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Effects of Environmental pH and Temperature on Embryonic Survival Capacity and Metabolic Rates in the Smallmouth Salamander, *Ambystoma texanum*

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Although the deleterious effects of acid precipitation on forest ecosystems (DOCHINGER 1976) and fisheries (LIKENS & BORMAN 1974; RUNN et al. 1977) have been documented, relatively little information is available on the effects of environmental pH in combination with temperature on the survival capacity and/or physiology of amphibians. In view of the aquatic nature of embryonic and larval amphibian life history strategies, these vertebrates can be especially sensitive to local changes in the pH of their aquatic habitats. Previous investigations on fish have indicated that increased acidity due to mine drainage or acid deposition can lead to mortality primarily through disruption of respiration and/or sodium balance (PACKER & DUNSON 1972). In amphibians, low pH can result in decreased survival capacity (PORTER & HARKANSON 1976; POUGH 1976), but the specific effects are not well understood.

POUGH (1976) reported that increased acidity resulting from acid precipitation common to the northwestern United States, can lead to increased mortality in the spotted salamander, Ambystoma maculatum. Egg mortality was found to be less than one percent in ponds near neutrality, but greater than 60% in ponds more acidic than pH 6.0 . Embryonic developmental anomalies such as gill asymmetry and swelling of the thoracic cavity (pH 5 to 6) as well as deformation of the posterior trunk region (pH 4 to 5) were shown to occur as well. Similar studies on A. jeffersonianum (POUGH & WILSON 1977) showed that this species was able to tolerate (50 to 89% hatching) a lower pH (as low as 4.0) than A. maculatum . SABER & DUNSON (1978) showed that the mortality of bullfrog larvae (Rana catesbiana) was significantly higher at pH 3.9 to 6.6 than it was at 6.6 to 6.9 . DUNSON & CONNELL (1982) reported that low pH resulted in a significant inhibitory effect on the hatching of Xenopus laevis embryos. Hatching was completely inhibited at pH 3.9 in sulfuric acid and at pH 4.3 in bog water.

In view of the potentially significant deleterious effects of stressful pH conditions on amphibian survivorship and the relative paucity of data on this subject, the present study was conducted in order to ascertain the combined effects of temperature and pH on embryonic metabolic rates and survival capacity in the smallmouth salamander, Ambystoma texanum Mathes. No previous data on these parameters are available for this species. This paper represents ongoing research on the effects of various environmental contaminants on the survivorship and physiology of herpetofauna (PUNZO et al. 1979).

MATERIALS AND METHODS

Eggs and larvae of Ambystoma texanum were obtained from a breeding laboratory stock of adult salamanders originally collected from ponds in Jackson Co., Illinois during March, 1979. Eggs, larvae and adults were maintained in aquaria at 17 deg C and provided regularly with pond water from their original collection sites. The pH of these waters was monitored with a Fisher portable pH meter at weekly intervals for 12 months and found to range from pH 6.4 to 6.7 .

Studies were conducted to evaluate the combined effects of temperature and pH on the hatching success of pre-gastrulation eggs during various embryonic stages. Embryonic stages were identified according to RUGH (1962). The procedure used to determine the effects of temperature and pH on embryonic development was similar to that described by POUGH & WILSON (1977). Tests were conducted in a Precision Model 816 incubator. Temperatures were controlled at 5 deg C intervals from 5 to 30 deg C. Four replications (each with 25 eggs) were tested at each combination of temperature and pH. Prior to testing, the eggs were removed from the aquaria and placed in ventilated plastic containers provided with filtered pond water. Desired pH test solutions were obtained by adding NaOH or H_2SO_4 to the pond water. Stock test solutions were obtained in advance and maintained at the desired test temperature. The pH of these test solutions was checked with a Coleman Metrion IV pH meter immediately prior to testing. Eggs were observed daily and developmental stages recorded according to RUGH (1962). Later developmental stages were identified on the basis of gill development (WILDER 1924). The criterion for reproductive success was embryonic development to the hatching and/or gill circulation stage characterized by the absence of developmental abnormalities. If neither gill circulation or hatching occurred after 100 days, the experiment was terminated and mortality recorded.

Studies were conducted to ascertain the effects of pH on embryonic metabolic rates at the temperature shown by the previous survival capacity studies to be "optimum" for hatching success (15 deg C). Oxygen consumption rates were recorded for 40 animals at each developmental stage studied. Stabilized oxygen consumption rates were determined at hourly intervals at 15 deg C in shallow water in a glass respirometer as previously described by SCHOLANDER et al. (1952). Respirometer vessels were provided with natural pond water at pH 6.6 (control) or pond water treated with H_2SO_4 or NaOH to obtain desired pH test solutions as described previously. The gas phase consisted of atmospheric air and CO_2 was absobed using filter paper strips treated with KOH (FITZPATRICK et al. 1972). Oxygen consumption measurements were converted to standard conditions (ul O_2 mg $^{-1}$ hr $^{-1}$) according to SCHOLANDER et al. (1952).

RESULTS AND DISCUSSION

The combined effects of temperature and pH on the hatching success of _A. texanum_ are listed in Table 1. Embryonic development was unsuccessful at 5 deg C and 30 deg C regardless of environmental pH. The optimum hatching success at all pH regimes occurred at 15 deg C for this species. This is in contrast with the results reported by POUGH & WILSON (1977) for _Ambystoma maculatum_ and _A. jeffersonianum_ . For _A. maculatum_ ,

Table 1. The combined effects of temperature and pH on the embryonic development (hatching success) of <u>Ambystoma texanum</u>

deg C	Percent Survival (%)							
	pH 4	5	6	7	8	9	10	11
5	0	0	0	0	0	0	0	0
10	13	21	75	83	81	71	69	19
15	17	30	95	92	90	78	73	29
20	9	20	64	74	66	59	50	2
25	6	14	30	24	27	19	7	0
30	0	0	0	0	0	0	0	0

optimal conditions for hatching were found to occur between 10 and 15 deg C at pH 7 to 9 . For <u>Ambystoma jeffersonianum</u> , optimal hatching conditions were achieved between 5 and 10 deg C at pH 5 to 6 . The results in Table 1 indicate that "optimal conditions" ($>$ 90% hatching) as defined by POUGH & WILSON (1977), for <u>A. texanum</u> in this study were observed only at 15 deg C within a pH range of 6 to 8 . Moderate hatching success (50 to 89%) was observed between 10 and 20 deg C within a pH range of 6 to 10. Severe mortality ($<$ 50% hatching) was found to occur at low (5 deg C) and high (30 deg C) temperatures at all pH values tested, and at 10 to 25 deg C at pH 4 to 5 and pH 11. A two-way analysis of variance showed the effects of temperature ($F = 4.81$, df 3/44, $p < .05$) and pH ($F = 4.51$, df 7/44, $p < .05$) to be significant. The most pronounced detrimental effects of pH on embryonic survival capacity were found at pH 4 to 5 and pH 11 over the temperature range of 10 to 25 deg C, suggesting that pH values greater than 10 and less than 5 represent conditions of pH stress for *A. texanum*.

The effects of pH on oxygen consumption rates (at 15 deg C) of various embryonic stages of *A. texanum* are shown in Table 2 . The highest rates were observed at pH 6.6 for all developmental stages (the value normally associated with the pond water in which this species is found). Significant differences were found between oxygen consumption rates of fertilized eggs observed at pH 4 and 6.6 ($t = 3.86$, $p < .01$), and between those observed at pH 6.6 and 11 ($t = 2.14$, $p < .05$). Significant differences ($t = 4.33$, $p < .01$) were also found between oxygen consumption rates of fertilized eggs observed at pH 4 and 5 for the dorsal lip, late gastrula and neural plate developmental stages.

The effects of environmental pH on the hatching success (Table 1) and metabolic rates (Table 2) of *A. texanum* embryonic stages reported in thi- study indicate that mortality in this species can be expected to increase markedly when the pH of pond water falls below 6.0 or exceeds 10.0 . This species is characterized by reproductive behavior that varies between populations. Some individuals attach egg masses to submergent pond vegetation whereas others are found to breed in streams and attach single eggs beneath rocks. Both pond and stream habitats are susceptible to changes in pH resulting from acid deposition, although pond habitats would tend to be more weriously altered and resultant pH changes would likely persist for longer durations. In Illinois, *A. texanum* breeds from late February throughout March and eggs have been reported from flooded fields, ditches, ponds and woodland streams (SMITH 1961). All of these breeding habitats are subject to pH alterations resulting from acid precipitation. Thus, it is of vital importance to assess the effects of environmental pH on the survival capacity and physiology of aquatic organisms. Further studies are needed along these lines with amphibians at all developmental stages. Recent studies although few, have suggested that acidification of breeding sites may represent an important factor in declining populations of British amphibians (PRESCOTT et al. 1974).

Table 2. Effects of pH on oxygen consumption rates (ul O_2 mg $^{-1}$ hr-1) of developmental stages of *Ambystoma texanum*, at 15 deg C.

Stage [a]	Oxygen Consumption Rates (ul O_2 mg $^{-1}$ hr $^{-1}$) at various pH				
	pH 4.0	5.0	6.6[c]	8.0	11.0
1	0.6 ± .01[b]	1.39 ± .03	1.48 ± .02	1.38±.01	0.9±.01
2	1.2 ± .04	2.10 ± .02	2.11 ± .11	2.03±.01	1.6±.31
3	1.5 ± .17	2.67 ± .10	2.72 ± .20	2.51±.16	1.9±.26
4	2.2 ± .23	3.45 ± .21	3.60 ± .46	3.47±.19	2.4±.17

[a] Developmental stages according to Wilder (1924) : 1 - fertilized egg; 2 - dorsal lip; 3 - late gastrula; 4 - neural plate

[b] Mean ± S.D. ; N = 40

[c] pH 6.6 represents the control group. The pond water from the collection sites ranged from pH 6.4-6.7 .

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