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Environmental influences on egg and clutch sizes in lentic- and lotic-breeding salamanders

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Abstract

Environmental influences on egg and clutch sizes in lentic- and lotic-breeding salamanders. Recent research indicates that social and environmental factors influence egg and clutch sizes in amphibians. However, most of this work is based on the reproductively diverse order Anura (frogs and toads), whereas less research has been conducted on Caudata (salamanders) and Gymnophiona (caecilians). Researchers have suggested that a relationship exists between social and environmental factors and egg and clutch sizes in salamanders, but studies controlling for phylogenetic context are lacking. We could not identify a sufficient number of comparisons for social influences on egg and clutch sizes; therefore, we focused on environmental influences for this study. Data on egg size, clutch size, environmental factors, and phylogenies for salamanders were assembled from the scientific literature. We used independent, pair-wise comparisons to investigate the association of larval salamander habitat and egg size and the association of larval salamander habitat with clutch sizes within a phylogenetic framework. There is a significant association between larval habitat and egg size; specifically, stream-breeding species produce larger eggs. There is no significant association between larval habitat and clutch size. Our study confirms earlier reports that salamander egg size is associated with larval environments, but is the first to use phylogenetically independent contrasts to account for the lack of phylogenetic independence of the traits measured (egg size and clutch size) associated with many of the diverse lineages. Our study shows that environmental selection pressure can be quite strong on one aspect of salamander reproduction—egg size.

Keywords: Caudata, larval environment, independent contrasts, lentic, lotic, egg site deposition.

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Resumo

Influência do ambiente sobre o tamanho do ovo e da desova em salamandras que se reproduzem em corpos d'água lênticos e lóticos. Pesquisas recentes indicam que fatores sociais e do ambiente influenciam o tamanho do ovo e da desova nos anfíbios. Contudo, a maioria desses trabalhos baseia-se na ordem Anura (anuros), que possui uma grande diversidade reprodutiva, e poucas pesquisas foram conduzidas nas ordens Caudata (salamandras) e Gymnophiona (cecílias). Sugeriu-se que existe uma relação entre fatores sociais e do ambiente e o tamanho do ovo e da desova em salamandras, mas não há estudos feitos dentro de um contexto filogenético. Não encontramos um número suficiente de comparações para a influência do comportamento social sobre o tamanho do ovo e da desova; portanto, neste estudo enfocamos a influência do ambiente. Os dados sobre tamanho do ovo e da desova, fatores do ambiente e filogenia das salamandras foram coletados na literatura. Usamos comparações *pair-wise* independentes na investigação da associação entre o habitat larval e o tamanho do ovo e entre o habitat larval e o tamanho da desova em um contexto filogenético. Encontramos uma associação significativa entre o habitat larval e o tamanho do ovo; especificamente, espécies que se reproduzem em riachos produzem ovos maiores. Não encontramos uma associação significativa entre o habitat larval e o tamanho da desova. Nosso estudo confirma relatos anteriores de que o tamanho do ovo das salamandras está relacionado ao ambiente em que as larvas se desenvolvem, mas é o primeiro a utilizar contrastes filogeneticamente independentes para explicar a ausência de independência filogenética das características medidas (tamanho do ovo e da desova) associadas a muitas das diferentes linhagens. Nosso estudo mostra que a pressão de seleção do ambiente pode ser muito intensa sobre um dos aspectos da reprodução das salamandras—o tamanho do ovo.

Keywords: Caudata, ambiente larval, contrastes independentes, ambientes lênticos, ambientes lóticos, local de ovipostura.

Introduction

Environmental factors and parental care play key roles in the evolution of offspring size and number (Shine 1978, Nussbaum and Shultz 1989, Clutton-Brock 1991, Stearns 1992). Shine (1978) proposed the "safe-harbor hypothesis," which predicted that typically, the evolution of parental care should precede the evolution of larger eggs. Other researchers argued that environmental factors may influence the number and size of offspring more than parental care (Nussbaum 1985, Morrison and Hero 2003). Specifically, Nussbaum (1985, 1987) proposed that the evolution of larger eggs typically precedes the evolution of parental care.

Amphibians, particularly anurans, provide an excellent system with which to evaluate relationships among parental care, egg size, and environmental factors. Recent comparative analyses of anurans confirmed a positive relationship between parental care and egg size (Summers *et al*. 2006, 2007). The authors found that egg size increased significantly with parental care. They also examined environmental factors and demonstrated that lotic- and montane-breeding anurans produce significantly larger eggs than their sister clades (Summers *et al.* 2007). These studies were the first to control for phylogenetic effects with regard to egg size in amphibian evolution.

Previous studies investigating relationships between environmental factors and egg and clutch sizes in salamanders lacked controls for phylogenetic effects (Salthe 1969, Kaplan and Salthe 1979). Coincidentally, Nussbaum's original proposal (1985, 1987) that the evolution of larger eggs should precede the evolution of parental care was based on salamander data. Nussbaum argued that larger eggs would be favored in lotic environments (flowing water such as streams and seeps) because newly hatched larvae would have to consume larger food items in contrast to larvae hatched in lentic environments (standing water such as ponds or lakes). Larger eggs take longer to develop, enforcing a tradeoff between offspring size and number. Longer development would lead to increased mortality in the embryonic stage unless selection favored behaviors (e.g., parental care) to reduce egg mortality.

Comparative analyses require well-supported phylogenies, and well-supported salamander phylogenies have not been available until recently. We used multiple phylogenies (Methods below) to construct a phylogenetic supertree within which to evaluate relationships among egg size, clutch size, and environmental factors. Social influences on egg and clutch sizes could not be tested due to a lack of phylogenetic independence. Because most salamander parental care has evolved in one family (Plethodontidae), we could not make valid comparisons within those clades (Wells 2007). Therefore, we focus here on the hypothesis that environmental factors influence egg and clutch sizes in salamanders with larval development in lentic and lotic environments. Specifically, we concentrated on determining whether the site of egg deposition affects egg size and clutch size in this subset of salamanders. Based on the work by Nussbaum (1985), we predicted that egg size would be greater and clutch size would be smaller in taxa inhabiting lotic environments than in taxa inhabiting lentic environments .

Materials and Methods

We constructed a phylogenetic supertree consisting of all salamander species for which we could obtain both phylogenetic information and response-variable data to test our hypotheses within a phylogenetic framework. The supertree was constructed with the same hierarchical approach as Summers *et al.* (2007). We used a small number of studies that addressed specific phylogenetic relationships (e.g., relationships within a genus). We preferentially chose studies that used DNA sequence data and maximum likelihood analyses. These studies were chosen because of (1) the quantity of sequence data available, (2) the thoroughness of the analyses, and (3) the well-found statistical rationale for the methods employed. The studies that were chosen consisted of the most recent studies of phylogenetic relationships of salamanders based on the same gene regions when possible.

The analysis utilized the following references for each group: Order Caudata (Wiens *et al.* 2005, Frost *et al.* 2006, Roelants *et al.* 2007); ambystomatids (Shaffer *et al.* 1991, Shaffer and Knight 1996, Weisrock *et al.* 2006a); salamandrids (Steinfartz *et al.* 2006, Weisrock *et al.* 2006b, Zhang *et al.* 2008); hynobiids (Zhang *et al.* 2006); plethodontids (Chippendale *et al.* 2004, Wiens *et al.* 2005).

Phylogenetic, pair-wise comparisons were used for our comparative analysis (Moller and Birkhead 1992, Maddison 2000, Summers *et al.* 2007). This method is used to compare continuous characters with discrete characters, and commonly is used in studies focusing on phylogenetic, pair-wise comparisons in which the traits of a focal group are compared to its sister clade (Gotmark 1994, Summers *et al.* 2007). Each phylogenetic pair-wise comparison was considered independent and counts of these points were analyzed with a χ^2 test with Yates correction when necessary.

Mean egg and clutch sizes were the continuous characters for this study. Egg size was defined as diameter of the ovum in millimeters after eggs were laid (including the jelly coat). The discrete characters in our analyses were larval salamander environments (lentic vs. lotic). Data on continuous characters (egg and clutch sizes) and discrete characters (larval environments) were taken from the primary literature for 31 species of salamanders (Appendices I and II); references from which data were extracted are listed in Appendix III. For species with multiple records for egg or clutch sizes, we averaged values (Appendix III). We also averaged egg and clutch values for sister species inhabiting similar larval environments to facilitate comparison with closely related species that occupy different larval environments. Phylogenetic, pair-wise comparisons were conducted only on species for which we had all of the above data. Female body size was excluded from our analyses because no significant relationship was found between egg size and maximum female body size ($n = 25$, $R^2 = 0.0238$, $F = 0.56$, $p = 0.4619$). This was also true for egg size and mean female body size ($n = 25$, $R^2 = 0.0195$, $F = 0.46$, $p = 0.5058$).

Results

Eleven comparisons were identified for pair-wise comparisons (when controlling for phylogenetic effects) between lentic- versus lotic-breeding salamanders (Figure 1). We detected a significant association between the larval salamander environment and larger egg sizes ($\chi^2 = 9.00$, df = 1, $p < 0.05$; Table 1). Specifically, we found that egg sizes are significantly larger in lotic larval environments. We did not find a significant association between larval salamander environment and clutch size ($\chi^2 = 2.88$, df = 1, $p > 0.05$; Table 1).

Discussion

Our results partially agree with previous results relevant to the association between larval salamander environment and egg and clutch sizes. This association has been documented previously, but not in a phylogenetic context (Nussbaum 1985, 1987, 2003). Nussbaum (1985) originally hypothesized that lotic environments would favor larger egg size, based primarily on prey items available for consumption by the larval salamanders; thus, lotic-breeding salamanders hatched at larger sizes to harvest the size classes of the most abundant food. This contrasts to the lentic-breeding salamanders with smaller embryos that hatch at smaller sizes and gorge on abundant, small zooplankton. Therefore the lotic environment lacking zooplankton would favor larger sizes of eggs and hatchlings. These results also are supported by recent work by Summers *et al*. (2007) with frogs in lotic environments that have larger egg sizes than those in lentic environments.

It is somewhat surprising that salamander clutch size was not found to be associated with the environment of larval salamanders in our study. This disparity may be the result of the small sample size for independent contrasts (9 comparisons). Nonetheless, salamanders that deposit eggs in lotic larval environments tend to have smaller clutch sizes than those that deposit eggs in lentic environments. This is in accord with previous work that hypothesized that adult female salamanders are subject to energy constraints. Thus, females that lay larger eggs deposit smaller clutches and females that lay smaller eggs deposit larger clutches (Nussbaum 2003). The variability of clutch sizes reported for some species may have contributed to the lack of association between larval environment and clutch size. Some species of salamanders in our study are widely distributed and exhibit geographic variation in egg and clutch sizes. For such species, we pooled data from across the range of the species, thereby increasing the variability of our data.

Table 1. Pair-wise comparisons of larval salamander deposition site and egg/clutch sizes.

	Sample Size	χ^2	*p*-value	Effect
Lotic versus Lentic – egg size	10 larger, 1 smaller	9.00	<0.05	Lotic larger
Lotic versus Lentic – clutch size	2 larger, 7 smaller	2.88	>0.05	—

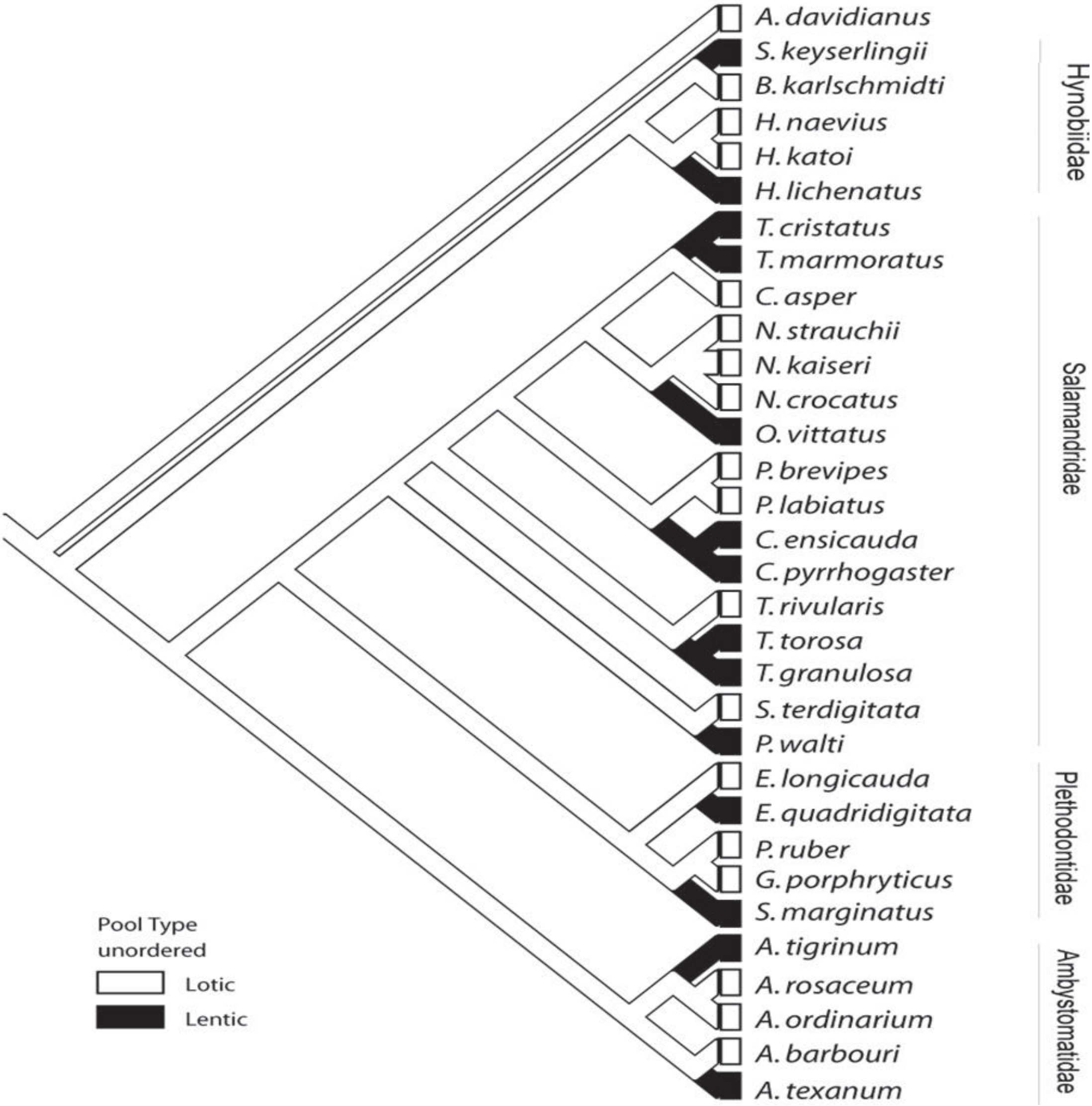

Figure 1. Salamander phylogenetic supertree reconstructed from recently published phylogenies. Black branches are lentic-breeding species and white branches are lotic-breeding species included in our analysis. Salamander genus and species names can be found in Appendix I.

Parental care also may influence egg and clutch sizes; however, we could only identify three independent comparisons. In general, a lack of independent phylogenetic contrasts limited valid comparisons. In some families (specifically Plethodontidae comprising 50% of extant salamanders), parental care is known for most species, making it difficult to compare social environments. Hence, for most of the taxa for which there are data on parental care, the comparisons were not phylogenetically independent. Data on presence or absence of parental care are lacking for many species, especially the several new taxa that have been described recently (Camp *et al*. 2009). Detailed natural-history data are needed for many species, especially for the Asian and New World tropical salamanders, to test the parental care hypothesis properly.

Egg and clutch sizes were not associated with the same factors; thus, larger egg size has a significant association with lotic environments, but clutch size lacks a significant association with the environment defined. Because clutch size tends to be smaller in lotic-breeding species, salamander egg and clutch sizes may be responding to similar environmental selective pressures.

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Appendix I. Salamander species in our analyses and their larval environmental categories.

Salamander taxon	**Larval environment**	
Hynobiidae		
Batrachuperus karlschmidti	Lotic-stream	—
Hynobius katoi	Lotic-stream	—
Hynobius lichenatus	—	Lentic-pond
Hynobius naevius	Lotic-stream	Lentic-pond
Salamandrella keyserlingii		Lentic-pond
Salamandridae		
Calotriton asper	Lotic-stream	—
Cynops ensacauda	—	Lentic-pond
Cynops pyrrhogaster	—	Lentic-pond
Neurergus crocatus	Lotic-stream	—
Neurergus kaiseri	Lotic-stream	—
Neurergus strauchii	Lotic-stream	—
Ommatriton vittatus	—	Lentic-pond
Pachytriton brevipes	Lotic-stream	—
Pachytriton labiatus	Lotic-stream	—
Pleurodeles walti	—	Lentic-pond
Salamandrina terdigitata	Lotic-stream	—
Taricha granulosa		Lentic-pond
Taricha rivularis	Lotic-stream	—
Taricha torosa	—	Lentic-pond
Triturus cristatus	—	Lentic-pond
Triturus marmoratus	—	Lentic-pond
Plethodontidae		
Eurycea longicauda	Lotic-stream	—
Pseudotriton ruber	Lotic-stream	—
Eurycea quadridigitata	—	Lentic-pond
Gyrinophilus porphryticus	Lotic-stream	—
Pseudotriton ruber	Lotic-stream	—
Stereochilus marginatus	—	Lentic-pond
Ambystomatidae		
Ambystoma barbouri	Lotic-stream	—
Ambystoma rosaceum	Lotic-stream	—
Ambystoma ordinarium	Lotic-stream	—
Ambystoma texanum	—	Lentic-pond
Ambystoma tigrinum	—	Lentic-pond

Appendix II. Salamander independent contrasts.

Lentic-pond	Lotic-stream
Salamandrella keyserlingii	*Batrachuperus karlschmidti*
Ambystoma texanum	*Ambystoma barbouri*
Ambystoma tigrinum	*Ambystoma ordinarium, A. rosaceum*
Pleurodeles walti	*Salamandrina terdigitata*
Taricha torosa, T. granulosa	*Taricha rivularis*
Cynops pyrrhogaster, C. ensacauda	*Pachytriton brevipes, P. labiatus*
Eurycea quadridigitata	*Eurycea longicauda*
Stereochilus marginatus	*Pseudotriton ruber, Gyrinophilus porphryticus*
Hynobius lichenatus	*Hynobius katoi, H. naevius*
Ommatriton vittatus	*Neurergus strauchii, N. kaiseri, N. crocatus*
Triturus marmoratus, T. cristatus	*Calotriton asper*

Appendix III. Salamander egg- and clutch-size data.

Taxon	Egg size	Clutch size	Authority
Cryptobranchidae			
Andrias davidianus	8.6	—	Haker 1997
Hynobiidae			
Batrachuperus karlschmidti	3.7		Liu 1945
Hynobius katoi	4.8–5.0		AmphibiaWeb 2010
Hynobius lichenatus	2.8–3.2	40–60	Takahashi and Iwasawa 1990, Goris and Maeda 2004, AmphibiaWeb 2010
Hynobius naevius	5.0	34–72	Goris and Maeda 2004, AmphibiaWeb 2010
Salamandrella keyserlingii	1.5–2.0		Tago 1931
Salamandridae			
Calotriton asper	3.5–5.0	20–30	Clergue-Gazeau 1999, AmphibiaWeb 2010
Cynops ensacauda	2.8		Tago 1931
Cynops pyrrhogaster	2.0		Anderson 1943
Neurergus crocatus	1.5–2.0		Schmidtler and Schmidtler 1975, Timofeev 1997
Neurergus kaiseri	1.5–2.0		Schmidtler and Schmidtler 1975
Neurergus strauchii	2.6–3.0		Steinfartz 1995
Ommatriton vittatus	1.8–2.3		Tarkhnishvii and Gokhlashvii 1999
Pachytriton brevipes	3.5		Thorn 1968
Pachytriton labiatus	4.7–5.3		Thiemeier and Hornberg 1998
Pleurodeles walti	1.7	150–1300	Gallien and Durocher 1957, AmphibiaWeb 2010
Salamandrina terdigitata	1.8	1–65	Zuffi 1999, Rocca *et al.* 2005
Taricha granulosa	1.8		Twitty 1936, Connon 1947
Taricha rivularis	2.8	10	Twitty 1935, 1936, Connon 1947, Riemer 1958
Taricha torosa	2.3	7–47	Twitty 1936, Connon 1947, Brame 1956, 1968, Mosher *et al.* 1964
Triturus cristatus	2.0	70–600	Thorn 1968, AmphibiaWeb 2010
Triturus marmoratus	2.0	200–380	Thorn 1968, AmphibiaWeb 2010

Appendix III. *Continued.*

Taxon	Egg size	Clutch size	Authority
Plethodontidae			
Eurycea longicauda	2.5–3.0	61–106	Hutchison 1956, Ryan and Bruce 2000, Minton 2001
Eurycea quadridigitata	1.8	7–62	Goin 1951, Semlitsch and McMillan 1980, Gibbons and Semlitsch 1991
Gyrinophilus porphryticus	3.5–4.0	16–106	Bishop 1941, Bruce 1969, 1978b
Pseudotriton ruber	3.3	29–130	Bruce 1968, 1978a
Stereochilus marginatus	2.0–2.5	16–121	Richards 1932, Schwartz and Etheridge 1954, Wood and Rageot 1963, Ryan and Bruce 2000
Ambystomatidae			
Ambystoma barbouri	2.4–3.8	~260	Petranka 1998
Ambystoma rosaceum	2.6		Anderson and Webb 1978
Ambystoma ordinarium	2.8	109	Anderson and Worthington 1971
Ambystoma texanum	1.6–2.5	550–700	Minton 1972, Licht 1989, Petranka 1998
Ambystoma tigrinum	3.0	421–7000	Bishop 1941, Petranka 1998