SALT TOLERANCES OF NATTERJACK TOAD (*BUFO CALAMITA*) EGGS AND LARVAE FROM COASTAL AND INLAND POPULATIONS IN BRITAIN

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ABSTRACT

(1) Eggs and larvae taken from coastal and inland natterjack populations were found to be similar in their sensitivities to salt water.

(2) Spawn was more susceptible than tadpoles to salt damage.

(3) The effects of salinities comparable with those experienced during a tidal inundation were rapid, mortality occurring within the first 1-2 hours of exposure.

INTRODUCTION

Survival rates during embryonic and larval development are commonly thought to be crucial factors governing overall sizes and age structures of amphibian populations. In the case of the natterjack toad *Bufo calamita*, two aspects of breeding site chemistry have so far been identified as having significant impacts on the reproductive success of wild populations. These are: (i) pH, which can fall low enough at some heathland sites to cause catastrophic spawn mortality (Beebee and Griffin, 1977); and (ii) total salinity, which can rise to lethal levels following tidal surges into exposed coastal pools and marshes. I have investigated the tolerances of spawn and tadpoles from two widely separated natterjack populations to various degrees of seawater contamination; one site was coastal in north-west England, with a small natterjack population breeding in a single, large pool close to the high-water mark and regularly subjected to salt spray as well as (winter) tidal inundation. The second was on heathland in southern England and separated from the sea by nearly 30 km. Two main aims of the work were: (i) to ascertain whether selection pressures had let to genetic differences between the two populations in the salt tolerances of their developmental stages. Such differences, or lack of them, could be relevant to future natterjack translocations. (ii) To establish whether it is possible to rescue spawn following tidal inundation, and how quickly this needs to be done for a high proportion of the embryos to survive.

MATERIALS AND METHODS

Spawn was collected from each of the two sites within 24 hours of deposition, and experiments started within a further 24 hours. Short (100 egg) sections were clipped from the ends of at least 4 separate strings at each site in each of the two study years, to ensure as good a genetic mix of material as reasonably possible. Both colonies are thought to contain fewer than 50 females. Seawater was collected from Brighton beach, filtered through a glass sinter and stored at 4° (for no longer than 1 month) prior to use. Conductivity measurements indicated that the salinity of undiluted seawater was equivalent to about 2.75 per cent (w/v) NaCl.

Natterjack spawn was cut into sections of 10 eggs, and batches from all strings sampled from a particular population mixed together. 10 eggs were then selected randomly, placed in plastic tanks containing 2 litres of various dilutions of seawater, and left to develop at ambient temperatures. Any particular tank therefore contained one section of spawn (10 ova) from one particular female. Controls were carried out allowing development in tapwater. Numbers of eggs or tadpoles surviving to particular stages of development, or for predefined times, were then noted together with the occurence of physical or behavioural abnormalities. Tadpoles were fed on rabbit pellets *ad libitum* (Beebee, 1983). At the end of the experiments, tadpoles or toadlets were released at their sites origin. In some cases, spawn was allowed to develop for fixed periods in water of particular salinities and then transferred to tanks with 2 litres of tapwater to monitor subsequent development.

RESULTS

In the first year of study, tolerance limits of spawn, small (12 mm) and large (20 mm) tadpoles from the two populations to various degrees of salinity were determined (Table 1). Differences between the two populations show no particular trend and are probably trivial. Spawn was killed by 15-20 per cent seawater (0.4-0.55 per cent NaCl), though often not until around the hatching stage. 25 per cent seawater (0.7 per cent NaCl) prevented all development of the embryos. Tadpoles were more resistant, some even surviving for a week in 30 per cent seawater (>0.8 per cent NaCl); there did not appear to be any major difference between small and large tadpoles in this regard, though the latter may have been marginally more tolerant of the salt.

Conditions	Population Locality					
	Coastal			Inland		
	ova	12 mm Larvae	20 mm Larvae	ova	12 mm Larvae	20 mm Larvae
Tapwater (control)	10	10	10	10	10	10
Seawater (as percentage of undiluted)						
5%	10	10	10	10	10	10
10%	8	10	10	8	10	10
15%	10	9	10	10	10	10
20%	0	9	10	0	10	10
25%	0	1	10	0	10	10
30%	0	0	5	0	0	0
35%	0	0	0	0	0	0
40%	0	0	0	0	0	0

TABLE 1. Effects of increasing salinity on egg and tadpole survival
10 ova or tadpoles were placed in each tank at the start of the experiments. Figures given are the numbers surviving 7 days later.

In the second year of the study, two salt concentrations known to be ultimately lethal to spawn (25 per cent and 75 per cent seawater) were used to probe further any possible differences between the two populations. Spawn was exposed for various periods before being removed and placed in tapwater to assess recovery during the remainder of development. 25 per cent salinity might result in a pond from a period of heavy storm spray; 75 per cent levels, or higher, can be reached following high tide inundation. Results of these experiments are shown in Fig. 1. Again, differences between populations appeared to be small and insignificant. Low-level salinity could be tolerated for at least 8 hours with no loss of spawn viability, though this had begun to drop by 24 hours. The effects of 75 per cent seawater were much more rapid. Only for the first 15 minutes or so was the spawn completely

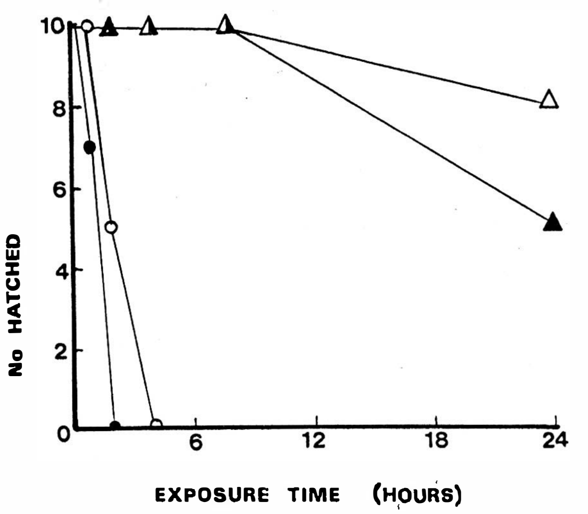

Fig. 1. Effects of salt exposure time on spawn hatch rates. Spawn sections were placed in either 25 per cent or 75 per cent (v/v) seawater, allowed to stand for various times, and transferred to tapwater tanks for subsequent development ▲, Coastal site spawn in 25 per cent seawater; ●, coastal site spawn in 75 per cent seawater; △, inland site spawn in 25 per cent seawater; ○, inland site spawn in 75 per cent seawater.

recoverable; within an hour it had been damaged to the extent that later hatch rates were reduced slightly (to 70-100 per cent normal) and subsequent development showed further reduced viability to 40-90 per cent. By 2 hours the hatch rate was down to 0-50 per cent and ultimate survival 0-30 per cent.

A tadpole abnormality associated with exposure of spawn to immediately sub-lethal salt conditions was also observed. It occurred in 10 individuals, only 1 of which later recovered and seemed to develop normally (Table 2). The condition involved a prolonged post-hatch phase in which the tadpoles grew broader than usual but remained essentially immobile, followed by more normal morphogenesis but with a severe (>90°) horizontal kink at the base of the tail. Afflicted tadpoles could not swim properly but nevertheless grew normally in terms of total bulk and body proportions, though in the wild they would no doubt have difficulty obtaining food and be highly vulnerable to predation.

DISCUSSION

The sensitivities of natterjack tadpoles to saline conditions observed in the two populations examined here was broadly similar to those found by others. Mathias (1971) used tadpoles from another coastal colony in north-west England and found malformation at 20 per cent seawater and complete kill at 25 per cent. Andren and Nilson (1979) investigated a Swedish island population and determined that larvae were killed by between 7-11 per cent salinity (25-39 per cent seawater). Neither of these studies compared the sensitivities of spawn and tadpoles, and evidently the former is the more vulnerable constituting the limiting factor for reproductive success in a brackish environment. It looks, however, as if selection has not operated to increase the salt tolerance of coastal populations relative to those inland. There are at least two possible explanations for this: (i) coastal natterjack populations usually have a variety of

Time of Spawn Exposure (hours)	Population Locality					
	Coastal			Inland		
25% Seawater:	Hatch	Abnormal	Large Tadpole	Hatch	Abnormal	Large Tadpole
0 Exposure	10	0	10	10	0	10
8 Hours	10	0	8	10	0	10
24 Hours	8	5	1	5	1	5
75% Seawater:						
0.25 Hours	10	0	10	10	0	10
1 Hour	7	3	4	10	0	9
2 Hours	0	—	—	5	2	3

TABLE 2. Survival of larvae after various spawn exposure times in saline media.
Experiments used batches of 10 ova, and figures refer to numbers of these surviving to hatch and to large (25 mm) tadpole stages. Numbers of tadpoles showing obvious signs of abnormal development are also given.

breeding pools at their disposal, many or most of which (in the case of dune slacks) are never inundated by the sea and remain completely fresh at all times. The colony selected for this study does not now have such a choice, but its restriction to one exposed breeding pond may be relatively recent following urban development in the surrounding area. Some other coastal colonies, notably in Cumbria, frequently experience inundation and total loss of spawn in particular ponds but these are only one or a few sites out of several or many used by the population as a whole, so selection is unlikely to be intense. (ii) Inundations are often followed by a rapid refreshening of the pools, within a day or two of the high tide, due to the continuous run-off of freshwater from higher land. This effect must be even more marked when only salt spray is involved, probably explaining the observations of Hardy (1974) of both *Bufo calamita* and *B. bufo* tadpoles apparently thriving at 1 per cent salinity in the coastal pond of the present study. Such salt concentrations would be lethal if maintained for many days or weeks, but tolerable for shorter periods. Mathias (1971) found common toad tadpoles to be only slightly less tolerant of salt than those of the natterjack, and Hagstrom (1981) noted *B. bufo* tadpoles thriving at 0.35 per cent NaCl in Norway.

Not only can temporary salination be accompanied by natterjacks, but such events may even be advantageous in some circumstances. The scouring of a pond by saltwater not only puts natterjack spawn at risk, but is also likely to kill off invertebrates (potential tadpole predators) and the tadpoles of other early-spawning anurans (potential competitors). The protracted breeding season of *B. calamita* means that if some spawn of this species is lost at the same time, there is every opportunity for more to be laid in a refreshed pond empty of predators and competitors. This kind of sequence of events certainly happens at some coastal natterjack sites in Cumbria.

It is doubtful whether other natterjack populations, at least in Britain, have been separated for longer (probably >1000 generations) or subjected to greater selective pressures than those compared here. On the basis of this particular parameter there would seem to be no reason why natterjacks could not be translocated from coastal to inland sites or vice-versa, though of course there could be other as yet unrecognised differences between populations on dunes and heaths. For more immediate conservation purposes, the rapid destruction of embryos caused by concentrated salt solutions comparable with seawater means that any rescue of spawn from inundated sites would need to be carried out more or less at the time of the event for it to be worthwhile. In some cases this may be possible, but there would clearly be little point in, for example, moving spawn in the morning following a tidal surge sometime the previous night.

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