Effects of Hydroperiod on Recruitment of Mole Salamanders (Genus *Ambystoma*) at a Temporary Pond in Vermilion County, Illinois

Christopher A. Phillips[1], Jarrett R. Johnson[1,2,4], Michael J. Dreslik[1,3],
and John E. Petzing[1]

[1]Illinois Natural History Survey, Center for Biodiversity, 607 E. Peabody Drive,
Champaign, IL 61820, USA
[2]Department of Ecology Ethology & Evolution,
University of Illinois at Urbana-Champaign, Champaign, IL 61820, USA
[3]Department of Natural Resources & Environmental Science,
University of Illinois at Urbana-Champaign, Champaign, IL 61820, USA
[4]Present address: Department of Biology, University of Missouri at Columbia,
Columbia, MO 65211, USA

Corresponding author: Christopher A. Phillips

ABSTRACT

We used a drift fence/drop can array during two years to examine the effect of hydroperiod on juvenile recruitment in four species of mole salamanders (*Ambystoma*). In a long hydroperiod year, size at metamorphosis was negatively correlated with day of emigration suggesting that salamander larvae inhabiting ephemeral ponds can take advantage of rare, long hydroperiod conditions. Abundance of metamorphs declined for polyploids in the long hydroperiod year, but increased for the other species. Average size at metamorphosis, species diversity, and species equitability were greater in the long hydroperiod year.

INTRODUCTION

Many amphibian species breed in ephemeral ponds that undergo daily fluctuations in water level and annual changes in hydroperiod. Ephemeral ponds present a trade-off between an environment free of predatory fish and a stochastic risk of larval mortality due to desiccation. Some amphibians such as *Scaphiopus hammondi* balance this trade-off with the adaptations of a shorter developmental period and accelerated metamorphosis in response to pond dessication (Denver et al., 1998). Anuran species such as *Hyla cinerea*, *H. gratisoa*, and *Rana* species, apparently lack these adaptations and either fail to recruit metamorphs from temporary ponds or suffer local extinctions after rapid drying events (Leips et al., 2000; Skelly, 1997).

Degree of pond permanence can affect both individual traits and community composition. At the individual level, models of amphibian transformation predict metamorphosis will occur between a minimum and maximum body size (Wilbur and Collins, 1973; Werner, 1986). In permanent ponds, larvae should remain until they attain the species specific maximum size for metamorphosis because a larger size is thought to be optimal for a reduction in predation (Werner, 1986), a reduced probability of desiccation (Spight, 1968; Licht and Bogart, 1990), a reduced time to reach sexual maturity (Semlitsch et al., 1988), and increased female fecundity (Semlitsch, 1987a; Semlitsch et al., 1988). With all individuals transforming at or near an optimal size, there should be no relationship between size and date of metamorphosis. In ephemeral ponds, models predict that timing of metamorphosis is determined by the recent growth history of individual larvae (Wilbur and Collins, 1973). When the hydroperiod is shorter, fast-growing larvae should transform earlier and closer to the optimal size whereas slow-growing larvae should transform later and closer to the minimum size. If this prediction holds, the result should be a negative relationship between size and date of metamorphosis. However, the models do not adequately account for the timing of metamorphosis in ephemeral ponds during rare, long hydroperiod years. If larvae are able to respond to the increased period, they should all transform at or near the optimal size (just as in a permanent pond) resulting in no relationship between size and date of metamorphosis.

Other predictions concerning the effects of hydroperiod are more straightforward. For example, both the abundance and size of metamorphs should increase with increased hydroperiod (Rowe and Dunson, 1995). They tested these predictions using *A. maculatum* and *A. jeffersonianum* in 84-day and 158-day hydroperiod treatments. Because no larvae metamorphosed in the 84-day treatments, this can be interpreted as support for both hypotheses. At the community level, extended hydroperiod should result in increased species diversity of amphibian larvae because the extra time allows transformation of species that breed later in the season. Data from ephemeral ponds in South Carolina support these relationships (Semlitsch, 1987b; Pechmann et al., 1989). In Rainbow Bay, South Carolina, the number of amphibian species metamorphosing was significantly related to the length of the hydroperiod (Semlitsch et al., 1996)

We examined the recruitment of four species of mole salamanders (genus *Ambystoma*) from an ephemeral pond in Illinois in two years with different hydroperiods. We compared the following parameters between the two years: 1) the relationship between size of metamorphs and date of metamorphosis (polyploids only), 2) mean metamorph size, 3) abundance of metamorphs, and 4) species diversity and equitability of metamorphs.

MATERIALS AND METHODS

Study Site
The study site consisted of a ridge-top basin bordered by mesic forest in Kickapoo State Park (KSP), Vermilion County, Illinois, USA. The basin was circular, approximately 30 m in diameter, and filled only after heavy rains, usually in late winter and spring. It typically retained water from late winter until mid-June but on several occasions over the past 20 years, it retained water year-round (Morris, 1981; pers. obs.). The basin was surrounded with a drift fence 40 cm high constructed of aluminum window screening that was buried a few cm in the ground. We placed drop-cans at 4.4 m intervals along the

inside and outside of the 127 m circumference fence. Plywood covers that allowed salamanders to enter, but discouraged predators from removing captured metamorphs, were placed over the drop-cans.

Study Organisms
Four species of ambystomatid salamanders breed in the ephemeral pond at the study site: the fall breeding marbled salamander, *Ambystoma opacum*, and the spring breeding spotted salamander, *A. maculatum*, smallmouth salamander, *A. texanum*, and unisexual polyploid members of the *A. jeffersonianum* complex. This complex consists of two diploid parental species, *A. jeffersonianum* and *A. laterale*, and two triploid clonal species that arose through hybridization of the parental genomes (Uzzell, 1964). Two sets of chromosomes from *A. jeffersonianum* are present in *A. platineum* whereas *A. tremblayi* has two set of chromosomes from *A. laterale* (Uzzell, 1964). The triploid hybrids reproduce gynogenetically, using the sperm of one of the parental species to trigger embryonic cleavage. No genetic material is normally contributed by the parental species (Macgregor and Uzzell, 1964). Some populations of *A. platineum* , such as the one in this study, exist outside of the range of either parental species and have adapted to parasitize another species of mole salamander, *A. texanum*. These *A. texanum*-dependent populations vary in ploidy composition, with some containing tetraploids and pentaploids resulting from fertilization of *A. platineum* triploid eggs with *A. texanum* sperm (Spolsky et al., 1992). The polyploids at KSP include the triploid *A. platineum* and tetraploid and pentaploid females (Phillips et al., 1997). In this study, no attempt was made to distinguish between these ploidy classes and they will be collectively referred to as polyploids.

The study site is the only known breeding location for members of the *A. jeffersonianum* species complex in Illinois. Members of this complex are listed as endangered in Illinois and an accurate estimate of recruitment over several years is needed to establish a long-term management plan for the salamanders and the surrounding forest, specifically as pertains to hyroperiod.

Procedures
In 1997, drop-cans were checked daily from 24 June to 22 July, except on 28 June, and 4, 5, 13, 16, 19, and 20 July. In 1998, drop-cans were checked at least every third day from 21 April to 13 August, 1998. During all visits, the depth and area of the pond were recorded and during the last visit in 1998, the pond was seined for 1 hour. For all metamorphs, snout to posterior end of vent (SVL) and total length (TL) were measured to the nearest mm with a plastic ruler. Tail injuries were noted and species were identified by gross morphological characteristics such as head width, body coloration, and size. Identifications were verified by measuring red blood cell (RBC) area and internarial distance (IND) on a subset of the total captures. Both of these measurements have been used to successfully distinguish members of the *A. jeffersonianum* complex from the morphologically similar *A. texanum* (Spolsky et al., 1992; Uzzell, 1964). RBC area was recorded for each individual as the average of ten cells from a dried blood smear using a light microscope at 400x (Uzzell, 1964). IND was measured to the nearest 0.1 mm using a dissecting microscope and plotted against SVL (Spolsky et al., 1992).

Analysis

We tested the relationship between size of metamorphs and date of metamorphosis using linear regression. In the first analysis, the independent variable was the number of days since the first metamorphs emerged, excluding days on which no emigration occurred. In the second analysis, all days were used to determine the independent variable. These two methods did not yield different relationships, so only the results of the second method will be presented, We examined differences in size of metamorphs between years for all species using t-tests. Abundance, Shannon Index of diversity, and equitability were compared directly.

RESULTS

Metamorphs of all four species were easily recognized by external morphological characteristics. *Ambystoma opacum* was distinguished by dark black coloration and light blue to white lateral spots. Polyploids were much lighter brown with yellow spots laterally at the base of their necks, long, spindle-like limbs, and long, wide snouts. *Ambystoma maculatum* were similar to polyploids but with shorter, stouter limbs, yellow to white flecking dorsally, and a broad snout. *Ambystoma texanum* displayed gray coloration with blue-gray flecks, short limbs, and a very blunt snout. The internarial distance (IND) and snout-vent length (SVL) of 3 *A. texanum* metamorphs and 10 polyploids captured in 1997 separated the two groups of juveniles as it has for adults of these species (Spolsky et al. 1992). While polyploids were easily distinguished from other *Ambystoma*, we could not identify specific ploidy levels using external characteristics alone. However, RBC area measurements of five of the polyploid metamorphs averaged 906 μ^2, well within the range given for triploids by Phillips et al. (1997) and Uzzell (1964).

In 1997, the hydroperiod was 127 days (2 March - 8 July), whereas in 1998 the hydroperiod was greater than 137 days (29 March – beyond 13 August). By 13 August 1998, there had been no emigrating metamorphs for ten days and after exhaustive seining produced no metamorphs of any salamander species, the fence was removed. Day of emergence is a significant factor in explaining the variation in the SVL of metamorphs in 1997 ($r^2= 0.118$; p=0.001), and SVL decreased with day of emergence, as predicted (Figure 1a). In 1998 there was no relationship between SVL and day of emergence ($r^2= 0.001$; p=0.84) as predicted (Figure 1b).

Average size for each species (SVL and TL) was greater in 1998 than in 1997 (Table 1) according to our prediction. Because some *A. opacum* larvae metamorphed and left the pond before we constructed our drift fence, we omitted them from the abundance and diversity analyses. From 1997 to 1998, the relative abundance of *A. texanum* and *A. maculatum* metamorphs increased (0.9 to 18.8% and 8.2 to 38.8%, respectively). The relative abundance of polyploid metamorphs decreased from 90.9% in 1997 to 42.5% in 1998. Species diversity increased for the long hydroperiod year (1997 = 0.336; 1998 = 1.04) as predicted. The equitability of the salamander community increased dramatically from 0.306 in 1997 to 0.951 in 1998. These trends are due both to the increase in *A. texanum* and *A. maculatum* metamorphs and the decrease in polyploid metamorphs in 1998.

DISCUSSION

The negative relationship between size at metamorphosis and time of metamorphosis in the short hydroperiod year suggests that the faster growing larvae attained optimum size, but the others could only reach minimum size, at best. The lack of relationship in 1998 indicates that all larvae, regardless of growth rate, metamorphosed at about the same size, which we suggest is the optimal size for the species. The difference in this size/timing relationship between the two years suggests that slower growing larvae can attain the optimal size given appropriate time, a rather straightforward result. Similar results have been observed for *A. talpoideum* (Semlitsch et al., 1988). Some species of amphibians, such as *Hyla cinerea* and *H. gratiosa*, appear to lack adaptations to drying ponds (Leips et al., 2000). This is consistent with the idea that there is an optimal size at metamorphosis (related to adult fitness) and there is intense selection pressure to reach that size (Wilbur and Collins, 1973).

Larvae of all species in this study metamorphosed at a smaller average size in 1997 compared to 1998. Again, this result is quite intuitive and straightforward. Overall, the lengths we recorded for newly metamorphosed ambystomatids were similar to those reported in the literature (see Petranka, 1998 for references). The exception was our 1997 *A. maculatum* metamorphs, which were much smaller than previously reported. This was undoubtedly caused by the early drying of the pond in that year. Throughout much of their range, *A. maculatum* adults are preceded in breeding by other members of the genus by a week or more (Mohr, 1930; Bishop, 1941; Cook, 1967; Nyman, 1991). A study on two vernal ponds in New Jersey revealed that *A. maculatum* eggs developed more slowly and hatched at a later stage compared to polyploid members of the *A. laterale-jeffersoni-unm* complex (Nyman, 1991). Further, polyploid members of the *A. laterale-jeffersoni-anum* complex larvae were larger at both ponds at any given time when compared to *A. maculatum* larvae (Nyman, 1991) Our 1998 data for *A. maculatum* metamorphs are similar to previously reported values (Hardy, 1952; Phillips, 1992). There are no size data for newly metamorphosed *A. texanum* in the literature.

The relative abundance of *A. texanum* and *A. maculatum* metamorphs increased in 1998, whereas the relative abundance of polyploid metamorphs drastically declined. Several factors could have contributed to this decline. Because the basin filled on 29 March 1998 (one month later than in 1997) and salamanders began moving in early February in both years (Phillips et al., 2001), polyploids may have had to retain their eggs for a longer period. It has been purported the longer *A. jeffersonianum* females retained their eggs, the greater the incidence of egg abnormalities (Clanton, 1934). However an analysis of Clanton's data using Spearman rank correlation does not support this conjecture ($r_s = -0.290$, $p = 0.577$, $n = 6$) and there are no other data available to support what Clanton (1934) termed the "over-ripening" of eggs. Another possibility is that female polyploids reabsorbed or released their eggs because of the prolonged interval between first movement and pond filling. Finally, the shortened amount of time available for mate location and selection by polyploid females may have influenced the decline in abundance. Because the polyploids parasitize *A. texanum*, the shortened mating period may have magnified competition with *A. texanum* females for *A. texanum* males, which are in low abundance at this site (Pollowy, 1992). Therefore the reduced number of available hosts may have caused or contributed to the decline. Other explanations include differences in

breeding population size, predation, and density of larvae between the two years, but we do not have the data to address these issues.

The higher diversity and equitability in the longer hydroperiod year are also straightforward results, but bring up an important point concerning design of created wetlands. Just a few weeks in hydroperiod can have dramatic effects on the amphibian community composition of a wetland. In the case of the state endangered silvery salamander, we have to consider not only its requirements but also those of its host, the smallmouth salamander. Other, less-obvious community relationships may also be present so the best strategy is to attempt to increase diversity and equitability. To this end, hydroperiod may be the most important variable and the easiest to manipulate if it is considered at the beginning of a project.

ACKNOWLEDGEMENTS

LITERATURE CITED

Bishop, S. C. 1941. The salamanders of New York. New York State Mus. Bull. 324:1-365.

Clanton, W. 1934. An unusual situation in the salamander *Ambystoma jeffersoniaum* (Green). Occ. Pap. Mus. Zool. Univ. Michigan 290. 15 pp.

Cook, F. R. 1967. An analysis of the herpetofuana of Prince Edward Island. Bull. National Mus. Canada. 212:1-60.

Denver, R. J., N. Mirhadi, and M. Phillips. 1998. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondi* tadpoles to habitat dessication. Ecology 79:1859-1872.

Hardy, J.D., Jr. 1952. A concentration of juvenile spotted salamanders, *Ambystoma maculatum* Shaw). Copeia 1952.181-182.

Leips, J., M. G. McManus, and J. Travis. 2000. Response of treefrog larvae to drying ponds: comparing temporary and permanent pond breeders. Ecology 81:2997-3008.

Licht, L. E., and Bogart, J. P. 1990. Comparative rates of oxygen consumption and water loss in diploid and polyploid salamanders (genus *Ambystoma*). Comp. Biochem. Physiol. A. 97:569-572.

Macgregor, H. C., and T. Uzzell. 1964. Gynogenesis in salamanders related to *Ambystoma jeffersonianum*. Science 143:1043-1045.

Mohr, C. E. 1930. The ambystomatid salamanders of Pennsylvania. Proc. Pennsylvania Acad. Sci. 4:50-58.

Morris, M. A. 1981. Taxonomic status, reproductive biology, and larval life history of two unisexual forms of *Ambystoma* from Vermilion County, Illinois. MA Thesis, Southern Illinois University, Carbondale, Illinois, U.S.A.

Nyman, S. 1991. Ecological aspects of syntopic larvae of *Ambystoma maculatum* and the *A. laterale-jeffersonianum* complex in two New Jersey ponds. J. Herpetol. 25:505-509.
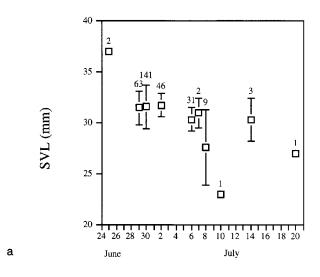
Pechmann, J. H. K., D. E. Scott, J. W. Gibbons, and R. D. Semlitsch. 1989. Influence of wetland hydroperiod on diversity and abundance of metamorphosing juvenile amphibians. Wetlands Ecol. Manage. 1: 3-11.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington. 587 pp.

Phillips, C. A. 1992. Variation in metamorphosisin spotted salamanders *Ambystoma macualtum* from eastern Missouri. Am. Midl. Nat. 128:276-280.

Phillips, C. A., M. J. Dreslik, J. R. Johnson, and J. E. Petzing. 2001. Application of population estimation to pond breeding salamanders. Trans. Illinois Acad. Sci. 94:111-118.

Phillips, C. A., T. Uzzell, C. M. Spolsky, J. M. Serb, R E. Szafoni, and T .R. Pollowy. 1997. Persistent high levels of tetraploidy in salamanders of the *Ambystoma jeffersonianum* Complex. J. Herpetol. 31: 530-535.

Pollowy, T. R. 1992. An endangered species investigation and recovery plan for the silvery salamander (*Ambystoma platineum*) in Illinois. Unpubl. Master's Thesis. University of Illinois, Urbana-Champaign, U.S.A.

Rowe, L. R. and W. A. Dunson. 1995. Impacts of hydroperiod and survival of larval amphibians in temporary ponds of Central Pennsylvania, USA. Oecologia 102:397-403.

Semlitsch, R. D. 1987a. Relationship of pond drying to the reproductive success of the salamander *Ambystoma talpoideum.* Copeia 1987:61-69.

Semlitsch, R. D. 1987b. Density-dependent growth and fecundity in the paedomorphic salamander *Ambystoma talpoideum.* Ecology 68:1003-1008.

Semlitsch, R. D., D.E. Scott and J.H.K. Pechmann. 1988. Time and size of metamorphosis related to adult fitness in *Ambystoma talpoideum.* Ecology 69:184-192.

Semlitsh, R. D., D. E. Scott, J. H. K. Pechmann, and J. W. Gibbons. 1996. Chapter 9: Structure and dynamics of an amphibian community. pp. 217-248. M. L. Cody and J. A. Smallwood (eds.) *In* Long-term studies of vertebrate communities. Academic Press, Inc. San Diego, California.

Skelly, D. K. 1997. Pond permanence and predation are powerful forces shaping the structure of tadpole communities. Am. Sci. 85: 36-45.

Spolsky, C., C. A. Phillips, and T. Uzzell. 1992. Gynogenetic reproduction in hybrid mole salamanders (genus *Ambystoma*). Evolution 46:1935-1944.

Spright, T. M. 1968. The water economy of salamanders: evaporative water loss. Physiol. Zool. 41:195-203.

Uzzell, T. 1964. Relations of the diploid and triploid species of the *Ambystoma jeffersonianum* complex. Copeia 1964:257-300.

Werner, E. E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. Am. Nat. 128:319-341.

Wilbur, H. M., and J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science 182:1305-1314.

Table 1. SVL and TL (mm) for metamorphosing salamanders caught in drop-cans in 1997 and 1998 at KSP.

Species	1997 Mean ±SD	1998 Mean ±SD	P
Ambystoma opacum	SVL: 29.35 ±3.75 TL: 50.91 ±6.27 n: 116*	SVL: 34.40 ±2.06 TL: 62.00 ±4.04 n: 352	<0.001 <0.001
polyploids	SVL: 31.36 ±2.12 TL: 54.44 ±4.20 n: 298	SVL: 39.26 ±4.08 TL: 70.47 ±5.85 n: 34	<0.001 <0.001
Ambystoma texanum	SVL: 17.33 ±0.58 TL: 29.33 ±0.58 n: 3	SVL: 34.67 ±4.27 TL: 61.73 ±6.53 n: 15	<0.001 <0.001
Ambystoma maculatum	SVL: 23.63 ±2.20 TL: 39.30 ±3.45 n: 27	SVL: 30.77 ±1.69 TL: 53.65 ±3.89 n: 31	<0.001 <0.001

* some *A. opacum* larvae metamorphosed prior to construction of our drift fence

Figure 1. SVL versus date of emergence for metamorphosing polyploids in (A) 1997 (127 day hydroperiod) and (B) 1998 (>137 day hydroperiod). Middle horizontal line is mean, box is ± one SD, whiskers indicate range, and number above is sample size.

Date of Transformation

Date of Transformation