. The temperature regime did however have some effect on survival to hatch. Spawn reared under relatively warm conditions in the laboratory showed an 80 per cent hatch rate at pH 4.0, whereas eggs outside exposed to uncontrolled environmental temperature fluctuations experienced 90 per cent mortality at pH 4.0, but 90 per cent hatch at pH 4.5. Moreover, hatch times and growth rates were directly related to pH. Eggs which did survive at pH 4.0 took 50 per cent longer to emerge from their jelly surrounds at environmental temperatures (in a rather cold spring) than those at pH 5.5 or above. Even under laboratory conditions growth rates of tadpoles at pH 4.5 were only 60 per cent of those seen at and above pH 5.5. Hatching tadpoles maintained at pH 4.0 in the laboratory failed to grow at all and eventually died; tadpoles from spawn hatched at pH 7.0 and then placed at pH 4.0 grew at only about one third the rate of siblings at pH 7.0 and also gradually died off.

The results of Table 3 show that acidification to pH 3.5 was a relatively slow killer of natterjack spawn. Exposure for up to 24 hours was tolerable without ill effects, though by 48 hours all the eggs were dead and would not recover when transferred to higher pH. Tadpoles hatching from spawn exposed to pH 3.5 for 24 hours grew and metamorphosed normally.

Another factor apparently influencing the susceptibility of natterjack spawn to low pH was its general condition at the start of the experiments. In cases of spawn strings with large numbers of dead eggs, even those apparently viable (as judged by shape and pigmentation) showed much reduced hatch rates below pH 5.0 (Table 4).

DISCUSSION

Natterjack toads are known to be susceptible to at least two kinds of chemical catastrophe in their breeding pools; high salt concentrations resulting from tidal inundations are not infrequent at some sites and the effects of this salination on spawn and tadpoles has been investigated (Mathias, 1971; Andren and Nilsen, 1979; Beebee, 1985). A second type of problem may be

Developmental Stage	Time of Exposure to pH 3.5 (hours)						
	0	0.25	1.00	4.00	24.00	48.00	72.00
Hatch	10	10	10	10	10	0	0
Metamorphosis	10	9	10	10	9	–	–

TABLE 3: Survival times of spawn at low pH. Tanks started out with 10 eggs, and these were transferred from water at pH 3.5 to neutral tanks at the times shown. Figures are the subsequent survival numbers to hatch and to metamorphosis. Experiments were at environmental temperatures.

encountered in inland sites, where heathland podsols often underlay acidic, nutrient-poor surface waters. Oligotrophic or dystrophic conditions are unlikely to have any serious direct consequences for natterjack development (Beebee, 1983) arising from low concentrations of inorganic ions or high concentrations of organic solutes, but the high levels of acidity can be very destructive of amphibian spawn (Beebee and Griffin, 1977; Strijbosch, 1979).

The results of this study show that the critical range of acidity for natterjack toads lies between pH 4.0 and 4.5. At or below pH 4.0 there is likely to be little survival even to hatch under field conditions of fluctuating temperatures, and many *Sphagnum*-dominated pools fall within this lethal range. Between pH 4.0 and 5.0 other environmental factors, especially temperature, may be crucial modulators of survival. Less mortality is likely under consistently warm conditions though even up to pH 5.5 the extent of acidity has a significant impact on tadpole growth rates irrespective of food supply. Longer development times increase mortality indirectly through higher risks of desiccation and predation (Banks and Beebee, in preparation). In the present study, natterjack development was found to be slightly less vulnerable to acid kill than was observed by Beebee and Griffin (1977), though the overall pattern was similar. The earlier study employed spawn containing a high percentage of initially non-viable ova, and the results presented here are probably a more accurate reflection of the tolerance of normal healthy spawn.

Low pH, at least down to 3.5, killed spawn much more slowly than tidal inundation (Beebee, 1985). It would clearly be worth moving fresh spawn from an acid pond to a more suitable one. A similarity to high salt toxicity was however observed in the critical lethal range of pH, i.e. around pH 4.0. At pH 3.5, as at high salt, eggs did not develop at all; at pH 4.0, close to the lethal limit, eggs developed slowly to hatch but subsequently died. Survival to hatch but not beyond was also seen near the lethal salt limit (Beebee, 1985).

This kind of experimental information should form a basis for understanding events in the field. Natterjacks certainly avoid acid ponds in Britain when a choice is available though occasional spawnings in ponds below pH 4.0 have been seen. In such circumstances the eggs have always died. In the Netherlands, attempts to spawn at low pH also result in very high spawn mortality but at least one site is known where a large population of natterjacks is maintained by a pond in the pH range 4.0-4.4 (Strijbosch, 1979; Hulswit and Mulder, 1984). Clearly there are situations in which *Bufo calamita* can survive at the edge of its pH tolerance despite the disadvantages of spawn and tadpole mortality which follow. It may be that the more continental climate enjoyed by the Netherlands, with warmer temperatures in the breeding pools, assists natterjack survival under these extreme pH conditions.

Some comparative information, particularly for North American species, is also available. *Bufo americanus* hatch rates drop precipitously below pH 4.6 and this species is also sensitive to low concentrations of inorganic Al (less than 50μg litre^{-1}) which can act cooperatively with low pH. Other amphibians tested (*Rana sylvatica* and *Ambystoma maculatum*) were less sensitive to low pH than the bufonid (Clarke and Hall, 1985). With the natterjack there seems to be a generally good correlation between pH effects seen in the laboratory and those observed in wild populations. There is therefore little need to consider secondary effects of low pH, such as mobilisation of Al. One exception to this may be the observation that large tadpoles have been seen to die in pondwater of pH 3.5-4.0 quite rapidly (unillustrated data) whereas they are tolerant of such acidity in buffered tapwater. Perhaps in this case heavy metal effects were also manifest, but for the most part H^+ probably acts directly on natterjacks to interfere with Na^+ uptake and secretion as has been shown for a number of North American amphibia (Freda and Dunson, 1984). Hopefully a better understanding of acid threats to amphibians will lead to improved conservation management procedures.

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Spawn Batch	pH of Trial Tank		
	4.0	5.0	6.0
1 (65-70% eggs dead at start)	0	95	100
2 (65-70% eggs dead at start)	0	0	70
3	100	100	100
4	80	90	100

TABLE 4: Susceptibility of different batches of spawn to low pH. Hatch rates of 4 separate batches of spawn, each derived from 2-4 strings, were compared at 3 pHs, and figures shown are percentages of apparently viable eggs which hatched successfully. Batch 1 was that of Beebee and Griffin (1977); Batch 2 was obtained in summer 1983 with a high proportion of dead ova thought to be caused by inefficient fertilisation (Banks and Beebee, in press); Batches 3 an 4 were from strings which later experienced more than 99% hatch.

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APPARENT LACK OF TERRITORIALITY DURING THE BREEDING SEASON IN A BOREAL POPULATION OF COMMON FROGS *RANA TEMPORARIA L.*

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ABSTRACT

The movements within a population of individually marked male Common Frogs *Rana temporaria* were studied during the breeding season. No signs of territoriality were found. The population was characterised by a high degree of disorder and internal movements. Site fidelity within the pond occurred, but was rare. Some other features of the reproductive biology of the species are also described.

INTRODUCTION

Anurans show a great interspecific variation in their behaviour at the breeding site. In most species, males seem to take the most active part in the activities in the breeding ponds. The ability of the females to actively choose a mate is somewhat difficult to prove, but has been discussed by Licht (1976) and Halliday (1983).

A survey of anuran reproductive and mating strategies is given in Wells (1977). As far as the temporal pattern is concerned, there seem to be two broad categories: *prolonged* and *explosive* breeders. Among the former we find species such as the Green Frog *Rana clamitans* and the Bullfrog *Rana catesbeiana*, which maintain well-developed social structures in their breeding ponds, and where male territoriality is an important aspect of the mating strategy (Emlen 1968, Emlen 1976, Howard 1978, Martof 1953 and Wells 1978).

The typical explosive breeders, on the other hand, have a short annual breeding period of one or a few weeks (Wells 1977). There seem to be no species within the group possessing territorial breeding pond behaviour.

The Common Frog, occurring widely in the cooler parts of the Palearctic, is considered a typical explosive breeder by Wells (1977). Its reproductive biology has been studied in Britain (Savage 1961, Ashby 1969), the Netherlands (van Gelder and Hoedemakers 1971, van Gelder, Evers and Maagnus 1978), Poland (Kozlowska 1971), Finland (Koskela and Pasanen 1975) and Sweden (Ericsson and Elmberg 1979, Elmberg and Ericsson 1980). The mating behaviour of its Nearctic relative the Wood Frog *Rana sylvatica* is described by Howard (1980).