

Short Notes

Application of anole lizard generic names proposed by Wagler, 1830 and Fitzinger, 1843

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Recent publications restrict the limits of the lizard family Polychrotidae to *Polychrus* and *Anolis* (sensu lato) (Frost et al., 2001) and provide additional support for the partition of *Anolis* (Jackman et al., 1999), although not exactly along the lines proposed by us (Guyer and Savage, 1986, 1992; Savage and Guyer, 1989, 1991). Most workers have followed our lead in utilizing the generic name *Norops* Wagler, 1830 (type species by monotypy *Anolis auratus* Daudin, 1802) for the beta section of *Anolis* Daudin, 1802 sensu Etheridge (1959) and Williams (1976a). However, a nomenclatural problem exists regarding the precedence of *Norops* over one available name proposed by Wagler (1830). As we attempted to resolve this issue it became apparent that similar questions of precedence are involved in the anole generic names proposed by Fitzinger (1843). The present paper aims to clarify these nomenclatural matters in the interest of stability.

Wagler (1830) proposed three generic names that are applicable to anoles, *Dactyloa*, *Draconura* (type species by monotypy *Draconura nitens* Wagler, 1830) and *Norops* (type species by monotypy *Anolis auratus* Daudin, 1802). Because these names all date from the same publication we acted as first revisers (Art. 24.2, International Code of Zoological Nomenclature, 1999) and selected *Norops* to have precedence over *Draconura* if these names are thought to be applicable to the same taxon (i.e. are synonyms).

Fitzinger (1843) designated *Anolis punctatus* Daudin, 1802 as the type species of *Dactyloa*. We incorrectly accepted this type designation in our previous publications (see Savage and Guyer, 1991). Fitzinger's action cannot stand because *Anolis punctatus* was not

among the species included originally in *Dactyloa* by Wagler (1830) (Art. 74 of the Code, 1999). Fortunately, a junior synonym of *A. punctatus*, *Anolis gracilis* Wied Neu-Wied, 1821 was included by Wagler (1830) as a species of *Dactyloa*. We act as first revisers (Art. 24.2 of the Code) and select *Anolis gracilis* as the type species of *Dactyloa*. Continuing as first reviewers we also select *Norops* to have precedence over *Dactyloa*, and *Dactyloa* to precedence over *Draconura* if these names are thought to be synonyms.

Fitzinger (1843) proposed 22 new generic and subgeneric names for species of *Anolis* (sensu lato). We recognized two of these names as valid genera (Guyer and Savage, 1992; Savage and Guyer, 1989), *Ctenonotus* (type species by original designation *Lacerta bimaculatus* Sparrman, 1784) and *Semiurus* (type species by original designation *Anolis cuvieri* Merrem, 1820). We later pointed out (Savage and Guyer, 1991) that *Xiphosurus* Fitzinger, 1826 is an objective senior synonym of *Semiurus* and must replace it. Again acting as first revisers (Art. 24.2.2 of the Code) we select *Ctenonotus* to have precedence over all other generic and subgeneric names applicable to anoles proposed by Fitzinger (1843).

Because the topology of the phylogeny for Polychrotidae is becoming known with greater certainty (Frost et al., 2001; Jackman et al., 1999), two other of Fitzinger's generic names seem likely to be removed from synonymy and raised to the generic or subgeneric level. We act as first revisers to establish the precedence of *Deiropyx* (type species by monotypy *Anolis vermiculatus* Cocteau in Dumeril and Bibron, 1837) and *Eupristis* (type species *Anolis equestris* Merrem, 1820 by monotypy) over all other generic and subgeneric names proposed by Fitzinger (1843), except *Ctenonotus*, applicable to anole genera. *Deiropyx* is here given precedence over *Eupristis* should the two names be considered applicable to the same taxon.

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Cost comparison of marking techniques in long-term population studies: PIT-tags versus pattern maps

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Conservation biology requires the assessment of demographic parameters and life-history data in wildlife populations. Such data can be gathered through capture-mark-recapture (CMR) of individuals. Marking techniques may vary for study organisms, research objectives and conditions, but all CMR studies have in common that the workload and expenditure is substantial, urging for cost-effective planning (Southwood, 1978; Hammer and Blankenship, 2001; Schmidt et al., 2002).

A common method for individual identification of wild animals is the recording of individual, unique spot patterns (by photographing, drawing, or photocopying: Hagström, 1973; Gill, 1978; Glandt, 1980; Hiby and Lovell, 1990; Heyer et al., 1994 — note that although pattern mapping is non-invasive we refer to it as ‘marking’). More recently, surgically implanted Passive Integrated Transponders (PIT-tags), small glass-encased electromagnetic coils bearing a unique alphanumeric code, were introduced to population studies of amphibians, reptiles and other small vertebrates (e.g., Sinsch, 1992; Zydlewski et

al., 2001). Various papers have already discussed the biological, legal and ethical issues of these methods (Henle et al., 1997; Braude and Cizek, 1998; Ott and Scott, 1999), but the cost-effectiveness of the use of pattern maps versus PIT tags has received no attention so far. We here develop an analytical model that identifies the marking technique that is best from an economic point of view. In particular, we investigate how costs per recapture data point relate to population parameters such as survival and population size, and parameters of study design such as duration and sample size. Note that capture histories such as '11' (two subsequent captures), '110' (two captures and a miss), '101', '1001' etc. all represent but a single recapture data point.

The model and its assumptions are developed with a focus on studies of single breeding populations of amphibians, but could be extended to other classes of deme-structured organisms. As opposed to toe clipping, for example, the methods considered here have in common that they do not pose an upper limit to the number of animals that can be individually recognized, and can thus be used in populations of any size.

There is now a vast body of literature discussing models for the analysis of data from CMR studies (e.g. Seber, 1982; Zar, 1996; Williams et al., 2002). To gain tractability, our model, in which we assume a population of constant size, is a somewhat simplified form of those commonly used. Our exposition uses the terminology for open populations, for which we therefore assume that immigration and emigration are both negligible and that births equal deaths. On each sampling occasion, a sample of predetermined size n is drawn with all animals in the population having equal probabilities of inclusion. The number of recaptures r depends on the sample size n , the population size N , the survival probability S and the study duration T , and, as such, is a function of human effort and the biology of the species.

The expected costs under our model are derived in appendix 1, and results for some illustrative parameter settings are presented below. Alternative parameter settings are easily explored with the help of a spreadsheet (in Microsoft Excel) available from the third author (JH) upon request.

We adopt the price of a PIT-tag as cost unit, in Europe currently at 4.30 Euros a piece when bought in quantity, such as with 'Trovan' electronic identification systems. The price of 100 pattern maps equals that of one PIT-tag and, in our hands, one PIT-tag matches the cost of one hour of work for 500 pattern map comparisons. Of course, parameter settings can be changed for more appropriate values, for example when PIT-tags become more affordable, or when photocopies are used instead of photographs. The price of a PIT-tag reader and a pattern map recorder (e.g. photocopier or camera) is not considered here. Similarly, the effort of injecting a PIT-tag equals that of recording a pattern map. Error in the identification of animals is assumed negligible for both methods.

The choice for pattern-mapping or PIT-tagging as a marking method is largely determined by a trade-off between costs of labor and consumables. Pattern maps are recorded cheaply, but the time spent on matching newly recorded patterns to archived ones is substantial since comparisons must be carried out "by eye", and the expected number of comparisons rises with the square of sample size (n^2 , see equation (22) in appendix 1). Conversely, PIT-tags are more expensive, but tagged individuals are recognized instantaneously, and the number of tags increases only linearly with the number of newly encountered individuals. In fig. 1 we show the conditions, in terms of population size N and sampling proportion $p = n/N$, for which the expected costs for pattern-mapping and PIT-tagging are equal (see also equations (14) and (19) in appendix 1). In a typical study of 3-year duration, with intensive sampling and medium annual survival ($T = 2$, $p = 0.9$, $S = 0.5$), pattern-mapping is the cheaper method for $N < 176$. A lower sampling pro-

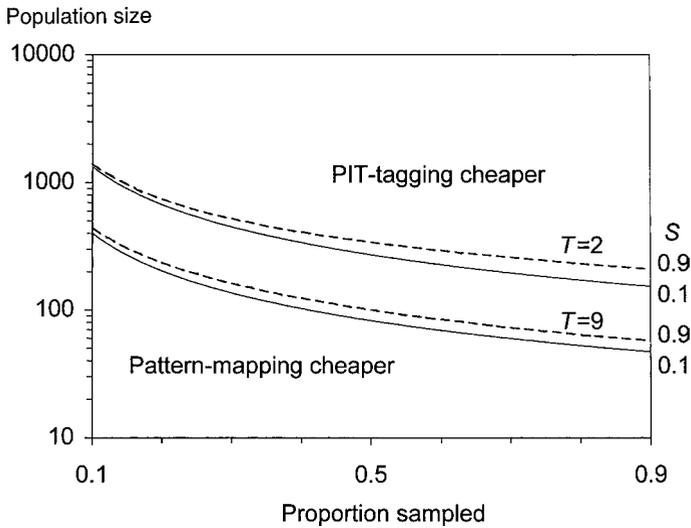


Figure 1. Break-even lines for the cost of individual animal recognition by pattern-mapping and PIT-tagging (using equations (14) and (19) of appendix 1), under varying conditions of population size (N), sampling proportion (p), survival ($S = 0.1$ shown by solid line, $S = 0.9$ shown by interrupted line) and study duration ($T = 2$, $T = 9$). Pattern-mapping and PIT-tagging are cost-efficient for small and large populations, respectively. For populations of medium size the choice for either technique is dependent on study duration.

portion renders pattern-mapping economical at a wider range of conditions ($p = 0.5$, $N < 297$; $p = 0.1$, $N < 1360$). In a 10-year study, PIT-tagging is more economic than pattern-mapping under a wide range of conditions. At high sampling and medium survival conditions ($T = 9$, $p = 0.9$, $S = 0.5$) the break-even point is at $N = 55$. Again, decreasing the sampling proportion increases the applicability of pattern-mapping ($p = 0.5$, $N = 92$; $p = 0.1$, $N = 412$). A large variation in survival ($0.1 < S < 0.9$) has a small effect on the conditions at which recognition techniques are in cost equilibrium (fig. 1). The cost per recapture data point (C_r) decreases with increasing study duration and survival (see equations (14), (19) and (20) in appendix 1), and varies by approximately one order of magnitude for $0.1 < S < 0.9$ in a 3-year study (fig. 2a, c, e), and up to two orders of magnitude in a 10-year study (fig. 2b, d, f). When S is low, the reduction in costs with increasing study duration is marginal (fig. 2a, b); when S is high the reduction in costs is substantial (fig. 2e, f).

We examined the effectiveness of pattern-mapping versus PIT-tagging and calculated the cost per recapture data point. Factors considered were population size, study duration, sampling proportion, and survival. The issue of statistical inference (i.e. accuracy of estimation) is not considered in this paper, and the two methods compared are considered equivalent from an inferential point of view. In general, we have limited this study to the exploration of the relative economic merits of two popular methods under fairly simple conditions and a range of plausible parameter values.

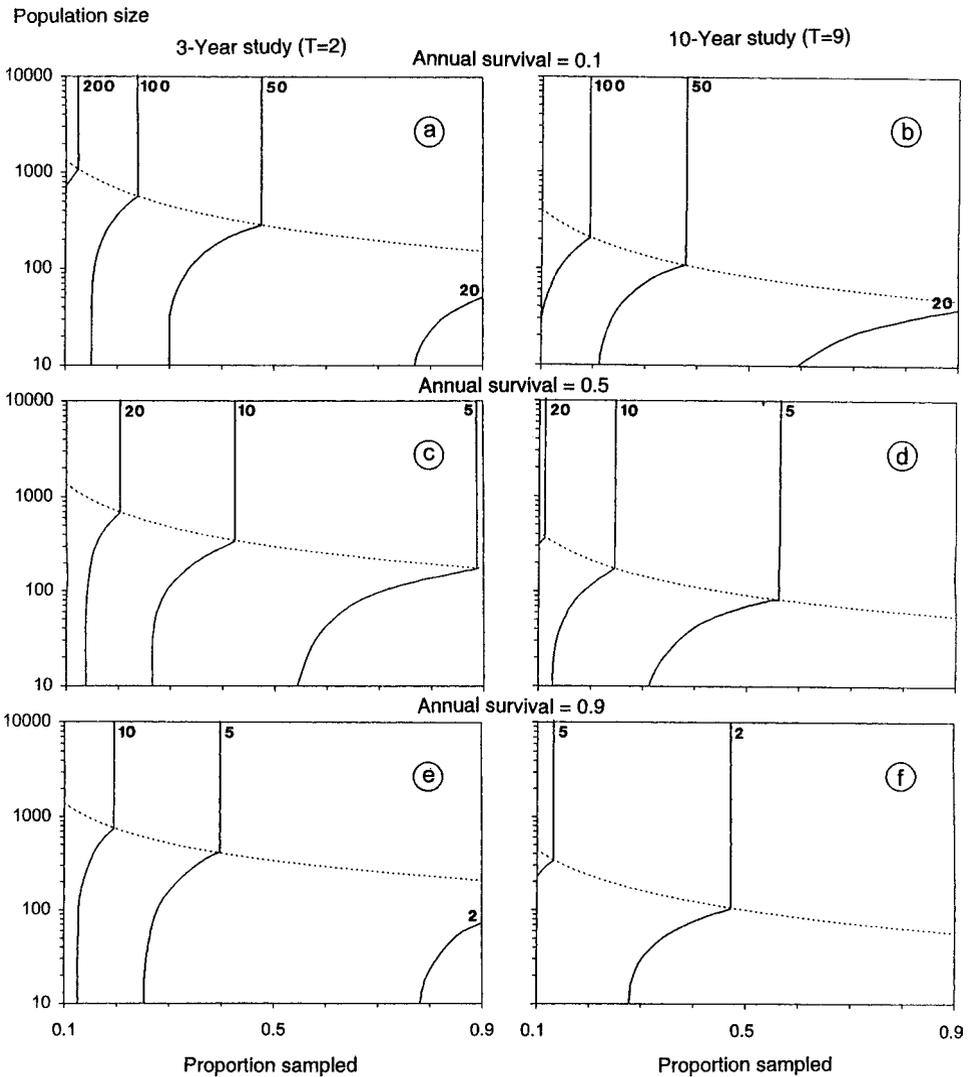


Figure 2. Contour diagrams of cost per recapture data point under varying conditions of population size ($N = 10$ - $10,000$), sampling proportion ($p = 0.1$ - 0.9), annual survival ($S = 0.1$ top row, $S = 0.5$ middle row and $S = 0.9$ bottom row) and study duration ($T = 2$ left hand column and $T = 9$, right hand column). One cost unit corresponds to the price of one PIT-tag (ca. 4.30 Euros). Note the correspondence with the choice for the pattern-mapping versus the PIT-tagging recognition method, indicated by the dotted line (from fig. 1).

Pattern-mapping is more appropriate for short studies on small populations, and PIT-tag marking pays off for long-term studies of large populations. At medium population size ($100 < N < 500$), the choice for either technique depends largely on study duration and sampling proportion, but not on survival. However, typically, the conditions under

which pattern-mapping outperforms PIT-tagging are those for which precision is also poor. Accordingly, pattern-mapping tends to apply to preliminary and ad hoc, low budget studies and when rough estimates are better than no estimates at all (as is frequently the case in conservation practice). Even limited information, such as obtained from a pilot study, would help to design an optimally cost-effective CMR research strategy.

Monetary cost for a population study may be substantial. At low sampling proportion the cost per recapture data point appears excessive. A high cost is also encountered when survival is low, especially in combination with short study duration. It is important to note, however, that scantiness of recapture data may represent important information, e.g. suggesting that survival is low or dispersal is high. Parameter values observed in a 3-year population study of the newt *Triturus cristatus* ($n = 1000$, $N = 1400$, $S = 0.88$; Arntzen et al., 1999) corresponded to the use of approximately 1400 PIT-tags (equaling 6000 Euros), or 2.6 Euros per recapture data point. In a 10-year study on the newt *T. dobrogicus* ($n = 90$, $S = 0.34$ — Ellinger and Jehle, 1997; $p = 0.76$, Arntzen et al., 1995) the cost was approximately 600 PIT-tags, or 4.3 Euros per recapture data point (note that in fact several recognition methods were used in combination).

The workload for pattern-mapping is overestimated in our model because once-matched pattern maps can be merged. A temporal search window would also reduce the workload. For some species it may be possible to split the collection of images into subgroups, according to gender or some basic feature of the pattern, lowering the number of comparisons to be made. Attempts are being made to recognize pattern maps by computer-aided image analysis (e.g., Hiby and Lovell, 1990; Sweeney et al., 1995; Streich et al., 1997). This would render the pattern-mapping technique applicable to large populations at negligible cost. Toe clipping is not considered in this study because the number of individual marks that can be applied is limited, and because it is by some considered to be unethical. A practical, low-cost alternative to PIT-tagging would be to combine pattern-mapping with group marking, such as with a panjet tattoo or the removal of a single toe. Only marked, recaptured individuals would require an image search, therewith securing the quality of the results. A toe clip has the additional advantage that the tissue can be used for genetic analysis (Gonser and Collura, 1996).

The lack of population demographic data for many organisms is crippling implementation of pressing conservation measures. More insights into the dynamics of natural amphibian populations are urgently required for addressing issues surrounding their observed global decline (Alford and Richards, 1999; Houlahan et al., 2000; Carey et al., 2001), and more fundamental research is required to improve our understanding of population processes (Halley et al., 1996). Although amphibians are relatively well studied, life tables from data covering at least one generation are still sparse. A further cost reduction and miniaturization of individual tags would generate a breakthrough in amphibian field research, but for the time being our research potential is restricted to studying a limited number of mostly adult individuals. A caveat to this end is that the study of adults in large,

healthy and accessible populations may not result in the gathering of the most urgently required or representative data.

Appendix 1. Derivation of expected costs for a population study employing PIT-tags and pattern maps

1.1. The Number of Marks in the Population in a T-year Study

We assume that, of the N animals in the population, exactly n are caught in years $0, 1, 2, \dots, T$. Marking takes place only in years $0, 1, 2, \dots, T - 1$, and analysis of marks only in years $1, 2, 3, \dots, T$. We denote by n_t the number of animals in the population which by the end of year t have been identified either by tagging or by the recording of their pattern maps. Since exactly n animals are identified in year zero, we set $n_0 = n$.

We assume that, independently of other animals, each animal survives from the end of the trapping season one year to the start of the season the following year with a constant probability S . Hence, conditional on the number n_{t-1} of animals identified at the end of year $t - 1$ ($t = 1, 2, 3, \dots, T$), the number s_t of identified animals surviving to the start of the season in year t has the binomial distribution given by

$$s_t | n_{t-1} \sim \text{Bin}(n_{t-1}, S), \quad t = 1, 2, 3, \dots, T. \tag{1}$$

Hence

$$E[s_t | n_{t-1}] = n_{t-1}S. \tag{2}$$

Let r_t denote the number of recaptured animals in year t ($t = 0, 1, 2, \dots, T$). As there can be no recaptures in year zero, we have $r_0 = 0$. For $t = 1, 2, 3, \dots, T$, the conditional distribution of r_t , given s_t , is the hypergeometric distribution $H(N, n, s_t)$ with probability function

$$P[r_t = r | s_t] = \binom{s_t}{r} \binom{N - s_t}{n - r} / \binom{N}{n}, \quad r = 0, 1, 2, \dots, n,$$

and conditional mean ns_t/N . By a standard property of conditional expectation (see, for example Grimmett & Stirzaker, 1992, p. 307), we have, for $t = 1, 2, 3, \dots, T$,

$$E[r_t | n_{t-1}] = E\{E[r_t | n_{t-1}, s_t] | n_{t-1}\} = E\{ns_t/N | n_{t-1}\} = nn_{t-1}S/N, \tag{3}$$

where the final equality follows from equation (2). At some points below it will be more convenient to consider the number of surviving marked animals that are not recaptured in year t , which is given by the random variable

$$v_t = s_t - r_t = n_t - n, \quad t = 1, 2, 3, \dots, T. \tag{4}$$

The conditional distribution of v_t , given s_t , is given by

$$v_t | s_t \sim H(N, N - n, s_t), \tag{5}$$

and its conditional mean is

$$E[v_t | s_t] = \beta s_t/S, \quad t = 1, 2, 3, \dots, T, \tag{6}$$

where $\beta = S(1 - n/N)$, the probability that an animal survives from one year to the next without being captured in the latter year. From (4), (2) and (3) we have

$$E[v_t | n_{t-1}] = E[s_t - r_t | n_{t-1}] = \beta n_{t-1}, \quad t = 1, 2, 3, \dots, T. \tag{7}$$

Thus, for $t = 1, 2, 3, \dots, T - 1$, we obtain, using equations (4) and (7)

$$E[n_t | n_{t-1}] = E[n + v_t | n_{t-1}] = n + \beta n_{t-1}.$$

If, for $i < t$, we let $\theta_{t,i} = E[n_t | n_i]$, it follows that

$$\theta_{t,i} = E\{E\{n_t | n_{t-1}, n_i\} | n_i\} = E\{E\{n_t | n_{t-1}\} | n_i\} = n + \beta\theta_{t-1,i}.$$

This recurrence relation implies that

$$\theta_{t,i} = \beta^{t-i}n_i + n(1 - \beta^{t-i})/(1 - \beta).$$

Now let $\theta_t = E(n_t)$ denote the expected number of identified animals at the end of year t ($t = 0, 1, 2, \dots, T - 1$). Since $\theta_t = \theta_{t,0}$, and $n_0 = n$, we get that

$$\theta_t = n(1 - \beta^{t+1})/(1 - \beta), \quad t = 0, 1, 2, \dots, T - 1.$$

Observe that $\theta_t = E(n_t)$ can be written as the sum, over $i = 0, 1, 2, \dots, t$, of $n\beta^{t-i}$, which is the expected number of animals captured at time i which survive without being captured up to time t . The conditional expectation $\theta_{t,i} = E[n_t | n_i]$ can be similarly interpreted, with $\beta^{t-i}n_i$ being the expected number of animals marked at time i which survive without being captured up to time t . We note also that

$$\theta_{t,i} = \beta^{t-i}n_i + \theta_{t-i-1}. \tag{8}$$

We can also use these results to find, for $t = 1, 2, 3, \dots, T$, both the expected number of identified animals surviving to the start of the season in year t and the expected number of recaptured animals in year t . If we let $\mu_t = E(s_t)$ and $\psi_t = E(r_t)$, it follows from (2) that

$$\mu_t = E\{E\{s_t | n_{t-1}\}\} = E(n_{t-1}S) = S\theta_{t-1}, \quad t = 1, 2, 3, \dots, T, \tag{9}$$

and from (3) that

$$\psi_t = E\{E\{r_t | n_{t-1}\}\} = (nS/N)E(n_{t-1}) = (nS/N)\theta_{t-1}, \quad t = 1, 2, 3, \dots, T. \tag{10}$$

1.2. The Cost of a PIT Tagging Study

The number of new marks m_t recorded in year t is

$$m_t = n - r_t, \quad t = 0, 1, 2, \dots, T - 1. \tag{11}$$

As there are no recaptures in year 0, we have $m_0 = n = n_0$. Recalling that no marking takes place in year T , the number of identified animals in the population at the end of the season in year t is given by

$$n_t = \begin{cases} s_t + m_t, & t = 1, 2, 3, \dots, T - 1; \\ s_t, & t = T. \end{cases} \tag{12}$$

If we are using PIT tags, the total number of tags used is

$$M_T = m_0 + m_1 + \dots + m_{T-1}, \quad T = 1, 2, 3, \dots \tag{13}$$

Thus, using (13), (12) and (9),

$$E[M_T] = n + \sum_{t=1}^{T-1} E(n_t - s_t) = \theta_0 + \sum_{t=1}^{T-1} (\theta_t - S\theta_{t-1}) = \theta_{T-1} + (1 - S) \sum_{t=0}^{T-2} \theta_t.$$

This intuitive result simply says that $E[M_T]$ is the sum of the mean number tagged at the end of the season in year $T - 1$ and the mean numbers failing to survive between each pair of adjacent years up until that time.

The total expected cost of using PIT tags is thus

$$C_1 = (T + 1)nC_e + C_t E[M_T], \tag{14}$$

where C_e is the cost of catching an animal in the field and C_t is the cost of a single tag. Note that we are assuming that the latter cost is the same for all members of the population and does not change over time.

1.3. Probabilistic Results

In §4 we will consider the cost of a pattern mapping study, which depends, in particular, on the expected number ϕ_t of comparisons made in year t between the images of animals caught in that year and those images previously obtained. The expression for ϕ_t that we will derive in §4 includes expectations of the form $E(r_t r_i)$, where $i < t$, and so the goal of the present section is to evaluate such expectations. The derivation requires us to obtain the second factorial moment of s_i , and the expectation of the product $r_t n_i$ ($i = 1, 2, 3, \dots, T$).

It follows from distributions (1) and (5) that the conditional second factorial moments of s_t given n_{t-1} and of v_t given s_t are given by

$$E\{s_t(s_t - 1) \mid n_{t-1}\} = n_{t-1}(n_{t-1} - 1)S^2, \quad t = 1, 2, 3, \dots, T, \tag{15}$$

and

$$E\{v_t(v_t - 1) \mid s_t\} = \gamma s_t(s_t - 1)/S^2, \quad t = 1, 2, 3, \dots, T, \tag{16}$$

where $\gamma = (N - n)(N - n - 1)S^2/[N(N - 1)]$.

Now set $\omega_i = E\{s_i(s_i - 1)\}$, the unconditional second factorial moment of s_i ($i = 1, 2, 3, \dots, T$). Using equation (15), we have

$$\omega_i = E[E\{s_i(s_i - 1) \mid n_{i-1}\}] = S^2 E[n_{i-1}(n_{i-1} - 1)] = S^2 E[E\{n_{i-1}(n_{i-1} - 1) \mid s_{i-1}\}],$$

$$(i = 2, 3, 4, \dots, T).$$

Hence, by (4), (16), (6) and (9),

$$\begin{aligned} \omega_i &= S^2 E[E\{(n + v_{i-1})(n + v_{i-1} - 1) \mid s_{i-1}\}] \\ &= S^2 E[E\{v_{i-1}(v_{i-1} - 1) \mid s_{i-1}\} + 2nE\{v_{i-1} \mid s_{i-1}\} + n(n - 1)] \\ &= \gamma E\{s_{i-1}(s_{i-1} - 1)\} + 2n\beta S E\{s_{i-1}\} + n(n - 1)S^2 \\ &= \gamma \omega_{i-1} + 2n^2 S^2 \beta(1 - \beta^{i-1})/(1 - \beta) + n(n - 1)S^2 \end{aligned}$$

Thus ω_i satisfies the recurrence relation

$$\omega_i = \gamma \omega_{i-1} - \lambda \beta^i + \alpha, \quad i = 2, 3, 4, \dots, T,$$

where $\lambda = 2n^2 S^2/(1 - \beta)$ and $\alpha = \beta \lambda + n(n - 1)S^2$, subject to the initial condition that $\omega_1 = n(n - 1)S^2$. Hence we obtain

$$\omega_i = \{\alpha(\gamma^i - 1)/(\gamma - 1)\} + \{\lambda\beta(\gamma^i - \beta^i)/(\beta - \gamma)\}, \quad i = 1, 2, 3, \dots, T.$$

Now let $\xi_i = E(r_i n_i)$. Using (4), we have

$$\begin{aligned} \xi_i &= E[E\{(s_i - v_i)(n + v_i) \mid s_i\}] \\ &= nE\{s_i\} + E\{(s_i - n - 1)E\{v_i \mid s_i\}\} - E[E\{v_i(v_i - 1) \mid s_i\}]. \end{aligned}$$

Applying equations (6) and (16) gives

$$\xi_i = n\mu_i + (\beta/S)E\{(s_i - n - 1)s_i\} - (\gamma/S^2)\omega_i = n\{1 - (\beta/S)\}\mu_i + \{(\beta S - \gamma)/S^2\}\omega_i.$$

If we set $\zeta = n(N - n)/[N(N - 1)]$, this simplifies to give

$$\xi_i = (n^2/N)\mu_i + \zeta \omega_i.$$

We can now obtain an expression for $E(r_t r_i)$ where $i < t$. Using equation (3), we obtain

$$E(r_t r_i) = E\{E(r_t r_i \mid n_{t-1}, r_i)\} = E\{r_i E(r_t \mid n_{t-1})\} = (nS/N)E(r_i n_{t-1}).$$

Now

$$E(r_i n_{t-1}) = E\{E(r_i n_{t-1} \mid r_i, n_i)\} = E\{r_i E(n_{t-1} \mid n_i)\}.$$

Hence, by (8),

$$E(r_t r_i) = (nS/N)E\{r_i(\beta^{t-i-1}n_i + \theta_{t-i-2})\} = (nS/N)(\beta^{t-i-1}\xi_i + \theta_{t-i-2}\psi_i). \tag{17}$$

1.4. *The Cost of a Pattern Mapping Study and Cost per Recapture*

In order to carry out pattern mapping on the population, the researcher must build up a “gallery” of images of animals. From the start the captured animals are compared with the images in the gallery. This can take a long time. The expected cost can be worked out as follows. For $t = 1, 2, 3, \dots, T$, the number of pictures in the gallery at the end of season $t - 1$ is the total number of animals newly encountered during that season or any previous one, namely $m_0 + m_1 + \dots + m_{t-1} = M_t$.

In year t , there are m_t animals caught for the first time, and, for each of these, the number of comparisons will be M_t , giving a total of $m_t M_t$ comparisons associated with newly encountered animals. For any recaptured animal, a match may be seen with the first image or may not happen until the last. More precisely, let u_i denote the number of comparisons required for the i^{th} recaptured animal ($i = 1, 2, 3, \dots, r_t$). The total number of comparisons in year t can then be written as

$$Y_t = m_t M_t + \sum_{i=1}^{r_t} u_i = (n - r_t) M_t + \sum_{i=1}^{r_t} u_i. \tag{18}$$

The total expected cost of using pattern comparison is thus

$$C_2 = (T + 1)nC_e + TnC_p + C_c \sum_{t=1}^T E[Y_t], \tag{19}$$

where C_p is the cost of photographing an animal, C_c is the cost of a single pattern comparison, and, as earlier, C_e is the cost of catching an animal in the field.

We approximate the cost per recapture by the expected cost of the study divided by the expected total number of recaptures:

$$C_r = C_X / \sum_{t=1}^T \psi_t. \tag{20}$$

C_X is C_1 given by equation (14) for PIT tags and is C_2 given by equation (19) for pattern-mapping. In general, this will not be the same as the expectation of the ratio of the total cost to the number recaptured, which is more difficult to derive.

The remainder of this appendix is devoted to finding an expression for $\phi_t = E[Y_t]$, the expected total number of comparisons in year t . Suppose now that we ignore entirely any changes to the gallery that could be made during the matching process in a given year. For example, if an individual \mathbf{Z} is recognized initially, time could obviously be saved by noting that other animals captured that year need not be compared with the image of \mathbf{Z} . Time could also be saved by making sure that images are arranged in last-in-first-out order, so that the first comparisons made are not with the images of the animals which are most likely to be dead. This will make an enormous difference if the study duration T is much longer than the expected lifetime of an animal. We suppose that no special time-saving devices are employed and that the duration T is not significantly longer than the animal’s lifetime.

In these circumstances it is reasonable to assume that the vector $(u_1, u_2, \dots, u_{r_t})$ constitutes a sample, drawn without replacement, from the set $\{1, 2, 3, \dots, M_t\}$. Thus, given M_t and r_t , the expected total number of comparisons associated with recaptured animals is

$$\sum_{i=1}^{r_t} E(u_i | M_t, r_t) = \sum_{i=1}^{M_t} \{i P(\text{match occurs with image } i)\} = \left(\frac{r_t}{M_t}\right) \sum_{i=1}^{M_t} i = r_t(M_t + 1)/2. \tag{21}$$

Thus, setting $\phi_t = E(Y_t)$, we have from (18) and (21) that

$$\phi_t = E\{E(Y_t | M_t, r_t)\} = E\{(n - r_t)M_t + \{r_t(M_t + 1)/2\}\}.$$

Hence by (13) and (11)

$$\begin{aligned}\phi_t &= E\{(2n - r_t)M_t/2\} + E(r_t/2) \\ &= E\left[\left\{n - \frac{r_t}{2}\right\}\left\{nt - \sum_1^{t-1} r_i\right\}\right] + \frac{1}{2}E(r_t) \\ &= \frac{1}{2}\sum_{i=1}^{t-1} E(r_t r_i) - n\sum_1^{t-1} \psi_i - \frac{(nt-1)}{2}\psi_t + n^2 t.\end{aligned}$$

This expression may now be evaluated, by recalling that ψ_i and $E(r_t - r_i)$ are given by equations (10) and (17) respectively. An approximation to ϕ_t can be obtained by noting that

$$\phi_t = \tilde{\phi}_t + \frac{1}{2}\sum_{i=1}^{t-1} \text{cov}(r_t, r_i),$$

where

$$\begin{aligned}\tilde{\phi}_t &= \frac{1}{2}E(r_t)\sum_{i=1}^{t-1} E(r_i) - n\sum_1^{t-1} \psi_i - \frac{(nt-1)}{2}\psi_t + n^2 t \\ &= \left(\frac{1}{2}\psi_t - n\right)\sum_1^{t-1} \psi_i - \frac{(nt-1)}{2}\psi_t + n^2 t.\end{aligned}\tag{22}$$

The approximation $\tilde{\phi}_t$ given by equation (22) to the expected total number ϕ_t of comparisons thus disregards the correlation between r_t and each of the random variables r_i ($i = 1, 2, 3, \dots, t-1$). Since $\tilde{\phi}_t$ depends only on the values of ψ_i for $i = 1, 2, 3, \dots, t$, it is easier to evaluate than ϕ_t .

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A preliminary study of the population ecology of *Vipera ursinii macrops* from eastern Montenegro

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Vipera ursinii is the most endangered viper in Europe. According to Nilson and Andrén (2001) the highland *V. ursinii* populations of Western and Central Balkan peninsula belong to the ssp. *macrops*. According to same authors (Nilson and Andrén, 1997), *V. u. macrops* is common in Bosnia and Herzegovina, and probably in Montenegro, rare in Croatia and Albania, and very rare in Serbia and the Former Yugoslav Republic of Macedonia. The presence of *V. u. macrops* on the north-western slopes of Bjelasica Mountain was detected in 1997 (Crnobrnja-Isailović, 2002), but no data are available as regards ecology and conservation. In this study we present preliminary data on the population ecology of *V. u. macrops* from the eastern Montenegro (Central Balkans).

Vipera u. macrops was studied at a mountainous site, approximately 1680 m a.s.l., on the south-western slope of Mt. Bjelasica (National Park "Biogradska Gora") in the eastern Montenegro (latitude 42° 54' 15" N, longitude 19° 37' 15" E). The study area was predominantly grassy, but with stone piles distributed haphazardly and several sites with bushes of juniper (*Juniperus communis* ssp. *sibirica*) and bilberry (*Vaccinium myrtillus*, *V. uliginosum*). Microhabitats in the study area were categorized according to Filippi and Luiselli (2002a, b) and modified to 8 types (1 — stones and *Juniperus* bushes between 4 and 6 m in diameter; 2 — stones and *Juniperus* bushes <4 m in diameter; 3 — stones and open grass; 4 — open grass; 5 — open grass and *Juniperus* bushes between 4 and 6 m in diameter; 6 — open grass and other bushes (*Vaccinium*); 7 — stones, *Juniperus* bushes <4 m in diameter and open grass; 8 — *Juniperus* bushes <4 m in diameter, open grass and other bushes (*Vaccinium*)). The total area surveyed covered approximately 4 ha, from 1651 to 1728 m altitude, including the south-western, southern and south-eastern slopes.

The study was conducted daily from July 19 - July 25 2002. Every day, the research lasted from hours 10.00 to hours 17.00. The snakes were captured by hand and sexed. In pregnant females, embryos were counted by gentle palpation of the abdomen. Subadults were identified by body length (less than 295 mm and 346 mm of the total length, for males and females of *V. u. ursinii* respectively — Baron, 1992). All vipers were photographed (pileus from above and from both sides, as well as dorsal colour pattern). Data about weather conditions (cloudiness, exposure, wind direction and speed), time, altitude and microhabitat type at capture site were taken. Body (cloacal), substratum (at the soil level) and air (measured at approximately 160 cm above the ground) temperatures were measured immediately after capture by digital thermometer, up to 0.1°C precision. Specimens were weighed by using Pesola balance (0.5 g precision). For each specimen, 13 morphometric measures were recorded: LTOT — total length; SVL — snout to vent length; BW — body width (at mid-body point); BH — body height (at mid-body point); TL — tail length; TW — tail width (at basis of tail); IN — internasal distance; ISO — intersupraocular distance; HL — head length (from the tip of the snout to the articulation point of the lower jaw and quadrate); HW — head width (across the widest part of the head); HH — head height (at the highest point of

Table 1. Descriptive statistics of morphometric and meristic characters of males ($n = 2$), females ($n = 12$) and subadults ($n = 7$). Morphometric measurements are given in mm. \bar{x} = mean value; $s_{\bar{x}}$ = standard error.

Characters	Males				Females				Subadults			
	\bar{x}	Min	Max	$s_{\bar{x}}$	\bar{x}	Min	Max	$s_{\bar{x}}$	\bar{x}	Min	Max	$s_{\bar{x}}$
LTOT	405.0	380.0	430.0	25.00	422.8	345.0	485.0	11.47	294.9	240.0	346.0	13.18
SVL	358.5	326.0	391.0	32.50	380.8	309.0	439.0	10.67	259.9	216.0	310.0	12.50
BW	17.5	15.1	20.0	2.42	17.8	13.6	20.7	0.64	11.8	9.6	16.9	0.90
BH	17.5	15.2	19.8	2.32	16.3	13.7	18.8	0.48	12.0	10.2	14.6	0.68
TL	46.5	39.0	54.0	7.50	42.0	34.0	48.0	1.36	35.0	24.0	47.0	3.07
TW	5.7	5.6	5.7	0.01	6.1	4.3	9.0	0.38	4.1	2.7	5.2	0.33
IN	3.4	3.3	3.6	0.17	4.1	3.5	4.5	0.08	3.4	3.1	4.0	0.11
ISO	4.3	3.7	4.8	0.52	4.4	3.9	4.9	0.10	3.9	3.3	4.5	0.17
HL	19.9	19.6	20.2	0.30	19.7	16.1	22.2	0.50	16.2	14.4	18.1	0.56
HW	13.5	13.4	13.5	0.04	14.1	12.7	16.3	0.35	11.4	10.0	13.3	0.50
HH	6.8	6.8	6.9	0.05	6.8	5.6	8.4	0.19	6.0	5.3	7.1	0.25
ML	16.2	15.8	16.5	0.38	16.3	13.0	18.6	0.44	13.2	12.1	15.2	0.47
MW	12.5	11.8	13.2	0.68	13.4	11.3	17.7	0.58	10.3	8.1	12.9	0.59
WEIGHT	57.0	42.0	72.0	15.00	67.8	42.0	100.0	4.81	19.9	10.0	36.0	3.15
D	19.0	19.0	19.0	0.00	19.0	19.0	19.0	0.00	19.0	19.0	19.0	0.00
V	119.0	115.0	123.0	4.00	125.2	117.0	129.0	0.98	123.4	118.0	126.0	1.15
S	26.5	21.0	32.0	5.50	22.1	19.0	25.0	0.58	24.1	21.0	30.0	1.37
A	1.0	1.0	1.0	0.00	1.1	1.0	2.0	0.08	1.1	1.0	2.0	0.14

the head); ML — mouth length; MW — mouth width. Four meristic characters were counted: number of dorsal scale rows (D), number of ventral (V) and subcaudal scales (S), and number of apical scales (A). Means are presented ± 1 standard deviation.

All vipers were individually marked by scale clipping, and were released after this procedure.

Twenty-one specimens were analysed, 2 were adult males, 12 adult females (6 of them pregnant) and 7 subadults. There was no recapture over the study period. All gravid females had 6 offspring.

Descriptive statistics for 13 morphometric and 4 meristic traits are given in table 1. Body temperature averaged $26.6 \pm 3.76^\circ\text{C}$ ($n = 21$) and ranged between 16.8 and 32.1°C , while air and substratum temperature were in average 22.1 ± 3.27 and 23.4 ± 3.49 , respectively. Snake SVL was not significantly correlated with body temperature ($r = 0.340$, ANOVA $F_{1,19} = 2.486$, $P = 0.131$). Body temperature was significantly correlated with both air temperature ($r = 0.515$, ANOVA $F_{1,19} = 6.857$, $P = 0.017$) and substratum temperature ($r = 0.592$, ANOVA $F_{1,19} = 10.251$, $P = 0.005$). Also, body temperature was correlated with the interaction of air and substratum temperatures (multiple $r = 0.596$, ANOVA $F_{2,18} = 4.969$, $P = 0.019$; $\beta_{\text{air}} = 0.120$, $\beta_{\text{substratum}} = 0.497$). Cloacal temperature depended much more on substratum than on air temperature.

The average body temperature of pregnant females was slightly, but not significantly higher, than that of non-pregnant females ($\bar{x} = 27.4 \pm 3.63^\circ\text{C}$, $n = 6$ and $\bar{x} = 25.4 \pm 4.77^\circ\text{C}$, $n = 6$, respectively; differences between the two samples $t = -0.82$, $df = 10$, $P = 0.429$). The air temperatures of activity of gravid females were not different

from those of non-gravid females ($\bar{x} = 20.8 \pm 1.71^\circ\text{C}$, $n = 6$ and $\bar{x} = 20.6 \pm 2.34^\circ\text{C}$, $n = 6$, respectively; $t = -0.99$, $df = 10$, $P = 0.923$), and the substratum temperatures of activity of gravid females were not significantly different from those of non-gravid females ($\bar{x} = 22.8 \pm 2.43^\circ\text{C}$, $n = 6$ and $\bar{x} = 21.2 \pm 2.81^\circ\text{C}$, $n = 6$, respectively; $t = -1.055$, $df = 10$, $P = 0.316$).

Analyses of multiple regression of the interaction of air and substratum temperature on snake body temperature showed non-significant correlation for both pregnant (multiple $r = 0.202$, ANOVA $F_{2,3} = 0.064$, $P = 0.940$, $\beta_{\text{air}} = -0.290$, $\beta_{\text{substratum}} = 0.47$) and non-pregnant females (multiple $r = 0.665$, ANOVA $F_{2,3} = 1.190$, $P = 0.416$, $\beta_{\text{air}} = -0.040$, $\beta_{\text{substratum}} = 0.689$).

Meadow vipers were mostly recorded between hours 11.00 and hours 13.00 (fig. 1a), and were more frequently found on the south-western and southern slopes, than on the south-eastern and eastern slopes (fig. 1b). Most specimens were found in habitat types 2 and 3 (fig. 1c).

The average clutch size in our sample was higher than that of *V. u. ursinii* from Italy (3-6 offsprings — Filippi and Luiselli, 2002a, b), Mont Ventoux and Montagnes de Lures (3.83 ± 1.53 — Saint Girons and Naulleau, 1981) and Mont Ventoux (4.03 ± 0.13 — Baron et al., 1996), but less than that of *V. u. rakosiensis* from Dabas Gyón (11.3 ± 3.1 — Újvári et al., 2000).

The total length of the adults in our study area was within the range for *V. u. macrops* (Nilson and Andrén, 2001) as well as for *V. u. ursinii* from France and Italy (Baron, 1992; Filippi and Luiselli, 2002a, b). The minimum recorded total length for a reproductive female was 345 mm (315 mm for *V. u. ursinii* from Mt. Ventoux — Baron, 1997). The maximum body mass of gravid females was 82 g, which is within the range of gravid females from Mt. Ventoux (84.5 g — Baron, 1992). The maximal weight of 72 g for adult males from Mt. Bjelasica exceeds the values reported for males from Mt. Ventoux (58 g — Baron, 1992). The number of dorsal mid-body scale rows and apicals mirrors data reported for *V. u. macrops* (Nilson and Andrén, 2001). On the contrary, the numbers of ventrals and subcaudals in both sexes were generally lower than those reported by Nilson and Andrén (2001).

Cloacal temperatures were similar to those recorded in France by Baron (1997) for both gravid and non-reproductive females, but lower than the optimum activity temperature reported for *V. u. rakosiensis* (32-33°C — Újvári and Korsós, 1997). The maximum cloacal temperature of a specimen from our study area (32.1°C) was recorded for a pregnant female captured at hours 11.26 (0% cloudiness, 0 m/s wind speed). It is expected that female snakes would tend to maintain higher body temperatures when pregnant (Luiselli and Akani, 2002), as Baron (1997) also observed in *V. u. ursinii* from France. The results on thermal ecology of *V. u. macrops* from this study are quite similar to those obtained by Luiselli and Zimmermann (1997) for *Natrix tessellata* from Italy and Austria: in both species, body temperature is significantly correlated with substratum temperature as well as with the interaction of air and substratum temperature, and the differences in average

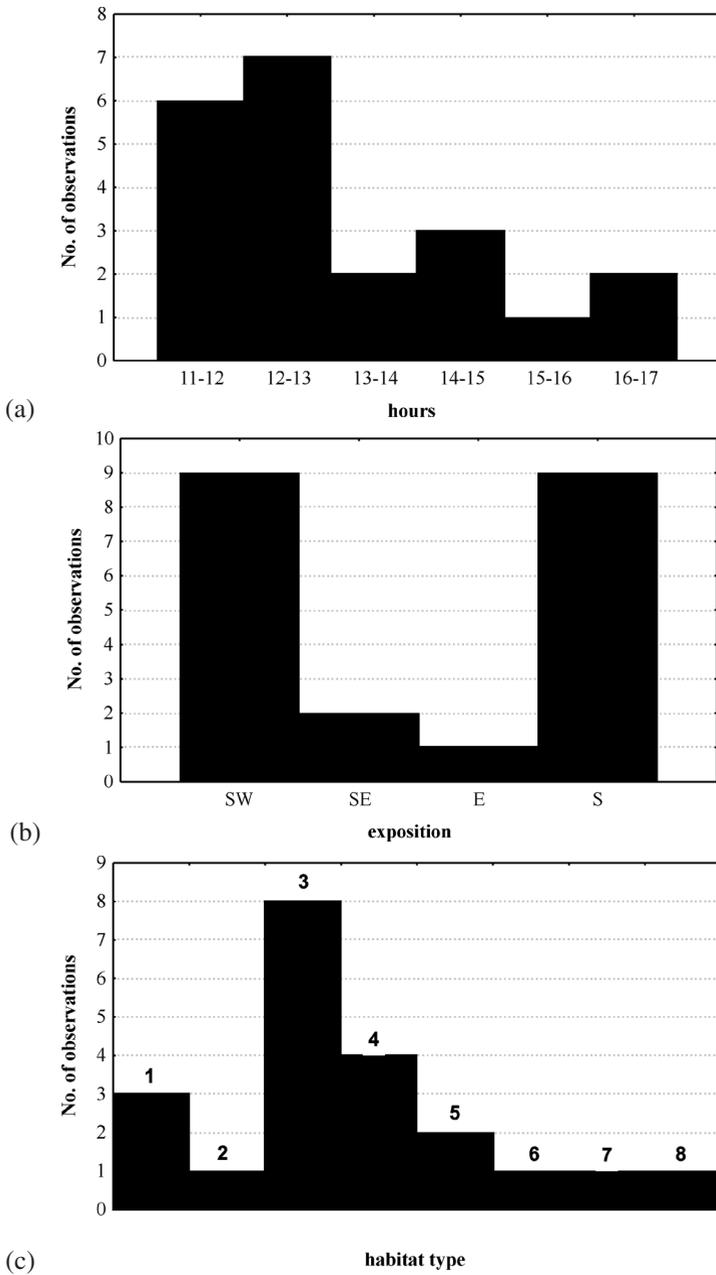


Figure 1. Histograms of daily activity (a), preferred expositions (b) and preferred habitat types (c) of analysed specimens (1 — stones and *Juniperus* bushes between 4 and 6 m in diameter; 2 — stones and *Juniperus* bushes <4 m in diameter; 3 — stones and open grass; 4 — open grass; 5 — open grass and *Juniperus* bushes between 4 and 6 m in diameter; 6 — open grass and other bushes (*Vaccinium*); 7 — stones, *Juniperus* bushes <4 m in diameter and open grass; 8 — *Juniperus* bushes <4 m in diameter, open grass and other bushes (*Vaccinium*)).

body temperature between pregnant and non-reproductive females were non-significant in both species, although pregnant females had slightly higher average body temperature. Our results indicate that in *V. u. macrops* the pregnant females may tend to have higher body temperature and selection of higher substratum temperatures than non-reproductive females, but additional data are undoubtedly needed.

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Death-feigning in *Eurolophosaurus divaricatus*: temperature and habituation effects

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The antipredator behavior of amphibians and squamates varies among and within species, and is influenced by many factors such as habitat structure (Bulova, 1994), predatory regime (Schall and Pianka, 1980; Dowley and Brodie, 1989), type of stimulus (Brodie, 1989), presence of toxic or noxious skin secretions (Brodie, 1977; Williams et al., 2000) and temperature (e.g. Rand, 1964; Hertz et al., 1982; Crowley and Pietruszka, 1983; Arnold and Bennett, 1984; Schieffelin and de Queiroz, 1991; Keogh and DeSerto, 1994; Brodie and Russell, 1999; Gomes et al., 2002). In lizards, known responses include fully active behaviors such as running, biting and other aggressive stationary behaviors, and non-aggressive stationary responses such as death-feigning (also called tonic immobility), a temporary and reversible motor inhibition with virtual suppression of responsiveness to environmental stimuli (Gallup, 1974). Because death feigning is usually elicited by physical contact between an animal and a potential predator, it is regarded as a final step in a sequence of possible antipredator responses (Ratner, 1967). This behavior has been described for ectotherms as diverse as fish (McKaye, 1981), beetles (Miyatake, 2001), anurans (Sazima, 1974, 1978; Zamprogno et al., 1998; Vrcibradic and van Sluys, 2000; Williams et al., 2000; Gomes et al., 2002), snakes (Burghardt and Greene, 1988; Rugiero, 1999), and several lizard families (Greene, 1988). Our aim is to quantify and discuss the ecological correlates of this behavior in the Neotropical lizard *Eurolophosaurus divaricatus*.

Eurolophosaurus divaricatus is a lizard species from the sand dunes of the west margin of the São Francisco River in the northeastern Brazil (Rodrigues, 1986). They are heliotrophic and maintain mean activity body temperatures close to 38.0°C (our data), but probably experience body temperatures as low as 20°C during the night. We collected by noose ten individuals of *Eurolophosaurus divaricatus* in Ibiraba, Bahia, Brazil (10°48'S, 42°50'W), between 12 and 15 November, 2001. Once in the laboratory at University of São Paulo, we measured the amount of manipulation required by individual lizards to exhibit death feigning, and the influence of temperature and number of experimental days on this variable. Animals were maintained in tanks with vermiculite and water ad libitum on a 10:14 light cycle and 27°C mean temperature, and had access to heat lamps to thermoregulate. They were fed crickets, cockroaches and *Tenebrio* larvae three times a week. Tests were conducted from 27 to 30 November 2001, as follows: animals were kept individually in cloth bags inside a climatic chamber and under controlled temperature for at least one hour. For a test, a bag was carefully removed from the chamber, opened, and then one of us (T.K.) suddenly grasped the lizard firmly by the neck. We then recorded the time elapsed from initial holding to the onset of death feigning. The test finished after two minutes

Table 1. Time to death feigning (sec) at three different temperatures (°C) and four days of experiments in ten individuals of *Eurolophosaurus divaricatus*. *n* is the number of trails on each condition.

	<i>n</i>	Mean	<i>s</i>	Minimum	Maximum
Temperature					
20	30	27.2	13.02	7	54
30	30	23.4	16.75	6	74
40	30	19.4	18.30	6	88
Day of experiment					
1	20	33.7	22.91	7	88
2	30	19.8	11.99	6	50
3	30	20.6	13.46	6	54
4	10	21.0	12.79	7	43

when an individual did not show death feigning. We performed up to three tests per day per individual until obtaining three recordings for each individual at each experimental temperature (20, 30 and 40°C). The order of temperatures was random.

The data were analyzed with an ANOVA in which temperature, individual and experimental day were introduced as factors ($n = 90$, $r^2 = 0.57$). Temperature influenced time to death feigning (table 1, $F_{2,75} = 11.611$; $P < 0.001$), which was longer in experiments at 20°C (Scheffé test, $P < 0.002$ in both comparisons), but similar between tests at 30°C and 40°C (Scheffé test, $P = 0.419$). These results are consistent with the view of temperature as one of the most important ecophysiological variables influencing the frequency of expression of antipredator behaviors in squamates. For example, stationary antipredator responses in lizards are more common at low temperatures, when fleeing is likely to be less effective given temperature-induced constraints on locomotor performance (Hertz et al., 1982; Crowley and Pietruszka, 1983; Schieffelin and de Queiroz, 1991). In *Eurolophosaurus divaricatus* death feigning might decrease injury in encounters with predators, and eventually increase the chances of after-capture fleeing, a more likely event for a warm lizard. Regarding the mechanisms by which temperature might influence death feigning we can only conjecture, as temperature has the potential to influence several components of the integrated regulatory mechanisms of this response.

Some individuals consistently required longer manipulation time than others before exhibiting death feigning (fig. 1, ANOVA: $F_{9,75} = 6.232$; $P < 0.001$). We are unaware of studies of individual variation in the antipredator responses of lizards, but there are some examples among amphibians. Stationary responses, for example, are more common in individual salamanders or anurans that exhibit comparatively modest locomotor performance (Dowdey and Brodie, 1989; Gomes et al., 2002). Therefore, future studies might find that quantifiable aspects of death feigning responses in Tropicuridae lizards are not only consistent among individuals, but also correlated to other behavioral, physiological or morphological traits. The number of individuals available at this point, however, is insufficient to test such hypotheses.

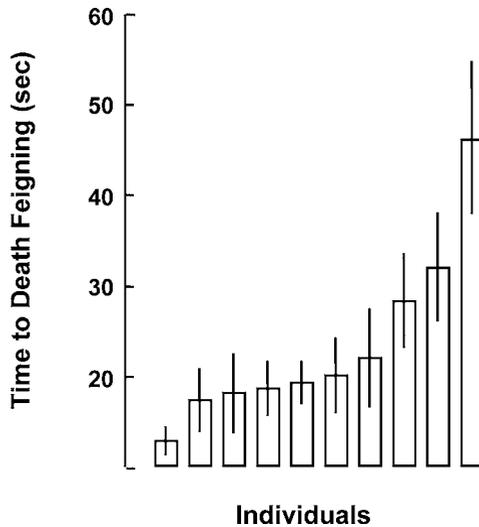


Figure 1. Individual variation in the time to death feigning (sec) of *Eurolophosaurus divaricatus* at three different temperatures unnecessary during four days of experiments. All individuals but number 8 ($n = 8$) were tested 9 times. Bars indicate standard errors.

Time to death feigning was longer the first day (table 1, $F_{3,75} = 12.620$; $P < 0.001$; Scheffé test, $P < 0.001$ in all comparisons involving day 1, $P > 0.245$ in other comparisons), suggesting some degree of habituation. This pattern, however, was influenced by the higher variance in time to death feigning that also characterized the first day of experiments (ANOVA: $F_{3,75} = 11.300$; $P < 0.001$), and is weakened if non-parametric tests are employed (Kruskal-Wallis ANOVA: $H = 6.209$, $df = 3$, $P = 0.107$). This pattern, then, might not be directly comparable to the stimulus-specific habituation in death feigning response has been reported for other squamates, for example the hognose snake (Platt, 1969; Burghardt, 1977).

In addition to *Eurolophosaurus nanuzae* (Galdino and Pereira, 2002) and *E. divaricatus* (Kohlsdorf et al., in press), death feigning has been anecdotally but consistently observed in several other related species, including *Uranoscodon superciliosum*, the most basal species in the phylogeny proposed by Frost et al. (2001), *Plica plica* and *P. umbra*, and the clade including *Eurolophosaurus nanuzae*, *E. amatithes* and *E. divaricatus* (M.T. Rodrigues, pers. comm.). Death feigning, then, might be a common and primitive character in tropidurids, although we have not observed this behavior in *Tropidurus hispidus* and *T. psamonastes*, not even after extensive manipulation (T. Kohlsdorf, pers. obs.).

Whether or not death feigning in Tropiduridae is adaptive is still open to discussion. One possibility is that the frequency of death feigning responses is more common in the more exposed populations to motion oriented predators, for example birds. Antipredator behaviors tend to be evolutionarily plastic (Ducey and Brodie, 1991; Bulova, 1994; Williams et al., 2000), and death feigning might be specially susceptible to rapid evolutionary changes

given the net of possible modulatory steps influencing this response. In mammals, for example, death feigning is modulated by the action of the cholinergic system on specific areas of the periaqueductal gray matter (Monassi et al., 1997), hypothalamus (Oliveira et al., 1997), and amygdala (Ramos et al., 1999), mediated by muscarinic and, possibly, nicotinic receptors (Monassi et al., 1997). Similar mechanisms in homologue brain areas have also been described for anurans (Gargaglioni et al., 2001) and lizards (Davies et al., 2002). Consequently, various simple changes, for example in the localization or quantity of cholinergic receptors in different control areas, could rapidly lead to the intensification or suppression of the death feigning response (sensu Harris-Warrick, 2000).

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Caudal autotomy does not influence thermoregulatory characteristics in the metallic skink, *Niveoscincus metallicus*

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Many species of lizard implement the strategy of tail autotomy as a means to escape from predators (Arnold, 1984, 1988). However, although caudal autotomy may facilitate escape from a potential predator, it may result in longer-term costs (Arnold, 1984, 1988). Following tail autotomy lizards may exhibit reduced levels of reproductive investment (Smyth, 1974; Dial and Fitzpatrick, 1981; Wilson and Booth, 1998), inhibited locomotor performance (Martin and Avery, 1998; Downes and Shine, 2001), restricted growth rates (Smith, 1996), diminished escape capabilities (Congdon et al., 1974; Downes and Shine, 2001), modified habitat use (Martin and Salvador, 1992, 1995), lowered social status (Fox and Rostker, 1982), and reduced survival in natural populations (Wilson, 1992; Fox and McCoy, 2000). However, tail autotomy does not invariably result in adverse consequences being inflicted upon the lizard. Several studies have reported tail loss to have no impact upon survival (Althoff and Thompson, 1994; Niewiarowski et al., 1997 [in some seasons]) or growth (Althoff and Thompson, 1994; Van Sluys, 1998; Fox and McCoy, 2000).

However, the impact of tail autotomy on the thermoregulatory characteristics or behaviour in lizards is poorly understood (Martin and Salvador, 1993; Wilson, 1994). The absence of the tail itself has the potential to modify the heat balance of the lizard through the alteration of its shape and surface area to volume ratio. More importantly, the reduction in activity levels (Formanowicz et al., 1990) and shifts in habitat use (Martin and Salvador, 1992, 1995, 1997; Downes and Shine, 2001) exhibited by several species following tail loss may reduce their basking opportunities or expose them to suboptimal basking environments. Consequently, tail loss has the potential to influence or inhibit thermoregulation in lizards. Such alteration in thermoregulatory characteristics or behaviour as a consequence of tail autotomy may also influence the speed with which an individual can re-grow its tail since the process of tail regeneration is sensitive to both temperature and photoperiod (Turner and Tipton, 1972; Bellairs and Bryant, 1985; Ndukuba and Ramachandran, 1988). Higher body temperatures are generally more conducive to faster rates of tail regeneration (Bellairs and Bryant, 1985). Increasing the mean body temperature, spending less time at cooler temperatures, or elevating the thermal setpoints are all ways to maintain high body temperature and therefore represent potential mechanisms through which tailless lizards

might enhance the rate of tail regeneration. However, simply maintaining normal thermal preferences during tail regeneration, whilst activity and habitat use is restricted (e.g. Formanowicz et al., 1990; Martin and Salvador, 1992, 1995, 1997), may also facilitate rapid tail replacement.

We investigated the effect of tail loss on the thermoregulatory characteristics of the metallic skink, *Niveoscincus metallicus*. Most natural populations of *N. metallicus* exhibit high frequencies of tail loss (60-80%; our unpublished data) and tail autotomy in this species has been demonstrated to impose energetic, reproductive and locomotor performance costs (Chapple and Swain 2002a, b; Chapple et al., 2002). Locomotor performance costs, at least, appear to be temporary and related to tail length in *N. metallicus* (Chapple and Swain, 2002b), therefore providing a meaningful opportunity to examine the effect of tail loss on thermoregulation. The potential alteration of thermoregulatory characteristics following tail loss (i.e. selecting different temperatures, altering thermal setpoints) was examined. Since almost all adult females of this species breed each year and are pregnant for most of the activity season at our study site (4 out of 6 months), we limited our study to male *N. metallicus* to avoid any confounding effects associated with pregnancy.

Study species and collection of animals. *Niveoscincus metallicus* is a small skink (45-65 mm adult snout-vent length; SVL) that is widely distributed over a range of habitats from sea level to sub alpine regions (1400 m) in Tasmania and southeast Victoria, Australia (Melville and Swain, 1999). It is a relatively cryptic species that occupies shaded microhabitats with medium to dense vegetation cover and thick litter (Melville and Swain, 1999). Predominantly a shuttling heliotherm with an active diurnal pattern, *N. metallicus* utilizes both rocks and logs close to the ground to behaviourally thermoregulate (Melville and Swain, 1997).

We collected 14 adult male lizards (i.e. SVL > 45 mm SVL; Swain and Jones, 1994) from around Clarence Lagoon (1000 m a.s.l.; 42°04'S 146°19'E), a small glacial lake on the Central Plateau of Tasmania, Australia, during late September and early October 1999. Lizards were captured by hand or noose and transported back to the laboratory where measurements (± 0.1 mm) were taken of SVL and tail length. All animals had original or completely regenerated tails. Animals were randomly assigned to one of two groups: control ($n = 6$) and experimental ($n = 8$). Initial body size (SVL $\pm s_{\bar{x}}$; mm) did not differ between lizards assigned to each tail loss treatment (Control Males: 54.0 ± 0.77 ; Experimental Males: 54.4 ± 0.80).

Housing conditions. Lizards were housed in an air-conditioned room maintained at 12-14°C under bright fluorescent tube lighting ($\sim 20\ 000$ lux) and UV lighting (14L:10D). They were housed in plastic terraria (20 × 30 × 10 cm) lined with an absorbent substrate. Basking surfaces were provided in the form of upturned terracotta pots, which also provided cover. The basking site was positioned under a 25 W basking light that provided 10 h of heat/light per day. A thermal gradient from 33°C at the basking site to ~ 14 °C in the remainder of each terrarium allowed the lizards to thermoregulate while the basking light was activated. Animals were fed 2-3 times weekly on a diet of mealworms (*Tenebrio* larvae), commercial catfood and mashed banana. Water was available ad libitum.

Experimental design. The thermoregulatory characteristics of each lizard were assessed upon arrival in the laboratory. Caudal autotomy was induced in the experimental animals within five days of the initial test and all lizards were re-tested within one week of this manipulation. The thermoregulatory characteristics of every individual were subsequently re-tested after four and twelve weeks. The length of tail regrowth (± 0.1 mm) for each experimental lizard was measured, using callipers, at each testing period. Consequently, the thermal preferences of lizards were assessed for the first three months of tail regeneration.

Tail autotomy was induced as described in Chapple and Swain (2002b). Visible tail regeneration was evident following a 'latent period' (e.g. Bellairs and Bryant, 1985) of 2-3 weeks. Control animals were handled in an identical manner to the experimental animals except that caudal autotomy was not induced.

A large wooden, open-topped terrarium ($1.2 \times 1.2 \times 0.3$ m) located in an air-conditioned room (ambient temperature $12\text{--}14^\circ\text{C}$) was used as the test arena during the study. The test arena was subdivided into eight separate sections (0.55×0.3 m) by wooden partitions, allowing up to eight lizards to be tested simultaneously each day. However, only one lizard was placed into each of the eight sections to avoid any possibility of social interactions influencing the thermoregulatory characteristics. A 3 cm layer of sand acted as the substrate. Basking sites (ceramic tiles) positioned on wooden blocks positioned under a heat source (25 W light bulb) were set-up in each of the eight sections. The set-up provided a temperature gradient of 15 to 38°C within each section allowing behavioural thermoregulation to occur. This temperature gradient encompassed the normal body temperature range of *N. metallicus* in the field (Melville and Swain, 1997; McCoull, 2001). The body temperature of lizards was recorded throughout the daily activity period (9am–5pm). Small temperature probes (0.5 mm diameter) were inserted into the cloaca and securely held in position with a strip of adhesive cloth tape around the tail. Each probe was connected to a data logger by a 1.5 m lead that allowed uninhibited movement around the arena, permitting shuttling heliothermy. The data logger was linked to a Macintosh computer that was programmed to record the body temperature of up to eight lizards simultaneously at five-minute intervals over the activity period (~ 100 data points per animal). Although up to eight lizards were tested on a given day, each lizard was tested individually in one of the eight partitioned sections of the test arena. Animals were familiarized with the testing arena with cloacal probes in place approximately half an hour prior to the commencement of each trial. Due to the potential effect of recent food intake on body temperature (e.g. Witten and Heatwole, 1978), animals were not fed during the 24 h prior to their trial. However, water was available ad libitum at all times.

For each animal the overall mean body temperature (average of the ~ 100 T_b measurements over the entire activity period), mean upper and lower basking setpoints (average T_b at which each basking event was initiated and ceased; see Tosini and Avery, 1993), and proportion of time (~ 100 T_b measurements) spent at low ($<25^\circ\text{C}$) temperatures was recorded. Repeated measures ANOVA was used to determine the effect of tail autotomy on each thermoregulatory characteristic of *N. metallicus* (tail condition as the factor and time as the repeated measure). The pre-autotomy testing period and the three post-autotomy testing periods were included in each analysis to determine whether the thermoregulatory characteristics of experimental (tailless) lizards differed significantly through time, as a result of autotomy and/or tail regeneration, compared to the control group.

Caudal autotomy did not significantly affect any of the thermoregulatory characteristics that were tested in male *Niveoscincus metallicus* (table 1), although experimental animals did appear to have re-grown substantial portions of their tail during the 12-week study, most having tails approximately half their original length at the completion of the study ($48.1 \pm 1.81\%$). Both mean body temperature and the lower setpoint varied significantly between testing periods, possibly due to seasonal fluctuations in thermoregulatory characteristics and behaviour previously demonstrated in this species (McCoull, 2001). However, these two thermoregulatory traits fluctuated in a similar manner in both control and tailless lizards (table 1). Tailless lizards maintained similar thermal setpoints to tailed individuals over the entire 12 weeks of the study (table 1). Following autotomy, *N. metallicus* did not modify the time spent at low body temperatures (table 1). Tail loss therefore does not appear to influence the thermoregulatory characteristics of *N. metallicus*.

Niveoscincus metallicus was not observed to alter any of the measured thermoregulatory characteristics as a result of tail autotomy. However, more importantly, lizards did not lower their thermal preferences following tail autotomy, which would have been detrimental to their rate of tail regeneration. Although our sample size is relatively small, our data clearly indicate that tailless *N. metallicus* maintain similar thermoregulatory characteristics to that of tailed individuals immediately following tail autotomy and throughout the first three months of tail regeneration (table 1). Despite our sample size, we found

Table 1. Thermoregulatory characteristics of control and experimental (tailless) male *Niveoscincus metallicus* during the 12 weeks of the study. Thermal characteristics prior to tail loss (initial) and during tail regeneration (1, 4, 12 weeks) are indicated. Mean thermal setpoints (SP; upper and lower) and mean body temperature (T_b) are presented as $^{\circ}\text{C} \pm s_{\bar{x}}$. The percentage of time ($\%$ $\pm s_{\bar{x}}$) spent at low ($<25^{\circ}\text{C}$) temperatures is also indicated. The results of the Repeated Measures ANOVA are presented. Asterisks indicate significance at $**P < 0.01$, $***P < 0.001$.

Trait	Treatment	Testing Occasion				ANOVA		
		Initial	1 week	4 weeks	12 weeks	Tail Loss	Time	Interaction
Upper SP	Control ($n = 6$)	34.7 \pm 0.7	35.2 \pm 0.4	35.3 \pm 0.9	35.7 \pm 0.3	$F_{1,12} = 0.54$	$F_{3,36} = 1.08$	$F_{3,36} = 0.54$
	Tailless ($n = 8$)	34.4 \pm 0.5	35.1 \pm 0.5	34.9 \pm 0.7	34.7 \pm 0.3			
Lower SP	Control ($n = 6$)	22.8 \pm 0.5	25.1 \pm 0.8	25.6 \pm 0.2	28.1 \pm 0.4	$F_{1,12} = 0.13$	$F_{3,36} = 32.92***$	$F_{3,36} = 1.01$
	Tailless ($n = 8$)	23.7 \pm 0.7	24.6 \pm 0.3	24.9 \pm 0.5	27.8 \pm 0.2			
Mean T_b	Control ($n = 6$)	30.1 \pm 1.2	30.3 \pm 0.7	30.7 \pm 0.7	31.9 \pm 0.4	$F_{1,12} = 0.50$	$F_{3,36} = 4.64**$	$F_{3,36} = 0.58$
	Tailless ($n = 8$)	29.3 \pm 0.7	30.6 \pm 0.5	29.9 \pm 0.7	31.2 \pm 0.2			
<25°C	Control ($n = 6$)	15.3 \pm 8.0	12.7 \pm 4.4	11.2 \pm 3.0	5.7 \pm 1.4	$F_{1,12} = 0.16$	$F_{3,36} = 2.80$	$F_{3,36} = 0.34$
	Tailless ($n = 8$)	19.5 \pm 4.7	10.0 \pm 1.7	11.7 \pm 2.5	8.3 \pm 1.1			

mean body temperature and lower thermal setpoints to fluctuate in a similar manner in both the control and experimental between testing periods. Such temporal fluctuation may have been the result of lizards changing their thermal preferences as a result of prolonged captivity or simply seasonal variation in thermoregulatory characteristics, as has previously been demonstrated for this species (McCoull, 2001). However, this temporal fluctuation highlights the need to compare changes in thermoregulatory preferences in tailless *N. metallicus* to that in the control group in order to fully gauge the impact of caudal autotomy on thermoregulatory characteristics.

Although thermoregulatory behaviour was not directly measured in our study, the thermoregulatory characteristics of tailed and tailless lizards enable some inference to be made concerning the thermoregulatory behaviour of lizards following autotomy. Previous studies that have examined the effect of caudal autotomy on thermoregulatory behaviour of lizards have produced some conflicting results; however, each has shown that tailed and tailless lizards are able to maintain similar body temperatures. Wilson (1994) found that the water skink (*Eulamprus quoyii*) did not alter its preferred body temperature or thermoregulatory precision following tail loss or during tail regeneration. Furthermore, both preferred body temperature and thermoregulatory precision were similar in tailed and tailless lizards. In contrast, Martin and Salvador (1993) reported that the Iberian rock lizard (*Lacerta monticola*) did modify aspects of its thermoregulatory behaviour following tail loss. Tailless *L. monticola* were found to increase the maximal duration of basking events, maintain lower body temperatures in the morning, and select rockier basking sites that were closer to a refuge. Increasing the maximal duration of basking events and the maintenance of lower morning body temperatures appears to have allowed tailless *L. monticola* to achieve the same thermoregulatory precision, albeit through subtle modification of thermal preferences, as tailed lizards (Martin and Salvador, 1993). This presumably enabled tailless *L. monticola* to maintain body temperatures conducive to tail regeneration despite their reduced locomotor performance and modified habitat use (Martin and Salvador, 1992, 1993, 1995, 1997). Consequently, this study and the other studies to date on the influence of caudal autotomy on thermoregulation have indicated that tailless lizards maintain similar thermoregulatory precision to that of tailed lizards. However, what consequences are the thermal preferences of tailless *N. metallicus* likely to have on the rate of tail regeneration and duration of costs experienced in this species?

Once a lizard has shed its tail it is unable to employ the strategy of caudal autotomy again until it has sufficiently re-grown its tail (Arnold, 1984, 1988). This is because both the length and movement of the shed tail determine the effectiveness of caudal autotomy as an escape strategy (Congdon et al., 1974; Dial and Fitzpatrick, 1983, 1984; Daniels et al., 1986; Downes and Shine, 2001). The extremely high incidence of tail autotomy in most natural populations of *N. metallicus* (60-80%; our unpublished data), suggests that tail loss is an extremely effective defensive strategy for this species. Given that many (but not all) lizard species experience restricted growth rates (Ballinger and Tinkle, 1979; Smith, 1996) and reduced survival (Wilson, 1992; Fox and McCoy, 2000) following caudal

autotomy, tailless lizards are exposed to many dangers until they are able to replace the tail. Indeed, tail loss in *N. metallicus* may reduce energetic reserves (Chapple and Swain, 2002a), decrease reproductive output (lower litter size; Chapple et al., 2002) and reduce locomotor performance (Chapple and Swain, 2002b), and consequently has the potential to significantly decrease fitness. Thus, rapid regeneration of the tail may be vitally important in *N. metallicus*.

Recent evidence indicates that the costs of caudal autotomy are only incurred until a certain proportion of the tail (~half) has been regenerated. Downes and Shine (2001) demonstrated that garden skinks (*Lampropholis guichenoti*) that experienced an initial reduction in sprint speed following autotomy regained their normal sprint performance once they had regenerated about half of their tail (~6 weeks). Similarly, tailless *N. metallicus* regain their locomotor performance once they have replaced about half of their tail, which takes approximately 6-12 weeks (Chapple and Swain, 2002b). *Niveoscincus metallicus* is capable of completely regenerating the tail within 3-5 months, whilst maintaining the thermal preferences presented in this study (Chapple and Swain, 2002b; our unpublished data). It is unknown whether increasing any aspect of thermoregulatory behaviour would significantly increase the rate of tail regeneration in *N. metallicus*. However, given that a large proportion of *N. metallicus* in natural populations have tails that have been substantially regenerated following previous tail loss events (Chapple et al., 2002a), it appears that this species is capable of enduring the short-term costs of autotomy until it is able to sufficiently replace its tail without the need to alter its thermoregulatory characteristics to assist the regeneration of the tail.

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Communal nesting of *Psammodromus algirus* (Linnaeus, 1758), under extreme environmental conditions

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On 9 July 2000, among the rear dunes of Lariño beach (Muros, A Coruña province, N Spain; 9°8'W-42°47'N, 5 m asl), a lizard nest containing 32 eggs was found. The nest, hidden beneath a stone, 60 × 45 cm in size, in loose siliceous sand with very little organic matter, averaged 4 cm in depth. All eggs were together but formed two sets of different size. The smaller eggs measured (mean ± one standard deviation): 12.9 mm ± 0.5 mm long (range 11.9-13.8 mm; $n = 18$) and 7.9 ± 0.3 mm wide (range 7.5-8.6 mm; $n = 18$). The larger eggs measured 13.8 ± 0.3 mm in length (range 13.6-14.0 mm; $n = 14$) and 10.6 ± 0.4 mm in width (range 10.0-11.5 mm; $n = 14$). The two sets attained significantly different sizes (unpaired t -test, $t = 5.20$ for the length of the eggs, $t = 17.28$ for the width of the eggs; $df = 30$ and $p < 0.001$ in both comparisons). These results suggest two sets to correspond to two different incubation stages (therefore to different laying dates), the smaller ones apparently corresponding to more recent clutches, and the larger ones apparently corresponding at a more advanced incubation stage (especially because of their larger width). The eggs were removed from the wild and young of *Psammodromus algirus* hatched under laboratory conditions, after 31-33 days for the larger eggs, and 52-59 days for the smaller ones. Newborns were released in the wild close to the nest.

On 16 July 2002, on the southern slope of Sierra Elvira (Atarfe, Granada province, S Spain, 3°42'W-37°14'N, 650 m a.s.l.), a lizard nest containing 42 eggs was found buried in

a pile of limestone sand intended for construction. The sand, covered by debris, retained high moisture content. Only a sample of the eggs was measured, because all were similar in size (mean \pm one standard deviation): 12.5 ± 0.9 mm in length (range 10.8-14.1 mm; $n = 14$) and 8.9 ± 0.9 mm in width (range 6.6-10.2 mm; $n = 14$). These dimensions match previously published data for *P. algirus*, and a hatchling confirmed the identity of this species. Thus, the eggs had been found in the final incubation stage.

Clutch size of *P. algirus* ranges from 2 to 11 eggs (Pérez-Mellado, 1998), although this number correlates with maternal body size (Pérez-Quintero, 1996). The mean clutch size has been established at 4.9 and 5.4 eggs in the south-western and central Iberian Peninsula, respectively (Díaz, 1994; Pérez-Quintero, 1996). Consequently, the two nests described in the present work correspond to the clutches of approximately six and eight females (A Coruña and Granada populations, respectively) and thus exemplify communal nesting.

Communal nesting has been described for reptiles in situations of high density (Shine, 1991), females attracted by eggs already laid (Petzold, 1982), high intraspecific social affinity (Swain and Smith, 1978), or the lack of appropriate nesting sites (Blázquez and Villafuerte, 1990; García-Márquez et al., 1996), situations in which females actively seek these places (Galán, 1994, 1996). The two study areas considered here are not heavily populated by *P. algirus*, implying that the communal nesting was not caused by population density. Because we have no direct observations on the behaviour of laying females, we cannot discuss the second situation. With respect to the third situation, in this species a single male territory can coincide with up to ten female territories (Salvador et al., 1995), making it plausible to find the combined clutches of six or eight females. Finally, with respect to the fourth possibility, precipitation at the southern site is rather low (430 mm of annual rainfall in the closest meteorological station) and temperatures during July are very high (mean maximum temperature for that month 38.5°C ; standard 30-year meteorological averages; MMA, 2001); the ombrothermic conditions would be even harsher in the exact nesting location, facing south. This appears to explain the selection of a soft, moist substrate as a nesting site. The northern communal nest reported here pertains to the northernmost population for this species throughout its western geographic range (Galán and Fernández, 1993; Carretero et al., 2002), marking the limit of the environmental conditions for the species. Conditions selected for the northern nest included less soil moisture (loose beach sand) and strong sun radiation, in a rather wet climate (1380 mm annual rainfall), with also mild temperatures (mean temperature of July 20.8°C ; 30-year standard meteorological averages; Martínez-Cortizas and Pérez-Albreti, 1999). This scenario supports the hypothesis of communal nesting due to a scarcity of suitable nesting places.

Although the breeding ecology of *P. algirus* has been intensively studied (see review in Pérez-Mellado, 1998), communal nesting has never been described for the species. The populations considered here are in diametrically opposed geographic positions in the Iberian Peninsula, and thus under markedly differing conditions that represent two limits of environmental conditions that the species can withstand (hot, dry south-eastern zone of

the Iberian Peninsula vs. the temperate, wet north-western zone). Despite these possible different ecological constraints, both populations seem to rely on the same communal nesting strategy. Therefore, we deduce that, under such adverse conditions, the search for a site favourable to egg incubation would induce communal nesting in *P. algirus*. However, only two communal nests were found, and more findings in the same study areas are necessary to support this hypothesis.

The stressful scenario at the distribution limit described here raises the issue of the conservation of this species. The southern population, in relying for nesting on an artificial substrate (a pile of sand for construction) with artificial conditions to conserve moisture (sand under debris), shows an unusual condition for nesting in Mediterranean ecosystems for this species. In fact, the site where the communal nesting was found is a degraded area because of the presence of stone quarries, cultivated fields, and coniferous plantations. Certainly, in the Iberian Peninsula, Mediterranean landscapes with complex and structured vegetation are shrinking in area from year to year, such habitats being preferred by *P. algirus* (Santos and Tellería, 1989; Díaz and Carrascal, 1991). The northern population considered here, inhabiting beach dunes, is threatened by encroaching construction and tourism (Galán, 1999). Therefore, conservation of Spanish Mediterranean reptiles requires a broad perspective that takes into account the overall biology and ecology of the species, and particularly its reproductive ecology. A reptile species becomes threatened when appropriate nesting places area are lacking in the wild (Castilla and Swallow, 1995), even though the all other habitat requirements remain suitable.

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Age structure in *Lacerta schreiberi* from Portugal

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Schreiber's green lizard (*Lacerta schreiberi* Bedriaga, 1878) is an endemic species of the Iberian Peninsula with a distribution restricted to the northwestern part of the peninsula and to the Iberian Central System (Estrela-Gredos-Guadarrama mountains) (Gasc et al., 1997). There are also some isolated populations in the south of Portugal and Spain (Barbadillo et al., 1997; Brito et al., 1998) (fig. 1). Usually, it inhabits mountainous regions and humid areas, being generally associated with streams with dense vegetal cover (Brito et al., 1996, 1998). This species is included in the Annex II of the Bern Convention and in Annexes II and IV of the Habitats Directive (Directive 94/43/EEC). In several areas,

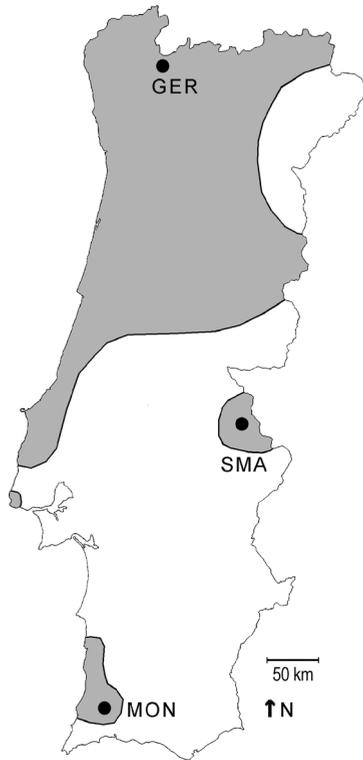


Figure 1. Distribution area of *Lacerta schreiberi* in Portugal (grey) and location of sampled populations. GER — Gerês; SMA — S. Mamede; MON — Monchique.

namely in the southern isolates, populations appear to have experienced serious declines and range reduction, probably since the last glaciations. However, human-induced habitat alterations have also been invoked as a cause for declines in non-mountainous areas (Brito et al., 1999).

Determining individual ages has been recognised as highly important for demographic and population viability studies (Ruggiero et al., 1994), as well as for species conservation and wildlife management (MacGuire et al., 1995). When direct age determination is not possible, skeletochronology is a very useful tool and has been widely used to evaluate ages of amphibians and reptiles (Castanet, 1985; Bastien and Leclair, 1992; Caetano and Leclair, 1996; Waye, 1999; Lima et al., 2001). The present study is part of a conservation programme for the populations and habitats of *Lacerta schreiberi* in Portugal that aimed to gather information on the biology and ecology of this species. The age structures of three populations, one from the main distribution range and two from the southern isolates, were estimated by skeletochronology.

Samples were collected between March and April, from 1994 to 1996, in three Portuguese populations: (1) Gerês (GER) in the North ($8^{\circ}34'W$ $37^{\circ}20'N$), at an altitude of 760 m (32 males, 46 females and 32 juveniles);

(2) S. Mamede (SMA), one isolated population in the Southeast ($7^{\circ}24'W$ $39^{\circ}19'N$), at an altitude of 510 m (47 males, 37 females and 32 juveniles); and (3) Monchique (MON), the southernmost isolate ($8^{\circ}08'W$ $41^{\circ}48'N$), at an altitude of 500 m (64 males, 36 females and 27 juveniles) (fig. 1). Lizards were measured (snout-vent length to the nearest mm) and were assigned to the stages of juvenile, sub-adult or adult, based on their coloration pattern (Galán, 1984).

The first finger of the left hand was clipped and preserved in 70% ethanol. The second phalanx was used for skeletochronology. The skeletochronological procedure was carried out as described by Castanet and Smirina (1990) and Rebelo and Caetano (1995). Phalanges from hatchlings born in captivity (11 from GER, 15 from SMA and 10 from MON) were used to check for the existence of a birth line (Castanet, 1985; Nourira, 1992). Lizards found dead in nature (6 males and 7 females), were used to test for the correspondence between the number of lines of arrested growth (LAG) present in different long bones (femora, humeri and phalanges) of the same animal. A digital image of the cross-section with the smallest medullary cavity was captured using a video camera installed in an optical microscope and connected to a computer. The bone areas corresponding to the cementing line of resorption, the first, second and third visible LAG, and the bone periphery, were measured using a Geographic Information System, TNTMips version 5.7 (MicroImages, 1997). The number of LAG omitted by endosteal resorption was evaluated using a back calculation technique described, among others, by Leclair and Castanet (1987) and Castanet and Smirina (1990): the mean values (\pm standard deviation) of the areas between LAG from hatchlings and juveniles born in captivity were used to estimate the number of LAG resorbed in each adult. To verify the periodicity of LAG formation, the methodology described, among others, by Hemelaar (1981) and Rebelo and Caetano (1995) was followed: the number of LAG was compared in 17 animals captured in consecutive years (4 from GER, 5 from SMA and 8 from MON), and to which a finger was cut each year.

For each population, the existence of differences between males and females in the area of endosteal resorption was tested through covariance analysis (ANCOVA) with body size (SVL) as covariate. The chi-square heterogeneity test (Zar, 1996) was used to: (1) verify if there was homogeneity among the age class distribution of the samples collected in each year; (2) test for differences in the distribution of males and females along the several age classes (within and among populations), and (3) test for differences in the distribution of individuals, within each population, among age classes. Size at birth (from clutches incubated in captivity), as well as adult and age-specific sizes were compared among populations with one-way ANOVA, followed, when significant differences were found, by Tukey HSD test for unequal sample sizes. Statistical tests were performed by hand or with STATISTICA 5.0 software package (Statsoft Inc., 1996).

In femora and humeri sections the transition between narrow and intensely coloured lines (LAG), and larger bands where colour was less intense (zones, sensu Castanet, 1985) was clearly distinguishable. In phalanges, LAG were less coloured and more diffuse than in femora (fig. 2). In 10 out of 13 cases we found an exact agreement between the number of lines counted in the several long bones of the same individual. Disagreements were caused by a higher endosteal resorption in femora, where fewer LAG were visible than in phalanges and humeri. The annual deposition of a LAG was verified in 15 out of the 17 analysed cases, confirming that only one zone and one LAG are formed each year and that winter corresponds to the period of LAG formation. In the two other cases it was not possible to identify any LAG deposited during the winter.

In the majority of the cases resorption was asymmetric, deleting partially one LAG in 107 cases (30%) and two LAG in 28 cases (8%). In a few cases there was a complete omission of one LAG (11 cases — 3%) or even two LAG (4 cases — 1%). The number of resorbed lines was different between populations. The population located at higher latitude and altitude (GER) had the highest resorption areas in both sexes (females: ANCOVA, $F_{2,134} = 11.7420$, $P < 0.001$; males: ANCOVA, $F_{2,155} = 3.1527$, $P < 0.05$). In this population there were 48 cases (44%) of partial resorption of LAG one and 17 cases (15%)

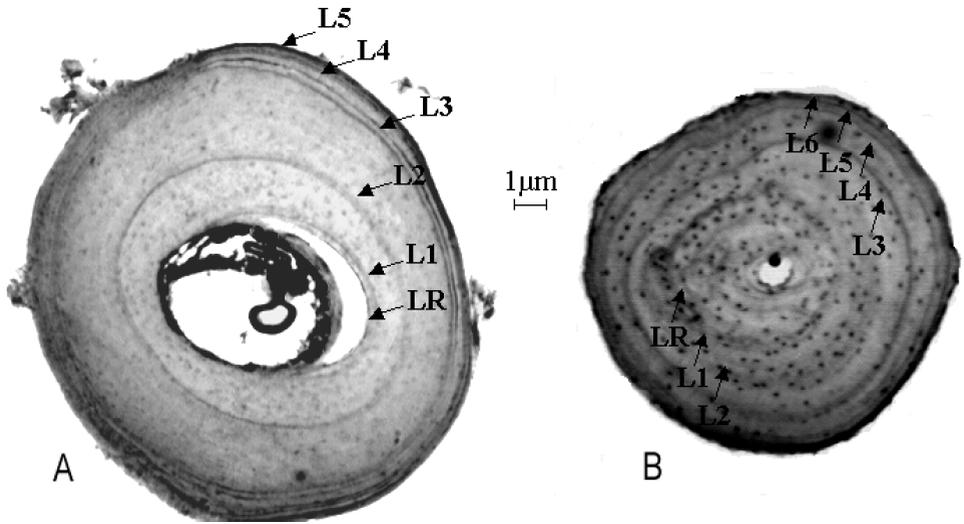


Figure 2. Histological sections from two bone pieces of the same animal (a male from MON). A — Femoral section; B — Phalangeal section; LR — Resorption line; L1 to L6 — Lines of arrested growth 1 to 6, respectively.

of LAG two, while at MON there were 36 cases (28%) of partial resorption of the first LAG and only one case (0.8%) of the second LAG. The first LAG was totally resorbed in six individuals from GER (1.6%), two individuals from SMA (0.6%) and three individuals from MON (0.8%); only in GER the LAG two was totally resorbed (4 cases — 4%).

Homogeneity of age distribution among the three sampling years was not rejected in any population (GER: $\chi^2_{0.05,16} = 17.99$, $P > 0.05$; SMA: $\chi^2_{0.05,14} = 16.71$, $P > 0.05$; MON: $\chi^2_{0.05,14} = 13.87$, $P > 0.05$), thus, data of the three years were pooled (fig. 3). The majority of the individuals (66%) were between 3 to 5 years old, in all populations. Longevity was also similar among the three populations (7 to 8 years), the oldest individuals being two 8-year old females from GER. Age structures of adult males and females were significantly different in GER ($\chi^2_{0.05,6} = 46.9$, $P < 0.001$) and MON ($\chi^2_{0.05,5} = 11.1$, $P < 0.05$), where females were older (fig. 3). Despite the large proportion of 3-year old males in MON and few 4-year old females in GER no significant differences were detected, among the populations, in adult (>2 years) age structures for both sexes (females: $\chi^2_{0.05,10} = 14.86$, $P > 0.05$; males: $\chi^2_{0.05,8} = 9.47$, $P > 0.05$). All of the 2-year old males were classified as sub-adults, based on their coloration pattern, whereas more than half of the 3-years old were already classified as adults (52% in MON, 60% in GER and 86% in SMA). A similar pattern was found for the females: most 3-years old were classified as adults (60% in SMA, 70% in GER and 75% in MON). However, some 2-year old females were already classified as adults (two out of four females in GER and one out of two females in MON). There were no interpopulational differences in body size (one-way ANOVA, $F_{2,40} = 1.43$, $P > 0.05$) among hatchlings born in captivity, as well as among adult females (one-way ANOVA, $F_{2,90} = 2.35$, $P > 0.05$). Adult males from MON were significantly smaller than those

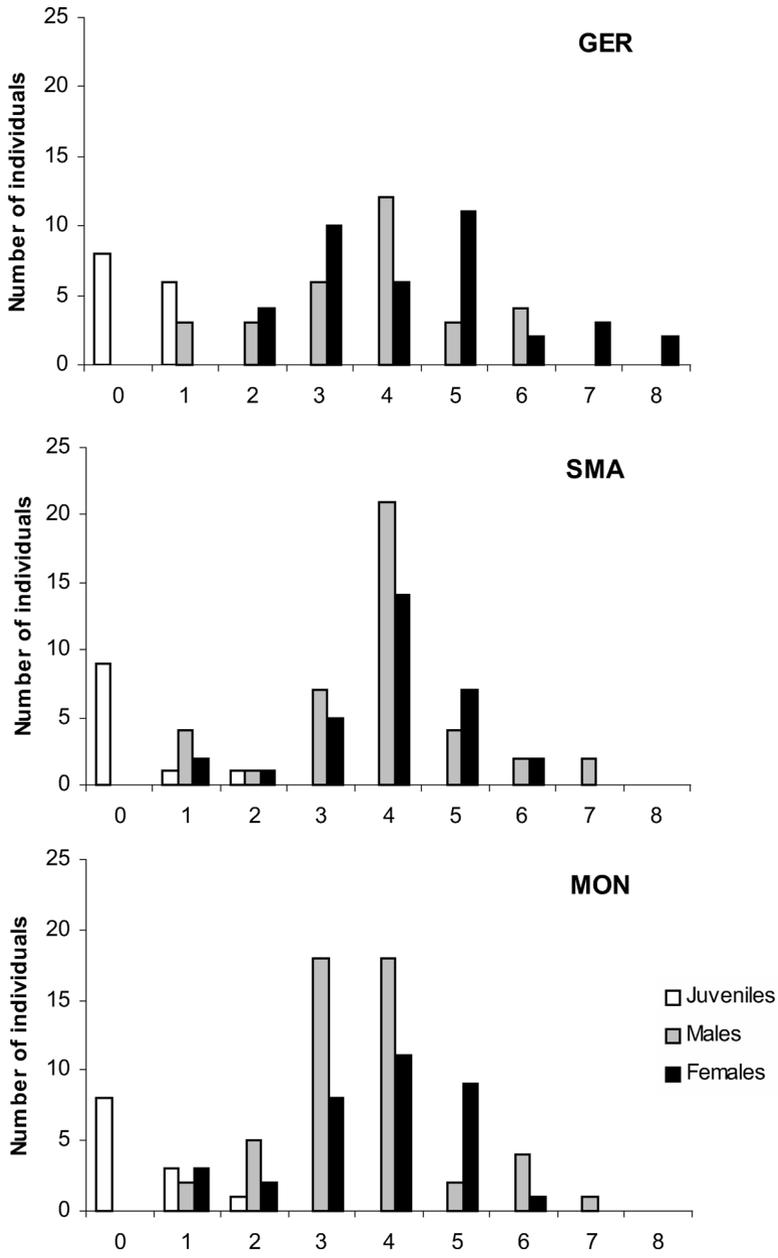


Figure 3. Age structures of *Lacerta schreiberi* from three populations. GER — Gerês; SMA — S. Mamede; MON — Monchique.

from the other populations (one-way ANOVA, $F_{2,102} = 8.35$, $P < 0.001$, followed by Tukey HSD test), but this is probably a consequence of a bias towards young individuals (3-years old) in this population (fig. 3). In fact, there were no interpopulational differences in age-specific size for the 3-, 4-, 5- and 6-year olds, in both sexes.

Despite the relative difficulty to assess LAG in phalanges, as opposed to other long bones such as the humeri, there were no major differences between the number of lines counted in several long bones (femora, humeri and phalanx) of the same individual, and in some cases femora showed a lower number of LAG due to a higher level of endosteal resorption. Therefore, the use of phalanges allows accurate age determination in *L. schreiberi* and does not imply the sacrifice of individuals from this protected species.

Using TNTMips a GIS, to calculate areas between different LAG allowed the estimation of the number of lines resorbed, without assuming bone circularity. We found a significantly higher endosteal resorption in the population located at a higher latitude and altitude, as well as with higher precipitation (GER). This may be related with local environmental conditions that may influence the processes of bone growth (Leclair, 1990).

The age structures of these populations are similar, with the 3, 4 and 5 year-old age classes being the most common. The acquisition of adult coloration, coincident with sexual maturation (Marco and Perez-Mellado, 1989), occurs mainly at 3 years of age in both sexes, but females may mature earlier than males, at age 2. The longevity detected in this study (8 years old), is slightly lower than the previously reported (10 years, Marco, 1995), but is similar to the longevities reported for lacertids of similar size from the paleartic region and from the Mediterranean basin (Castanet, 1986-1987; Bauwens and Díaz-Uriarte, 1997; Castilla and Castanet, 1986).

Results seem to indicate that geographical location and temporal isolation do not influence, in a determinant way, the age and size structures of *L. schreiberi*. These demographical traits may thus have become fixed in this species. This invariance may also be a consequence of the high selectivity that *L. schreiberi* presents towards specific habitats, as it occupies homogenous habitats, always near river streams, independently of its geographic location (Brito et al., 1996; Brito et al., 1998).

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Density dependence in stream-dwelling larvae of fire salamander (*Salamandra salamandra*): a field experiment

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Numerous laboratory and field studies have demonstrated that larval growth and development is negatively affected by density in several amphibian species (e.g. Wilbur, 1977; Petranka, 1984; Newman, 1994; Scott, 1990; Walls, 1998). General responses to increased larval density included reduced growth rates, smaller body sizes at metamorphosis, lower survival, longer larval period, and skewed body size distribution. The mechanism generally claimed behind this process is food limitation (e.g. Petranka and Sih, 1986; Scott, 1990).

We conducted a field experiment to test this negative effect of density on the larval development of fire salamander (*Salamandra salamandra*). Fire salamander is an ovoviviparous species, which deposits larvae in streams, mostly in stream-fed pools (Dely, 1967). This species has never been involved in such an experiment, and field experiments with manipulated larval density in stream-dwelling species are sparse in the literature.

The experiment was carried out in the Aggtelek National Park in northeastern Hungary from May to July in 1999. Eight enclosures were constructed in the bed of Lakatos-creek, a first-order stream. The enclosures were made of a metal frame fitted with mesh screen. Enclosures were 2 m long, 0.2 m high, and 0.7 m wide, and were divided into two halves in the middle, perpendicular to the long side. Enclosures were embedded in the streambed with their 2-m side parallel to the direction of flow. The water depth in the stream was never more than 0.2 m, thus, larvae were able to swim to the water surface for oxygen. Distances between enclosures ranged between 8 and 30 m according to the morphology of the streambed. The average width of the stream was nearly equal to the size of the enclosures.

Mesh size (0.5 cm) allowed the inflow of the makrozoobenthos, which provided continuously available food for larvae within the enclosures. Enclosures were covered with the mesh screen to exclude predators. *Neomys* sp., a probable predator of fire salamander larvae (Haberl, 2002), was observed around the enclosures.

Experimental populations were introduced into the enclosures on 2 May in 1999, two weeks after the enclosures had been placed to the stream, when enough larvae became available. Larvae were collected only from the Lakatos-creek. Larvae were assigned randomly to experimental treatments to assure the initial homogeneity and all injured individuals were excluded.

Table 1. Description of developmental stages developed for fire salamander larvae. Notes: sometimes the whole larva is black even in Stage 5, and gills can be large and well developed even on Stage 3 or 4 larvae.

Stage	Overall color	Yellow pattern	Gills	Tail fin
1	light gray, nearly transparent	very lightly on the proximal part of limbs	small	small, transparent
2	light gray with dark gray marbled pattern	on the proximal part of limbs	large, red	gray and thicker
3	dark gray	on the parotid gland and upper eyelid	large, red	superior part of the fin, narrow
4	dark gray with black pattern	starts to appear on the entire body	large, red	narrow, the tail becomes thick, no end point
5	glistening black	characteristic pattern on the whole body	reduced or no	no fin, the tail is cylindrical

Larvae were placed to the enclosures in two densities. The high-density treatment consisted of 30 individuals per enclosure half, while the low-density treatment only 10 individuals. Both densities occur under natural conditions (K. Csilléry, personal observation). The treatments were randomly assigned to enclosure half pairs, i.e., one half of an enclosure was in the high-density treatment, while the other half was in the low-density treatment.

Every second week after larvae were introduced to the enclosures the total length (to the nearest 0.1 mm, venire caliper), and body mass (to the nearest 0.1 g, OHAUS balance) of larvae were measured after drying the larvae with a towel to remove excess surface water. The stage of development was also recorded using criteria developed in previous years of the study (table 1). Developmental stages have been developed previously for the fire salamander by Juczyk and Zakrzewski (1981). Although their definitions of larval stages are very similar to ours, we prefer our staging, which is independent of size and concentrates on the morphological changes. When finding larvae close to metamorphosis (stages 4 or 5) or on pieces of rock or wood we moved them out of the enclosures after measuring them. Water depth was also recorded each time as an average of nine measurements taken at nine evenly spaced points in each half enclosure with a ruler.

Larvae initially introduced to the treatments did not differ between treatments and between enclosures (ANOVA of log-transformed body length: $F_{1,317} = 0.199$, $P = 0.656$; $F_{1,317} = 0.405$, $P = 0.525$, on body weight: $F_{1,317} = 0.854$, $P = 0.356$; $F_{1,317} = 0.602$, $P = 0.438$, for treatments and enclosures respectively).

In order to detect the effect of the density treatment half-enclosure means were used for further analysis, because individuals within an enclosure-half cannot be treated as statistically independent observations (Hurlbert, 1984; Wilbur, 1987). Repeated measures analysis of variance was used to test the effect of larval density and location in the stream (enclosure) on body length and log-transformed body mass as response variables and mean water depth as covariate. The enclosure effect was non-significant, thus we excluded it from the analysis. Type III model was used and data met the assumptions of analysis of variance. Larvae at metamorphosis (stage 5, see table 1) were first recorded on the week 8. After any metamorphosis the half enclosure means of the remaining populations are biased, so data were used only until week 6. One enclosure which was torn apart was excluded.

The repeated measures analysis of variance of body length revealed that larvae increased their body length on average more in the high density treatment than in the low density (table 2, fig. 1). Body mass was not influenced by the density treatment, but by water depth (table 2, fig. 1); with increasing mean water depth the mean body mass decreased. Larvae experienced a decreasing water level throughout the whole experiment (linear regression, $t = -10.597$, $P < 0.0001$), and this decrease did not differ between treatments (water level by treatment interaction: $t = 0.720$, $P = 0.472$).

A paired test was conducted for the proportion of individuals in each developmental stage on week 6 to compare the effect of the two density levels on the rate of development.

Table 2. Repeated measures analysis of variance in body length and body mass. Salamander larvae were kept in two different densities (initially 10 and 30 individuals per enclosure half, respectively) in enclosures ($n = 7$) placed in a first-order stream.

Source	SS	df	F	P
Body length				
density	9.206	1	5.842	0.034
water depth	3.317	1	2.105	0.175
residual	17.33	11		
Body mass				
density	0.004	1	2.172	0.169
water depth	0.016	1	8.463	0.014
residual	0.020	11		

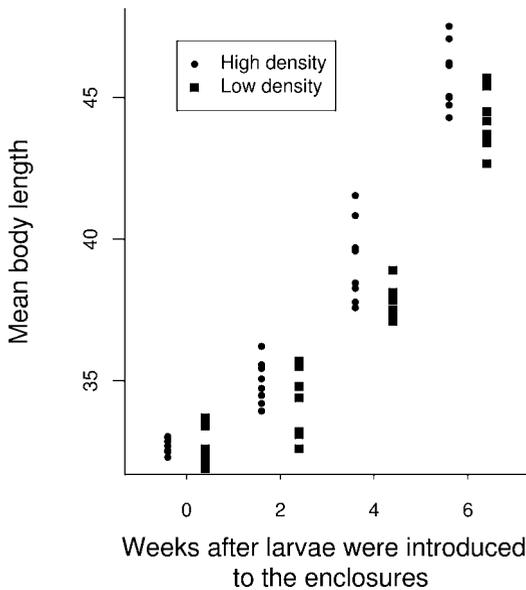


Figure 1. Mean body length (mm) for each high and low density enclosure-half from the introduction of larvae to the enclosures to the sixth week. *Salamandra salamandra* larvae were kept in two different densities with initially 30 and 10 individuals per enclosure half placed in a first-order stream.

The proportion of larvae in stage 1 was higher in the low density treatment (fig. 2, Wilcoxon test, $Z_6 = -2.197$, $P = 0.028$) than in the high density treatment, whereas there was no difference in the proportion of larvae in stage 2 between density treatments (Wilcoxon test, $Z_6 = -1.022$, $P = 0.307$). There were more larvae in stage 3 in the high-density treatment (Wilcoxon test, $Z_6 = -2.028$, $P = 0.043$), and stage 4 larvae appeared only in the high density treatment on week 6.

The difference in size among larvae is a well-known parameter in promoting cannibalism. Under high density the ratio of the body length of the smallest to the largest larva was

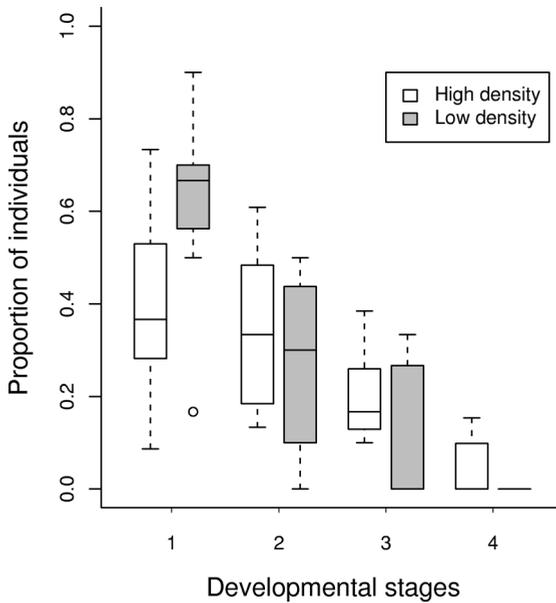


Figure 2. Proportion of individuals in each developmental stage after six weeks the introduction of the experimental populations to the enclosures. Data are combined from all seven enclosures. *Salamandra salamandra* larvae were kept in two different densities (initially 30 and 10 individuals per enclosure half, respectively) placed in a first-order stream. Boxes represent the interquartile range with median line.

higher than that under the low density on week 6 (Wilcoxon test, $Z_6 = -2.36$, $P = 0.018$). Larval survival was estimated as proportion of individuals surviving until week 6 for each half-enclosure and compared with a paired test. Larval survival until the sixth week did not differ between density treatments (Wilcoxon test, $Z_6 = -0.42$, $P = 0.674$). Number of larvae in the enclosures on week 6 were 23, 30, 29, 24, 15, 30, 13 in the high density treatment (initially 30), and 10, 8, 3, 10, 10, 10, 6 (initially 10) in the low density treatment. Dead larvae were not found, most likely because *Gammarus* species quickly consume any organic matter in the stream.

Data on larvae at metamorphosis were available for a total of 46 individuals collected on weeks 8 and 10, of which 31 (67.4%) were from the high density and 15 (32.6%) from the low-density treatment approximately evenly distributed over all enclosures. This proportion did not differ from the 3:1 expectation (continuity corrected $\chi_1^2 = 1.420$, $P = 0.2334$). Body mass of larvae differed between the high and low density treatments, whereas body length did not (t-test for body mass: $t_{45} = 2.494$, $P = 0.016$; body length: $t_{45} = 1.837$, $P = 0.073$). Body length and mass of larvae at metamorphosis were the following with normal based confidence intervals in parenthesis: body length at high density was 52.68 mm (51.43, 53.93), whereas at low density 55.25 mm (53.81, 56.69); body mass at high density was 1.14 g (1.05, 1.23), whereas at low density 1.38 g (1.29, 1.47).

Salamandra salamandra larvae in this experiment responded to high density by increasing their body length. Since body mass did not change with density in the first six weeks of the experiment, we might conclude that larvae became longer under the high density not because they experienced better conditions, but because they wanted to escape from the more stressful environment, which might include competition, aggression, and food limitation, as it has been reported for larval amphibians before (e.g. Scott, 1990; Newman, 1994; Walls, 1998). This explanation is in agreement with our other results. Larvae developed faster in high density, in other words, they were closer to metamorphosis six weeks after the introduction to enclosures than larvae in the low density treatment. Similarly data on larvae at metamorphosis suggests that larvae developed in low density are heavier at metamorphosis, thus more likely to survive in the terrestrial habitat and reproduce (Scott, 1990; Walls, 1998).

Alternatively, larvae at the high density at the beginning of their development treatment may have experienced better conditions in presence of more conspecifics, as they were longer, which has been shown for other larval amphibians (Wilbur, 1977). If under our experimental conditions the food was not limited (mesh size allowed food renewal), crowded larvae could have a higher feeding success in a stream full of fallen leaves and other organic material.

Larvae in this experiment and under natural conditions as well, experience decreasing water level due to the gradual desiccation of the stream, which is common for most first-order streams in this region. We found that larvae were heavier in shallower water than in deeper. This suggests that larvae accelerated their metamorphosis in shallower water. Both field and laboratory studies provided evidence that larval amphibians subject to water volume reduction or short pond duration accelerate their metamorphosis and actively avoid desiccation (Denver et al., 1998; Newman, 1989, 1994).

Larval survival was not effected by the treatment. It is interesting to note this though the experimental conditions inhibited mortality due to predation and larval drift, which can also be an important mortality factor (Petranka and Sih, 1986) especially if larvae are drifted to fish-dominated downstream areas (Thiesmeier and Schuhmacher, 1990; Thiesmeier 1994). Thus the mortality measured on this experimental population should arise from other sources, which might include consumption by *Gammarus* species after injuries (observed) or cannibalism.

The more pronounced size differences among larvae under high density after six weeks indicate an unequal resource partitioning among larvae. The size differences are also a well known factor in promoting cannibalism (Walls, 1998). We did not observed cannibalism directly, but cannibalism is well-documented among fire salamanders (Degani et al., 1979; Warburg, 1992; Bressi et al., 1996). We observed a larva out of the enclosures, perhaps a cannibalistic form, in a deep pool of the stream, with a size of 80.23 mm. It was obviously an individual that stayed in stream over at least the previous winter, which emphasizes the high degree of plasticity in this species.

The facts that no strong effects of density and water level were observed in our experiment, as opposed to experiments carried out in ponds (see Wilbur, 1987), may support the idea that natural streams should be viewed as dynamic patch mosaics (Pringle et al., 1988; Townsend, 1989; Lake, 2000). In such systems the effect of density may be important locally, but is likely to be less intense in general. Thus, in natural streams it may be more difficult to find evidence of density-dependence of salamander larvae.

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The male reproductive cycle of the North American salamander *Ambystoma macrodactylum columbianum*

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For male vertebrates, proximate regulation at multiple neural and endocrine levels of the brain-pituitary-gonadal axis should be fine-tuned by both natural and sexual selection to maximize or optimize reproductive success (Crews and Moore, 1986). In many vertebrates, especially birds and mammals, males engage in behavioural-ecological breeding activities and produce mature sperm at the same times seasonally (Lombardi, 1998). However, this association may be absent in certain taxa of amphibians and reptiles; in these there exists a clear temporal dissociation between breeding and spermatogenesis. Crews (1987) suggests that proximate mechanisms responsible for such dissociation may be favoured by selection when environmental conditions necessary for breeding and for gametogenesis are not coincident in time.

Such a dissociated pattern of male reproduction has been described for a number of species of newts and salamanders (Amphibia: Caudata) of the temperate zone (reviewed by Lofts, 1984; Verrell et al., 1986; Houck and Woodley, 1995; Verrell, 2003). In these, spermatogenesis exhibits a temporal pattern in which mature gametes are produced between, not during, consecutive breeding seasons. This dissociated pattern of temperate species contrasts with the more or less continuous pattern of sperm production seen in certain tropical species residing in aseasonal habitats (e.g., Chan, 2003).

The Columbia long-toed salamander, *Ambystoma macrodactylum*, subspecies *columbianum*, is a common ambystomatid salamander of northwestern North America, including southeastern Washington state (Nussbaum et al., 1983). Adults reside on land in more or less inaccessible subterranean burrows when not breeding (Verrell and Davis, 2003). In late winter-early spring (January to March), both sexes migrate to aquatic sites such as ponds within which they court, mate and lay eggs (Beneski et al., 1986; Verrell and Pelton, 1996; Verrell et al., 2001). Individual males may remain in the water for a few weeks, females for no more than a few days (Verrell and Pelton, 1996). Southeastern Washington is highly seasonal climatically; this region's cold winters and hot, dry summers may be challenging energetically for small ectotherms such as salamanders in terms of the acquisition of energy necessary for gametogenesis (Verrell and Davis, 2003). Thus, I suspected that males of this species might exhibit a pattern of spermatogenesis that is dissociated from the time of breeding (the latter of brief duration).

In this paper I present data on the temporal pattern of spermatogenesis in *A. m. columbianum* obtained from males sampled in all but one month of the year. These data show a clear dissociation between spermatogenesis and breeding in this species.

All salamanders used in this study were obtained from a single pond and adjacent terrestrial habitat in the city of Pullman, Whitman County, Washington, in 1994. Breeding males ($n = 38$) were captured in water from January 19 to March 14 using wire minnow traps. Non-breeding males were captured on land, necessarily opportunistically, from March 29 to June 8 by excavating piles of soil and broken basaltic rock ($n = 5$).

Of the 38 males trapped in water, 18 (47%) were sacrificed within 24 h of capture (see below). The remaining 20 males were placed into one of two semi-natural enclosures to provide reliable access to terrestrial samples outside of the breeding season (see table 1). Each enclosure was a 200 L plastic garbage bin, the bottom of which was punctured to allow drainage. Each bin was filled to a depth of approximately 1 m with a mixture of rock rubble, potting soil and humus, and leaf litter, and was seeded with earthworms, pill bugs (terrestrial isopods), wax worms (moth larvae), house flies and *Drosophila*. Each bin was covered by a perforated lid that allowed penetration of rain, light and small invertebrates, but which excluded predators such as snakes, birds and small mammals. Both bins were placed in the shady portion of a managed garden amid shrubs and trees on the campus of Washington State University, and were searched from April to December (except for October) to obtain terrestrial males.

Salamanders were sacrificed within 24 h of collection from the three types of habitats by immersion in saturated chloretone solution. Each was blotted dry and weighed to the nearest 0.1 g. A ventral incision was then made, and the right testis and 4 mm of the right vas deferens proximal to the gonad were separately removed. The testis was blotted dry and weighed to the nearest 0.01 g, and then was placed directly into 70% ethanol solution together with its accompanying segment of vas deferens.

Testes were examined in order to determine the temporal pattern of spermatogenesis using standard histological techniques (Humason, 1979). Each testis was embedded in

Table 1. Numbers of males sampled from three 'habitat types' across months (except October) in 1994.

Month	Numbers per habitat type		
	Water	Land	Bins
January	7		
February	2		
March	9	1	
April		1	1
May		2	1
June		1	4
July			3
August			4
September			2
October			
November			3
December			2

paraffin wax and a minimum of three midsagittal sections was cut, each approximately 8 μ m in thickness. These were then stained with hematoxylin and eosin, and mounted permanently. Four types of tissues were identified microscopically (see Armstrong, 1989; Aranzabal, 2003): (1) cysts containing spermatozoa, (2) cysts containing spermatogonia, (3) cysts containing spermatocytes, and (4) tissue formed from ruptured cysts after evacuation of spermatozoa. The proportions of each of three sections comprised of each type of tissue were determined, and a mean score for each tissue type for each male was calculated. Vasa deferentia were macerated in distilled water and viewed microscopically to determine the presence of spermatozoa.

Single and discrete right testes were observed both macro- and microscopically in all of the males examined. This is in contrast to some plethodontid and salamandrid caudates, in which each testis may consist of several separate lobes joined in series along a cephalocaudal axis by cords of connective tissue (Lofts, 1984). A distinct fat body, yellow in colour, was associated with the testis of most males examined, including those taken from bins (indicating adequate availability of food). This fatty tissue carefully was trimmed away before testes were weighed and preserved.

Internally, the testes (or lobes thereof) of caudates consist of more or less spherical nests of cells, or cysts, within which spermatozoa develop from progenitor stem cells. Cysts may be separated into more or less distinct groups at comparable stages of germ cell development by connective tissue to form lobules (Lofts, 1984). Organization of cysts into lobules was apparent in the testes of most of the males in this study.

Mean proportions of four distinct types of tissues in testes were highly variable across months, and it is apparent that sperm maturation was dissociated temporally from breeding (fig. 1). Males compete for and mate with females in water from January to March when cysts containing spermatozoa were few in their testes. Most (and sometimes all) of the testes of breeding males were comprised of cysts containing spermatogonia and

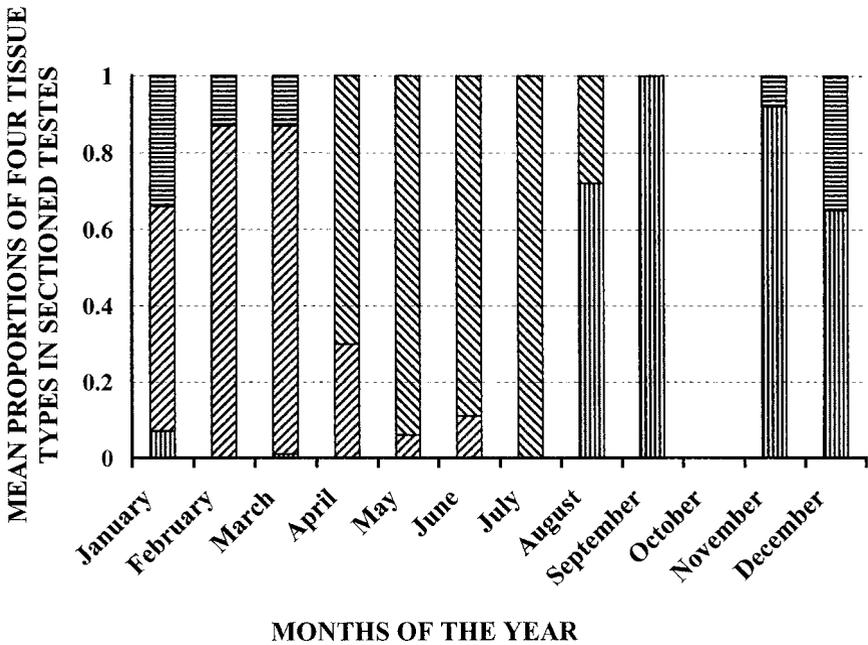


Figure 1. Monthly variations in the mean proportions of four types of tissues in sectioned testes of males sampled in water, on land and from bins in 1994 ($n = 43$) in every month except October. Vertical lines: cysts containing spermatozoa; right diagonal lines: cysts containing spermatogonia; left diagonal lines: cysts containing spermatocytes; horizontal lines: evacuated tissue formed from cysts that are empty of spermatozoa.

of evacuated tissue. This histological profile characterizes the breeding season of *A. m. columbianum*.

Males migrate to terrestrial habitats after breeding. The testes of males sampled on land and from bins between March and July showed a decline in the proportion of testicular tissue consisting of cysts containing spermatogonia as their testes became packed with spermatocytes. Meiosis apparently is bracketed by the months of April and August. This histological profile characterizes the post-breeding season of *A. m. columbianum*.

Spermiogenesis, during which time spermatids develop elongated flagella, was first observed in the testes of males sampled in July. Males sampled from August onward exhibited considerable numbers of cysts containing spermatozoa, and evacuated tissue was present in November and December. This histological profile characterizes the pre-breeding season of *A. m. columbianum*. Spermiation is the release of spermatozoa into the vas deferens, with the resultant formation of evacuated tissue in the testis (see above). Spermatozoa were present in the vasa deferentia of 7 (39%) of 18 males sampled in water during the breeding season (January to March). None of 14 males sampled terrestrially in the post-breeding season (March to July) had spermatozoa in their vasa deferentia (Zalisko and Larsen, 1989, observed that males discharge unused spermatozoa from their cloacae at the end of the breeding season). Spermiation apparently was initiated on land

Table 2. Relative testes masses (calculated as percentage of body mass) of males sampled during the breeding season (January to March in water), the post-breeding season (March to July on land and in bins) and in the pre-breeding season (August to December in bins).

Season	Median relative		
	<i>n</i>	mass	Range
Breeding	18	2.7	1.4-3.6
Post-breeding	14	3.4	2.7-4.2
Pre-breeding	11	4.5	2.9-6.9

late in the pre-breeding season, for two (18%) of 11 males sampled from bins from August to December exhibited sperm in their vasa deferentia (one in November and one in December).

To obtain a measure of relative testes mass, the mass of the right testis of each male was doubled and combined testes mass was calculated as a percentage of a male's total body mass. Relative testes mass was variable across months, ranging from a minimum of 1.4% in February to a maximum of 6.9% in November.

Data on relative testes mass were combined for males sampled in each of the three seasons identified histologically (table 2). Significant heterogeneity in median relative testes mass was found among these seasons (KW = 22.98, 2 df, $P < 0.001$, two-tailed Kruskal-Wallis one-way ANOVA). Three pairwise comparisons were then made among these three seasons using the method of Siegel and Castellan (1988). These revealed significant differences in relative testes mass between the breeding and post-breeding seasons (difference in mean ranks = 13.0, critical difference = 10.71, $P < 0.05$) and the breeding and pre-breeding seasons (difference in mean ranks = 22.5, critical difference = 11.49, $P < 0.05$). No significant difference was found between the post-breeding season and the pre-breeding season (difference in mean ranks = 9.5, critical difference = 12.06, $P > 0.05$). Thus, testes appear to be of lightest relative mass during the breeding season, when cysts containing spermatozoa are few (or absent) and the bulk (or all) of the testis is comprised of evacuated tissue and cysts containing spermatogonia.

Male *A. m. columbianum* in southeastern Washington clearly exhibit a temporal dissociation between behavioural-ecological breeding activity and the production of spermatozoa. Courtship and mating occur in water from January to March (Verrell and Pelton, 1996) using sperm that were produced in the previous year. Males breed when their testes are light in relative mass and when the only immature germ cells present are spermatogonia; spermiogenesis begins in July. Similar dissociated reproductive cycles occur in males of several other temperate caudates, including paedomorphic individuals of the congeneric tiger salamander, *A. tigrinum* (Norris et al., 1985).

Some additional details can be added to provide a finer resolution to the coarse-scale male reproductive cycle summarized in fig. 1. First, the timing of spermiogenesis (and spermiation: see below) suggests that males potentially could produce spermatophores in late autumn-early winter (the pre-breeding season). However, I have never captured adult

salamanders in water except in the early months of the year, and so autumnal breeding in nature likely does not occur in southeastern Washington.

Second, spermiation apparently occurs before the onset of behavioural-ecological breeding activity, for two of five males sampled terrestrially in November and December had sperm in their vasa deferentia. Thus, males may be ready to deposit spermatophores for females as soon as they enter the water from January to March. Pre-breeding spermiation might be sexually selected if intermale competition for mates is intense, as likely is the case for caudate taxa that have brief breeding seasons (Arnold, 1976; Verrell, 1989; Sullivan et al., 1995). As suggested by Verrell and Pelton (1996) and Verrell et al. (2001), scramble competition for females among male *A. m. columbianum* likely is frequent, especially early in the brief breeding season of this species (a time when males may outnumber females considerably in terms of operational sex ratio: see Dyson, 2000).

Third, testicular tissue formed from cysts that have evacuated their contents of spermatozoa first appears in November. This tissue then persists, albeit in varying amounts, until it is replaced completely with cysts containing spermatogonia by April (the start of the post-breeding season). Thus, the presence of tissue formed from evacuated cysts encompasses the whole time when males are competing for and mating with females. Histochemical evidence for a number of caudate taxa indicates that evacuated tissue is steroidogenic (Kikuyama et al., 2003). I predict that levels of plasma androgens (and, perhaps, other sex hormones) are elevated during the period when evacuated tissue is present in the testes of male long-toed salamanders. It is likely that the sexual behaviour patterns of males of this species are regulated at least partially by testicular androgens.

Fourth, clear spatial clustering of similar cyst types into more or less distinct lobules was common but not invariant among individuals for which multiple types of tissue were apparent simultaneously in their testes. Also, in many, but not all, caudates, the degree of maturity of germ cells increases as a 'spermatogenic wave' moves along the testis in a cephalocaudal direction (Lofts, 1984). Such zonation was apparent in most individuals containing multiple types of cysts that I sampled, but was not invariant.

Finally, while an annual cycle of testicular activity appears obvious at a coarse temporal scale, fig. 1 masks variations in testicular histology among males sampled over narrower windows of time. For example, for five males sampled in water from March 10 to 14 (toward the end of the breeding season), only one possessed testicular cysts containing mature sperm. The proportions of the testes of these males that contained evacuated tissue ranged from 0.1 to 0.3 and of spermatogonia from 0.6 to 0.9. Similarly, for three males sampled on August 3 (early in the pre-breeding season), proportions of cysts in their testes that contained mature sperm ranged from 0.5 to 1.0. Such fine-scale variation likely results from idiosyncrasies in how individuals respond to environmental factors that influence the 'microtiming' of breeding and spermatogenesis.

The proximate causes and ultimate consequences of the dissociated reproductive cycle of male *A. m. columbianum* are obvious targets for future research. In the first context, amphibians now are recognized as model systems for investigating mechanisms that

control the development of vertebrate spermatozoa (Pierantoni et al., 2002). In the second context, I note that the dissociated pattern of reproduction present in the newt *Triturus vulgaris* (similar in timing to that described here: see Verrell et al., 1986) appears to explain certain aspects of male sexual behaviour in this species. Thus, post-mating refractory periods (Verrell, 1986a) and mate choice for larger, more fecund females (Verrell, 1986b) in smooth newts may arise due to constraints placed on males by limited availability of sperm due to temporal dissociation between breeding and spermatogenesis.

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