

ACID PRECIPITATION AND REPRODUCTIVE SUCCESS
OF *AMBYSTOMA* SALAMANDERS

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ABSTRACT

The two species of mole salamander that occur in the Ithaca, New York, region (*Ambystoma maculatum* and *A. jeffersonianum*) breed in temporary ponds that are formed by accumulation of melted snow and spring rains. Water in many of these pools during the breeding season is acid; pH values as low as 3.5 have been measured. In laboratory experiments *A. maculatum* tolerated pHs from 6-10 and had greatest hatching success at pH 7-9. *Ambystoma jeffersonianum* tolerated pH 4-8 and was most successful at pH 5-6. Mortality rose abruptly beyond the tolerance limits. The pH optimum shifted upward with increasing temperature for *A. jeffersonianum* and downward for *A. maculatum*.

Judging from our laboratory studies, the acidity measured in breeding ponds should cause mortality in *A. maculatum* and permit normal development in *A. jeffersonianum*. In a four-year study of a large acidic vernal pond, 938 adult *A. maculatum* produced 486 metamorphosed juveniles (0.52 juvenile/adult), while 686 adult *A. jeffersonianum* produced 2157 juveniles (3.14 juveniles/adult). Because the effects of acid precipitation on the salamanders' breeding ponds are cumulative from year to year, profound changes in the salamander populations can be anticipated.

INTRODUCTION

Certain species of amphibians may be the vertebrate animals most immediately and directly affected by acid precipitation. Their vulnerability to this form of pollution derives from their reproductive habits. Especially in temperate regions, many species of amphibians congregate at ponds to lay eggs which hatch into aquatic larvae. This pattern is particularly characteristic of frogs and toads; 90% of the

species in the United States adhere to it (Salthe and Duellman 1973, Table 8). Salamanders show more diversity of reproductive modes but half of the terrestrial salamander species of the United States lay eggs in water. (In tropical regions a majority of amphibians lay eggs out of water. In some of these species the aquatic larval stage is also omitted.)

Temperate-zone amphibians are not only largely aquatic in their reproduction, but many species breed in temporary pools formed each year by accumulation of rain and melted snow. Approximately 50% of the species of frogs and toads in the United States regularly breed in ephemeral pools of this nature. Calculating a similar percentage for salamanders is more complicated because there are permanently aquatic species as well as completely terrestrial ones. One-third of the species that have aquatic eggs and larvae and terrestrial adults breed in temporary pools.

These temporary pools are usually completely empty at some time during the year and are refilled by rain and/or melted snow shortly before the amphibians enter them. For many species of amphibians the heavy rains that fill the pools are also the stimulus that initiates breeding activity. Because there is no water in the pools at the end of the dry season, the precipitation that fills them is not diluted by water already present as is the case in a permanent pond. Furthermore, most of these pools are small and collect drainage from a limited area. As a result, precipitation has relatively little contact with soil buffer systems before it enters the pools. In many regions amphibians breed early in the spring, before deciduous trees have sprouted leaves, so precipitation is not buffered by passage through the forest canopy. Because of these characteristics of amphibian breeding sites, the acidity of the water in the pools is strongly influenced by the pH of the precipitation that fills them and the pools are more acid than adjacent permanent bodies of water. During the spring amphibian breeding season in 1975, pH was measured in ponds used by frogs or salamanders near Ithaca, New York. The average pH of 12 temporary ponds was 4.5 (range 3.5-7.0). Permanent ponds are less acidic, the average of 6 used as amphibian breeding sites was pH 6.1 (range 5.5-7.0) (Pough, in prep.). Amphibian eggs and larvae in the temporary pools are exposed to these acid conditions.

There is virtually no information about the effect of pH on amphibian embryonic development, especially in an ecological context.

The single study we have found, Gosner and Black (1957) reported that only acid-tolerant species of amphibians could breed in sphagnum bogs in the New Jersey Pine Barrens. Less tolerant species were excluded from the Pine Barrens or occurred only in more alkaline pools, most of which were products of human activity.

Temperature is another potential environmental stress to which amphibian eggs are exposed. Some amphibians breed when pools are partly ice-covered and water temperatures near freezing. Sunlight

penetrating the leafless forest canopy can raise water temperatures to 25 C or higher before the eggs hatch. In contrast to the paucity of information about the effects of pH on amphibian development, there is a massive body of information about the effects of temperature. Both the ecological and evolutionary significance of rate of development and tolerance limits of particular species have been extensively considered. (See Zweifel 1968 for a recent review.) We know of no study that has considered the effects of temperature and pH in combination, and data of this sort are essential in predicting the effects of acid precipitation on amphibian populations.

MATERIALS AND METHODS

Two species of mole salamanders widely distributed in eastern North America were studied. The geographic range of the spotted salamander (*Ambystoma maculatum*) extends northward from the Gulf of Mexico into southern Canada. The Jefferson salamander (*A. jeffersonianum*) has a more northern distribution extending southward from the Canadian border to northern Kentucky and Virginia. Both species occur in the Ithaca region, although the Jefferson salamander is found only in local populations. Both species breed in vernal ponds formed by accumulation of melted snow and rainwater, or in small permanent ponds. Freshly laid eggs were collected in the field or inseminated females were brought to the laboratory and held at field temperature until they laid their eggs. Eggs and salamanders were obtained from three sites, all in Tomkins County, New York: a small pond on the Cornell University Golf Course (N 42° 27' 30" W 76° 28' 00" pH 7-8), the Lloyd-Cornell Wildflower Preserve (N 42° 27' 00" W 76° 22' 26" pH 5-6), and several pools on Connecticut Hill (N 42° 22' W 76° 40' pH 5.5-6.5). Only eggs that had not reached gastrulation were used, and every combination of temperature and pH was tested at least once with eggs in the temperature-sensitive stages of early cleavage.

Incubation temperatures were controlled (± 1 C) at 5 C intervals from 5 to 35 C. The eggs were placed 40-60 cm from a 20 W cool white fluorescent tube and a 12-hour photoperiod centered on noon EST was maintained. Sulfuric acid or NaOH was added to dechlorinated tap water to produce pH values from 4 to 11. The ionic content of the water approximates that of water in the breeding ponds. Sulfate is the predominant anion in these ponds (average concentration 3.9 mg/l, range 2.9-4.9, n = 5; Pough, in prep.). Nitrate concentration in the breeding ponds is low because of biological activity.

Solutions were prepared in advance and stored at the test temperature, pH was checked with a pH meter before the solutions were used. For each temperature/pH combination 20-30 eggs were placed in 300 ml of solution in a capped, ventilated plastic dish (9.5 x 9.5 x 6.5 cm). After eggs were added the pH of these unbuffered solutions drifted as

much as 0.5 pH units toward neutrality in the intervals between daily changes. Critical temperature/pH combinations were tested repeatedly with eggs from different clutches, and total sample sizes for different combinations range from 20 to over 100. We tried to retain the eggs within the gelatinous mass in which they were deposited, but handling expelled some eggs from the jelly. Eggs outside the mass appeared to develop slightly faster than those that remained in the jelly, but no other effects of separation were noted and no distinction was made in the analyses between individual eggs and eggs *en masse*.

Eggs were examined daily and development recorded by reference to the Harrison stage (Rugh 1962) except at 5 C where examinations were made on alternate days. We had no difficulty comparing different temperature/pH combinations until the later embryonic stages were reached. At that point developmental stages are defined largely on the basis of gill development, and gills of individuals at high temperatures were hypertrophied while those of individuals at low temperatures were small. Our criterion of success was development of an embryo without gross abnormalities to the gill circulation or hatching stage. Hatching sometimes preceded gill circulation, but in every case in which one occurred the other followed. If neither had been achieved in 100 days the experiment was terminated. Larvae that appeared normal were released in the breeding ponds.

Field temperatures were measured with calibrated Wesco Museum Special thermometers or calibrated thermistors. Pond water pH was measured in the field with a Fisher portable pH meter or with LoIon pHDrion paper. Data were graphed on an IBM 1120 computer using a program written by Dr. Donald Allen.

RESULTS

HATCHING SUCCESS

We have followed previous authors in distinguishing several levels of hatching success. "Extreme" conditions never produced successful development. "Severe" mortality was < 50% hatching, and "moderate" mortality was 50-89% hatching. "Optimal" conditions produced $\geq 90\%$ hatching. The optimal conditions were different for the two species. For *A. maculatum* these were pH 7-9 at 10-15 C, while for *A. jeffersonianum* the greatest success was achieved at pH 5-6 at 5-10 C (Figure 1). There was a similar but less pronounced difference in conditions that produced moderate and severe mortality. As temperature increased, the pH giving the greatest success increased in *A. jeffersonianum*, but not in *A. maculatum*.

A two-way analysis of variance was performed on the ungrouped data. For *Ambystoma maculatum* the effects of temperature and of pH were

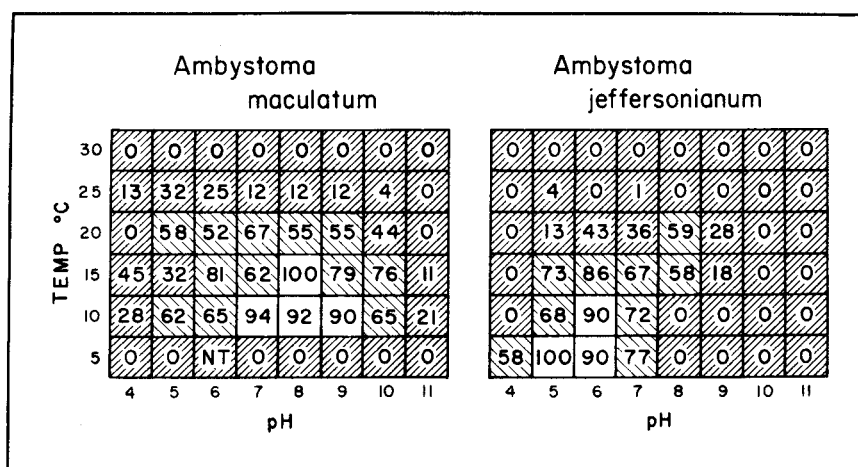


Figure 1. Hatching success of *Ambystoma maculatum* and *A. jeffersonianum* at different combinations of temperature and pH. The figure in each square is the percentage of eggs tested under those conditions that developed successfully.

significant (Table I) but the interaction between temperature and pH was not significant.

For *Ambystoma jeffersonianum* significant effects of temperature and of interaction between temperature and pH were demonstrated (Table I). Additional comparisons involving cell means were computed. At 5 C survival at pH 5 and 6 was significantly greater than survival at pH 4, 7, 8, or 9 ($F_{1,53} = 30.43$, $p < 0.001$). At 10 C survival at pH 6 was significantly greater than survival at pH 4, 5, 7, 8, or 9 ($F_{1,53} = 5.763$, $p < 0.025$).

SUBLETHAL EFFECTS

Temperature profoundly affected the rate of development. The average time from gastrulation (stage 10) to hatching is shown in Figure 2. *Ambystoma jeffersonianum* developed more rapidly than *A. maculatum* at all temperatures; the difference was greatest at low temperatures. *Ambystoma maculatum* did not develop successfully at 5 C, and *A. jeffersonianum* did not tolerate 25 C. Neither species survived at 30 or 35 C.

The pH had little effect on rate of development (Figure 3). The experiments illustrated were conducted at 10 C which is within the optimum temperature range for both species. There is some variation in the stage of development of different pHs on a given day, but development was not consistently faster or slower than the average at any pH

Table I. Two-way analysis of variance of survival
at different temperatures and pHs.

Source	SS	df	MS	F
<u>Ambystoma maculatum</u>				
Temperature	1.266	3	0.422	3.802*
pH	2.791	7	0.399	3.596*
Temperature x pH	1.835	21	0.087	0.784 n.s.
Error	4.896	44	0.111	
<u>Ambystoma jeffersonianum</u>				
Temperature	1.640	3	0.547	4.675**
pH	0.640	5	0.128	1.094 n.s.
Temperature x pH	5.741	15	0.383	3.274***
Error	6.189	53	0.117	

n.s. = $p > 0.05$

* = $p < 0.05$

** = $p < 0.01$

*** = $p < 0.001$

and the daily variation resulted largely from the stepwise character of the Harrison stages. In each species only one pH produced hatchlings in this experiment (pH 6 for *A. jeffersonianum*, pH 8 for *A. maculatum*) but development proceeded as rapidly at pHs that were to prove lethal as it did at the optimal pHs.

STAGES AT WHICH ABNORMAL DEVELOPMENT APPEARED

Under extreme conditions of temperature or pH death occurred within a few hours of the eggs' being placed in the test solutions. In some cases development ceased at the stage at which the egg was put into the solution, in others irregular cell divisions continued for several hours. These eggs became mottled without a differentiation between the animal and vegetal poles. By blastula (stages 7-9) the eggs were shriveled and no further development occurred. Mortality produced by high temperatures occurred in these early stages. Lethal effects of acidity were expressed at different developmental stages depending upon the severity of the stress. Under severe pH stress developmental anomalies appeared during late gastrulation. Yolk plugs failed to retract or retracted incompletely. Some embryos died at this stage, others

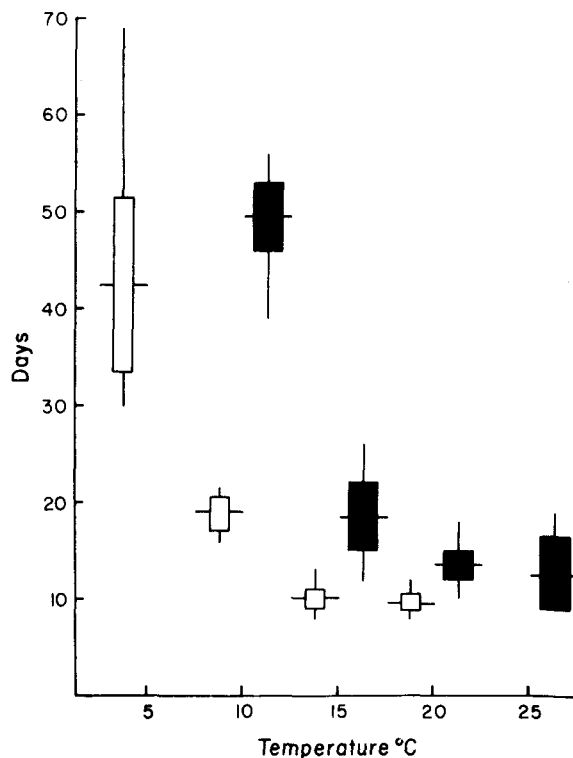


Figure 2. Effect of temperature on rate of embryonic development in *Ambystoma maculatum* (dark symbols) and *A. jeffersonianum*. The vertical axis shows the time in days for development from gastrulation to hatching. At 5 C *A. maculatum* eggs did not hatch, and 25 C was lethal to *A. jeffersonianum*.

survived with deformations of the spine and tail. The body usually arched dorsally. Those embryos that hatched retained this deformity, rested on their sides, and were barely able to swim. Moderate mortality was associated with abnormalities appearing late in the embryonic period, especially at gill development (stages 37-40). A swelling on the body wall near the heart was often seen, and the gills were asymmetric, sharply kinked, and stunted.

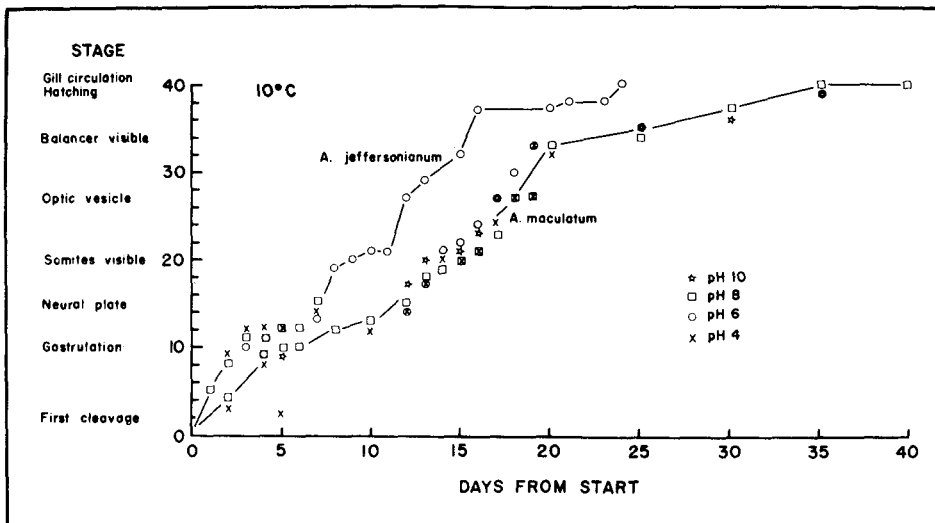


Figure 3. Effect of pH on rate of embryonic development of *Ambystoma maculatum* and *A. jeffersonianum*. Although only one pH produced hatchlings in each species, development proceeded at the same rate at all pHs to the point of embryonic death.

SPECIAL CASES

At 5 C *A. maculatum* eggs progressed to stage 37 at all pHs that permitted development, but did not hatch nor initiate gill circulation. If the eggs were moved to warmer temperatures they hatched after several days.

At pH 4 *A. maculatum* egg membranes shrank, forcing embryos into a sharp curve. Development of some embryos was apparently normal except that they had little space to move and did not engage in the muscular activity that normally characterizes late embryos. These embryos did not hatch spontaneously, and when they were removed from the egg membranes they were unable to straighten their bodies to swim. Although this occurred in every trial of *A. maculatum* eggs, it was seen only once in *A. jeffersonianum*.

DISCUSSION

TEMPERATURE TOLERANCE AND RATE OF DEVELOPMENT

The temperature relationships of the two species of *Ambystoma* conform to Moore's (1939) conclusions concerning anurans; northern forms that breed at lower water temperatures than southern forms have lower minimal and maximal temperatures for development than the southern forms, and develop more rapidly at low temperatures. Normal development was confined to a temperature range of only 10-15 C in the salamanders compared to a 22-24 C range in the frogs Moore studied.

Anderson (1972) studied six populations (four species) of *Ambystoma*. He concluded that rate of development and temperature tolerance reflected adaptation to life history pattern and the climate of a species' range rather than its geographic location *per se*. Inasmuch as *A. maculatum* and *A. jeffersonianum* breed in the same pools in the Ithaca region, Anderson's conclusions do not appear to apply to this case.

TOLERANCE OF pH EXTREMES

The most interesting results of the present study concern the effects of pH on hatching success. Mortality resulting from acid conditions can be distinguished from that produced by temperature extremes; high temperatures killed eggs at early developmental stages while low temperatures (in *A. maculatum*) permitted development but inhibited hatching. Furthermore, temperature profoundly affected the rate of development. In contrast, stressful pHs appeared to act as a series of hurdles in the embryonic process. Mortality occurred at specific developmental stages and development proceeded normally between these stages. Embryonic deformities produced by stressful pHs appear identifiably different from those resulting from temperature-induced chromosomal abnormalities (Fankhauser 1945), low oxygen (Hall 1924), and metal salts (Deuchar 1966). Some mutations described in a related salamander, the Mexican axolotl (*A. mexicanum*) are grossly similar to the deformities seen at stressful pHs, especially mutations *f*, *g*, and *v* (Malacinski and Brother 1974). They differ from pH stress in morphological detail and time of expression (Humphrey 1948, 1959, 1960, 1962; Briggs and Humphrey 1962; Justus and Humphrey 1964; Tompkins 1970). The similarities that do exist probably indicate only that both the mutations and stressful pHs cause profound cellular and subcellular rearrangements at early developmental stages. For example, acid or alkaline stress causes rearrangement of RNA in amphibian eggs (Brachet 1960) similar to that produced by mutation *v* (Briggs and Humphrey 1962), and varying the

acidity of the medium changes surface charges differentially in different germ layers (Schaeffer et al. 1973).

In 1957 Gosner and Black published a study of the effect of pH on development of several species of anurans that occur in or near the New Jersey Pine Barrens. They concluded that the low pH (3.6-5.2) associated with sphagnaceous pools in the Pine Barrens limited the breeding sites of most anurans to less acidic areas such as grassy ponds and gravel pits. Only *Hyla andersoni* and *Rana virgatipes*, species endemic to the Pine Barrens, could breed successfully in sphagnaceous pools. Ten of 12 amphibian breeding sites in temporary pools near Ithaca, New York, had pHs below 6 (the approximate lower limit of tolerance of *A. maculatum*). Six of the 12 pools were pH 5 or lower. On the basis of laboratory results, these acidic pHs should cause significant mortality in *A. maculatum*. Egg masses with a high proportion of embryos dead were frequently observed in this study, as in previous work with *Ambystoma* by other investigators (Anderson et al. 1971, Shoop 1974). Recent field work conducted by the senior author (Pough, in prep.) indicates that mortality of *A. maculatum* in natural breeding ponds closely corresponds to mortality seen at similar pHs in the laboratory. In a series of ponds ranging from pH 4.5 to 7.0, hatching success fell from > 90% at pH 6.5 and above to 40-60% at pHs lower than 6.5. An abrupt transition from high to low success occurred between pH 7 and pH 6 in the field as it does in the laboratory.

Relative reproductive success of the two species of *Ambystoma* at a common breeding site, an acidic (pH 5.0-6.5) pond in the Lloyd-Cornell Wildflower Preserve, is consistent with the hypothesis that there is an environmental stress that affects *A. maculatum* more severely than *A. jeffersonianum*. From 1968 through 1971 the pond was enclosed by a fence and virtually every adult entering the pond and juvenile leaving it was counted (Wilson 1976). In the four years of the study, 938 adult *A. maculatum* produced 486 juveniles, an average of 0.52 juvenile/adult. In the same period 686 *A. jeffersonianum* (most of which bred biennially) produced 2157 juveniles, an average of 3.14 juveniles/adult.

PROBABLE ECOLOGICAL EFFECTS OF ACID PRECIPITATION

We suspect that acid precipitation has already had a deleterious effect on the reproduction of *A. maculatum* in the Ithaca region. The annual mean pH of precipitation of Ithaca, New York, in 1970-1971 was 3.98 (Likens and Bormann 1974). Likens (1972) calculated that 1 meter of precipitation at pH 4 leaches the equivalent of 50 kg CaCO_3 /hectare. This represents the approximate annual loss of soil buffering capacity in the Finger Lakes region of New York. The effect is cumulative, and in terms of amphibian breeding sites is increased by the fact that the water comes in contact only with leaf litter and superficial layers of the soil before entering the pools. It seems inevitable that the pH of

the breeding pools will continue to fall toward the pH of incident rain and snow. If the pH in the pools reaches those levels, *A. maculatum* will be unable to reproduce and *A. jeffersonianum* will experience significant mortality. Lack of precise information on water pH and reproductive success of any *Ambystoma* before the advent of acid rain prevents documentation of its effects. The data presented here will provide a baseline for future study.

Presently *A. maculatum* is more abundant than *A. jeffersonianum* in the Ithaca region. We know of many sites at which only *A. maculatum* breeds, but none utilized exclusively by *A. jeffersonianum*. This situation probably reflects human land use in the 18th and 19th centuries; there is little indication of direct competition between the species (Wacasey 1961, Wilson 1976). A century ago central New York was largely farm and pasture land. Now much of that land is reverting to woods, and second growth forest is widespread. *Ambystoma maculatum* breeds in overgrown pastures and second growth woods, but in our experience *A. jeffersonianum* occurs only in or adjacent to areas of relatively undisturbed woods. The pH of breeding sites does not explain the present distribution of *A. jeffersonianum*. They breed successfully in ponds in undisturbed woods near Ithaca at pHs of 7.0 and 7.25 as well as in ponds with pHs from 4.5 to 6.0 in the Wildflower Preserve, but are absent from ponds in second growth woods with pHs of 4.5 to 7.0, and from ponds in meadows (where *A. maculatum* breed) with pHs of 6.0 to 7.0.

We believe that *A. maculatum* was able to tolerate land clearing associated with agriculture, maintaining populations in hedgerows and small patches of woodland, while *A. jeffersonianum* could not utilize these habitats and persisted only in undisturbed woods. As farms were abandoned and succession returned fields to woodland, the small populations of *A. maculatum* formed nuclei to invade the restored habitats, but *A. jeffersonianum*, confined to a few limited populations, has not been in a position to exploit second growth woods. Strips of cleared land between woodlots are probably a greater barrier to a dispersal of *A. jeffersonianum* than they are to *A. maculatum*. Attempts to reintroduce *A. jeffersonianum* into apparently suitable habitat by moving egg masses or larvae from pond to pond would probably be successful. One population was established this way in 1969 in a small permanent pond (pH 7.0-8.0) with larvae produced in the experiments described here. It is still in existence and appears to be growing. Before that attempt is made on a large scale, however, more should be known about the ecological relationships of *A. jeffersonianum* and *A. maculatum* to ensure that introduction of *A. jeffersonianum* does not add to the stress to which *A. maculatum* is subjected.

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