

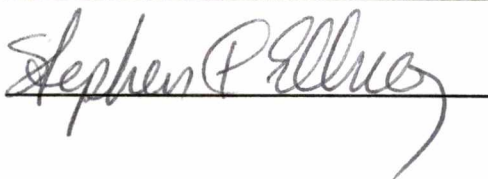
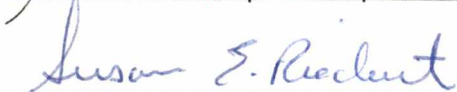
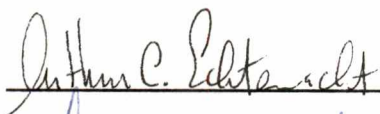
To the Graduate Council:

I am submitting herewith a dissertation written by Carole Louise Hom entitled "The Reproductive Ecology of Female Desmognathus fuscus: Field, Laboratory, and Modelling Studies." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology.



Thomas G. Hallam, Major Professor

We have read this dissertation
and recommend its acceptance:



Accepted for the Council:



Vice Provost
and Dean of the Graduate School

THE REPRODUCTIVE ECOLOGY OF FEMALE DESMOGNATHUS FUSCUS:
FIELD, LABORATORY, AND MODELLING STUDIES

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Carole Louise Hom

August 1986

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ABSTRACT

Fecundity in Desmognathus fuscus is positively correlated with body size and reproduction is annual. Females in a Southern Appalachian population oviposited beneath moss during mid summer; hatching occurred 40 to 60 days thereafter. Based on laboratory studies, females probably defended their eggs against other salamanders but not against snakes. Reproductive success over the entire population was low in all three years of the study and varied between years. Successful nests usually hatched over 70 percent of the original clutch. The major source of egg mortality probably was predation.

Females in the natural population usually did not increase body length from year to year. Females given high levels of food in the laboratory grew, accumulated visible fat deposits, and contained what seemed to be fully developed ova earlier in the spring than females in the natural population. Carcass lipids probably were mobilized for energy during summer brooding and overwintering.

Analysis of a control theory model of Desmognathus reproductive allocation predicted that female salamanders should grow if enough food is available and always should provide maternal care during egg brooding. Numerical analyses suggest that quantitative results are sensitive to conversion rate and parameters that affect clutch mortality. Large model salamanders had high fecundity and reproductive success; small females had low reproductive success and limited growth because of the energetic demands of reproduction.

...To see a salamander, one has to be a philosopher...never forget that you have seen a salamander. It's a sign that you are going to be a scientist, perhaps even a magician.

- Anatole France
"La Rotisserie de la Reine Pedauque"

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CHAPTER 1

INTRODUCTION

Understanding the life history attributes of organisms -- that is, processes that involve reproduction and mortality -- is central to understanding ecology at the population level. Life history theory seeks to explain patterns in phenomena that include total reproductive effort, age at first reproduction, frequency of reproduction, and the number and quality of offspring (Stearns 1976, 1977); the ultimate goal of such efforts is to gain insight into how natural selection has shaped organisms (Stearns 1984b). Mathematical modelling traditionally has had a substantive role in the development of this theory. By making simplifying assumptions about population structure, demography, and environmental parameters, theoreticians have predicted suites of life history traits that maximize individual fitness (e.g., Cole 1954, Charnov and Schaffer 1973, Schaffer 1974, Hastings 1978). At the same time, empiricists have collected extensive data on the life history attributes and reproductive ecology of individual species. However, rigorous empirical tests of the theoretical predictions and assumptions on which they are based are less common (Stearns 1976, 1980; however, see also Stearns and Crandall 1981, Chiarello and Roughgarden 1984, Beuchat and Ellner 1986).

One of the standard assumptions in life history theory is that the particular combinations of life history traits observed in nature are coadapted and arose through differential reproductive

success of phenotypes. Modelling techniques that use mathematical optimization in some form are natural approaches to investigating such phenomena. These methods (e.g., Stearns 1977, Oster and Wilson 1978) and the concept of optimality in organisms as a whole (e.g., Gould and Lewontin 1979, Gould 1980) have been criticized. The critics point out that, first, morphogenetic or physiological limitations may impose constraints upon the optimal design of organisms (Gould and Lewontin 1979, Gould 1980). Secondly, natural selection operates on entire organisms rather than on isolated traits. Consequently, life history attributes may not be free to vary independently of one another (Stearns 1980, 1984a). These comments are valid, but, for several reasons, the optimization approach remains a useful methodology for studying life history traits. In response to the first criticism, Maynard Smith (1982) has pointed out that an essential step in mathematical optimization is specification of the set of phenotypes among which optima may be found. This is equivalent to specifying constraints dictated by the biology of the organism. Thus, application of optimality theory is justified so long as it is not naive. In response to the second criticism, several investigators recently have emphasized that phenotypic optima (representing a set of life history attributes) might be either canalized or plastic (e.g., Stearns 1980, 1982a, Wake et al. 1983). Canalization of traits would allow more than one genotype to produce the optimum phenotype; plasticity would enable the organism to respond to variations in environmental conditions. Indeed, one of the strengths of

mathematical optimization is that it predicts phenotypes (i.e., sets of life history attributes) that may maximize the fitness of an organism under certain specified conditions; these phenotypes and conditions can be checked against empirical observations, and the model or general theory modified accordingly.

Another implicit assumption in many investigations of life history is that the amount of energy available to an organism for physiological processes is finite and results in competing needs in allocation of this energy (e.g., Cody 1966, Williams 1966). Thus, both empirical and theoretical studies of life history traits often examine compromises in allocation: reproduction versus somatic growth, or reproduction versus survivorship. Intuitively, postponing reproduction in favor of growth would be advantageous if pre-reproductive mortality were low and if increased body size conferred benefits in terms of higher adult survivorship, fecundity or reproductive success.

Salamanders in the genus Desmognathus (Plethodontidae) are well suited to the study of life histories. They are large and fairly conspicuous, it is possible to quantify many of their reproductive characteristics, and several aspects of their behavior related to reproduction are amenable to study in the laboratory. Moreover, because of potential relationships between female body size, growth, maternal care, female survivorship, fecundity, and reproductive success, they exemplify some of the compromises implicit in the evolution of life history traits.

In this dissertation, I examine the life history characteristics of a plethodontid salamander, Desmognathus fuscus, with specific regard to schemes for resource allocation that maximize the fecundity and reproductive success of an individual female. The dissertation is organized as follows. In Chapter 2, I describe the natural history of Desmognathus fuscus and discuss the reproductive ecology of female desmognathines. The reproductive ecology of a population of D. fuscus in the Southern Appalachians is explored in greater detail in Chapter 3 with emphasis on phenomena that may influence reproductive effort and reproductive success of females in natural populations. In Chapter 4, I discuss cover object use by gravid and non-gravid Desmognathus fuscus in natural populations, and compare these results to observations of microhabitat choice in the laboratory. Chapter 5 presents preliminary observations of clutch defense in female D. fuscus. Lipid cycling and the relationships between growth and food availability in female D. fuscus are described in Chapter 6. A control theoretic model of reproduction in Desmognathus is developed and analyzed in Chapter 7 to determine schemes of reproductive allocation that maximize the reproductive output of an individual female. Chapter 8 presents the quantitative predictions of this model using specific values of the model parameters. In Chapter 9, I integrate the qualitative and quantitative modelling predictions with empirical results and present implications for the reproductive ecology of desmognathine salamanders and life history theory in general.

CHAPTER 2

NATURAL HISTORY

The plethodontid genus Desmognathus is one of the most ecologically diverse groups of salamanders in the world (Wake 1966). These lungless urodeles have radiated from what probably was an aquatic ancestor (Dunn 1926, Wake 1966) and the modern genus includes representatives in stream, streambank, and terrestrial environments. Parallel trends toward abandonment of aquatic larval stages, attainment of direct development, and increasing terrestriality in adults have been well documented in Desmognathus (e.g., Hairston 1949). In terms of habitat use, body size, and reproductive characteristics, the dusky salamander, Desmognathus fuscus, is at the middle of this aquatic-terrestrial continuum.

Desmognathus fuscus is a highly polytypic and wide-ranging species. It is distributed over much of southern Canada and the eastern United States from Quebec and Nova Scotia to the Fall Line (Figure 1). Disjunct populations also occur in Arkansas, Mississippi, and Florida. The preferred habitat of D. fuscus appears to be seepages and the bed and banks of wooded headwater streams (Hairston 1949, Organ 1961, Means 1974, Krzysik 1979, Keen 1982, Southerland 1986a, 1986b), but it also may be found in small muddy streams and river floodplains in the Southeast (Means 1974).

The general patterns of reproduction in D. fuscus are similar across the range of the species, although geographic variation in

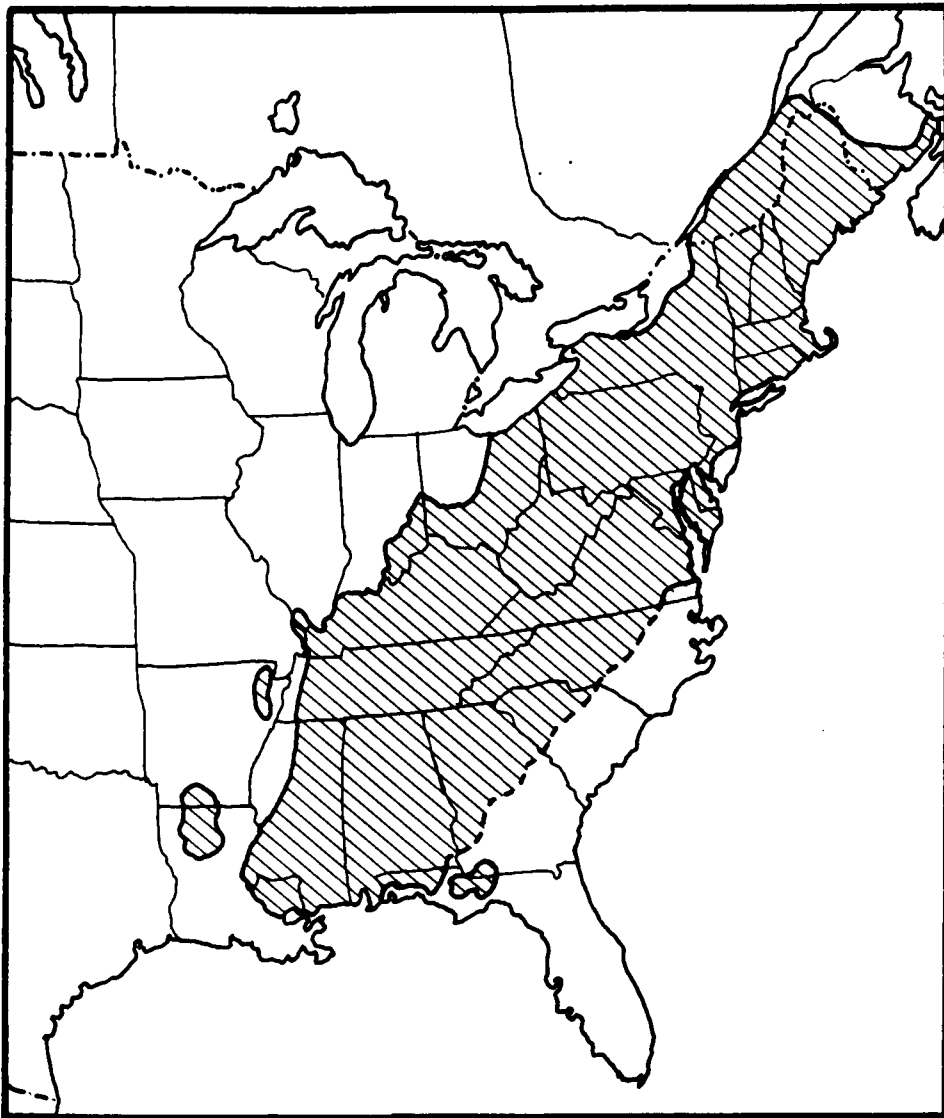


FIGURE 1. Geographic distribution of *Demognathus fuscus*, adapted from Means (1974), Conant (1975), and Denman (1982). Dashed line indicates an uncertain range limit.

phenology and microhabitat use may exist. Female D. fuscus become sexually mature at four to five years of age and appear to reproduce annually thereafter (Tilley 1968). Courtship and oogenesis take place in the late fall prior to the onset of cold weather and again throughout the spring (Organ 1961). Oviposition in southern Appalachian populations usually occurs in the late spring through summer (Organ 1961, Folkerts 1968, E. Juterbock personal communication); synchrony of oviposition has not been reported. At oviposition, each female deposits a single mass of eggs in an individual nest site along the banks of small, slow-moving streams (Danstedt 1975, Organ 1961). Nests usually consist of smooth, shallow depressions in moist soil beneath a cover object. This cover may be a rotting log, a rock, a mat of moss, or leaf litter in populations from the Southern Appalachians (R. L. Jones personal communication, Organ 1961); Desmognathus fuscus in Ohio and Pennsylvania usually nest under rocks (Davic 1977, Dennis 1962, Krzysik 1980a). It appears that a female broods her clutch from oviposition until hatching, which usually spans 45 to 60 days in the southern portion of the species' range (Danstedt 1975, Organ 1961, Wilder 1917). Brooding in Ohio populations may require up to 80 days (Dennis 1962). Female D. fuscus do not leave the nest until after their eggs hatch (Krzysik 1980b, Organ 1961); subsequently, they may remain active throughout the autumn and early winter until surface water freezes.

Fecundity in female Desmognathus is positively correlated with body size (Tilley 1968), but variance in body size leaves considerable

variance in clutch size unexplained (Tilley 1968, 1973, 1980). Tilley (1980) demonstrated that clutch size in D. ochrophaeus did not increase with age. Other variables that may influence fecundity include age at first reproduction, nutritional state of the female while yolking eggs, and genetic differences in fertility.

Size may affect other aspects of the reproductive ecology of Desmognathus. In behavioral interactions, small individuals appear to be attacked and injured more frequently than larger conspecifics (Keen and Sharp 1984). Injury may reduce the reproductive potential of a female salamander (Maiorana 1976) or impair her chemosensory ability (Jaeger et al. 1982). Small animals or juveniles may shift to microhabitats that are not used by larger Desmognathus (Southerland 1986a, 1986b). These microhabitats usually are more terrestrial than the sites used for nesting.

Several lines of evidence suggest that reproduction is energetically expensive in Desmognathus. Organ (1961) and Krzysik (1980b) suggested that females do not leave the nest to forage during egg brooding. Opportunistic feeding on insects that intrude into the nest does occur (Montague and Poinski 1978). This implies that females may remain concealed to avoid detection of their clutch by potential predators (Krzysik 1980b). The cost of reproduction in Desmognathus therefore includes energy required for physiological maintenance during brooding in addition to the cost of egg production. Fitzpatrick (1973) estimated that these expenditures represent approximately 48 percent of the annual energy budget of D.

ochrophaeus. This high investment has been hypothesized to limit growth of sexually mature females (Organ 1961, Fitzpatrick 1973, Tilley 1973, 1980) and thereby to preclude the possible advantages of larger body size.

Although it has not been studied in D. fuscus, extensive maternal care during egg brooding has been reported in other plethodontid species. Females consume those eggs infected with pathogenic fungi and defend the clutch against potential egg predators (Snyder 1971, Forester 1978, 1979, 1983, Bachmann 1984). Females that defend their eggs, however, do so at potential risk to themselves since most of the oophagous predators in streambank habitats also consume salamanders. These include crayfish (R. L. Jones personal communication; J. Holomuzki personal communication), larger plethodontids (e.g., D. quadramaculatus, Gyrinophilus porphyriticus), conspecifics, and small snakes (e.g., Diadophis punctatus).

Thus, compromises appear to exist throughout the reproductive biology of Desmognathus. These trade-offs include questions of reproductive allocation, the timing of reproduction versus growth, and investment in maternal care.

Because of the positive correlation between body size and fecundity, it apparently is advantageous for a reproducing female salamander to be as large as possible. Postponing reproduction in favor of growth is one means by which this may be accomplished. Longer postponement of reproduction, however, may decrease the probability that a female will survive to reproduce. As a result,

there should be some size at which the advantages of growth are outweighed by the risk of mortality.

The timing of reproduction and choice of nest site may influence the probability that eggs will survive to hatch. Sites very close to a stream may be susceptible to predation by larger aquatic salamanders (Tilley 1972) or inundated by floods following severe thunderstorms; clutches in sites far from a stream or that are laid very late in the summer may dry out before hatching. Further, if the ability to obtain a nest site in a suitable location is related to body size, then small females, as well as having smaller clutch sizes, would have another penalty in fitness imposed upon them. Alternatively, their size would not be disadvantageous if small females were able to nest in narrow crevices not accessible to larger females.

In addition, a female salamander may face a paradoxical situation while brooding her eggs. If she abandons her clutch, then all the eggs surely will succumb to fungal infestation or be eaten by a predator and she will lose her reproductive investment for that year. If, on the other hand, she remains with and cares for her clutch, then she may be killed by that same predator and lose both her future and her current investment. Current theories of maternal investment are contradictory. Trivers (1972) argues that the amount of care provided by a parent to offspring should be positively related to the amount the parent already has invested in those offspring, thus abandonment should not occur. Kaplan and Crump (1978), however, suggest that

selection should favor defense until the point at which the gain in fitness resulting from maternal care is exceeded by the cost in fitness of those behaviors, after which abandonment should be favored.

CHAPTER 3

REPRODUCTIVE ECOLOGY

Introduction

It has long been suggested that the distribution and abundance of plethodontid salamanders may be affected by predation (Organ 1961, Tilley 1968, Hairston 1980). Recently, Hairston (1986) demonstrated that predation is an important interaction in Desmognathus assemblages. Removal of D. ochrophaeus, a species with small body size, from experimental plots resulted in decreases in abundance of D. monticola and D. quadramaculatus, two larger congeners. Removal of D. monticola caused increases in the abundance of D. ochrophaeus and D. quadramaculatus. Hairston concluded that the two larger species are competitors and that the distribution and abundance of the smaller D. ochrophaeus is affected by predation pressure from both congeners. Similarly, the presence of large congeners can result in shifts in microhabitat choice (Southerland 1986a, 1986b, 1986c), or lower juvenile survivorship (Southerland 1986c) in smaller Desmognathus. These effects have been attributed to the larger species acting as predators on the smaller species, rather than as competitors.

Desmognathus fuscus is a medium-sized plethodontid that is distributed over much of eastern North America. It occurs primarily in the interface between lotic and terrestrial habitats and appears to prefer the banks and beds of wooded headwater streams (Hairston 1949,

Organ 1961, Means 1974, Krzysik 1979, Keen 1982) over most of its range. Desmognathus fuscus, however, may be absent from portions of the interior of its range within the Ridge and Valley Province of Tennessee and Virginia (Hairston 1949, Martof and Rose 1963, Huheey 1966, Tilley 1968). Tilley (1968) has hypothesized that this distributional pattern is a result of intrageneric predation pressure. Other Desmognathus species may have at least a partial refuge from predation by virtue of adult size (D. quadramaculatus, D. monticola) or terrestriality (D. aeneus, D. wrighti). Desmognathus fuscus, however, may be vulnerable because of its intermediate size and habitat affinities. In particular, brooding females and egg clutches could be subject to particularly high rates of predation because of their immobility and location near stream margins. Predation on nests can cause mortality of a fraction or all of the eggs in a clutch (e.g., Forester 1979), thus decreasing recruitment to the juvenile class, and may also result in injury or death of the brooding female.

This study investigated the reproductive ecology of Desmognathus fuscus in the Southern Appalachians at the edge of the Ridge and Valley Province. My primary goal was to examine the variables that may affect reproductive effort and reproductive success of females in natural populations. Reproductive success in D. fuscus may be influenced by the size, weight, general physical condition, and fecundity of a female, weather, biological interactions both within and between species, and characteristics of the nest site. Viewed from a different perspective, these can be divided into two

categories: variables that the female may be able to influence, and variables that she cannot control. I call the former category decision variables and the latter, constraints. Decision variables include nest site parameters (e.g., depth of the cover object, distance from the nest to the stream) and date of oviposition. They also may include female weight, which depends on foraging behavior, and clutch size, which might be altered if the female yolked fewer eggs than her maximum capacity. In contrast, the female cannot change the year or her snout-vent length at the time that she oviposits. Thus, a biologically informative approach to considering the reproductive ecology of dusky salamanders is to examine how female reproductive success is affected by 1) all variables, and 2) decision variables conditioned on year and female size.

Materials and Methods

Field and Laboratory Methods

Observations of a natural population of Desmognathus fuscus were made in six small first-order streams in the Reed Creek watershed, Chilhowee Mountain, Tennessee (U.S.G.S. Kinzel Springs and Wear Cove Quadrangles)(Figure 2). The streams are fairly low gradient, range from 50 to 300 m in length, and are fed by permanent springs. Four of the streams (3, 4, 5, 6 in Fig. 2) flow through a closed-canopy cove hardwood forest with heavy growths of Rhododendron shading the streams. The other two streams (8, 9) are in forests that are

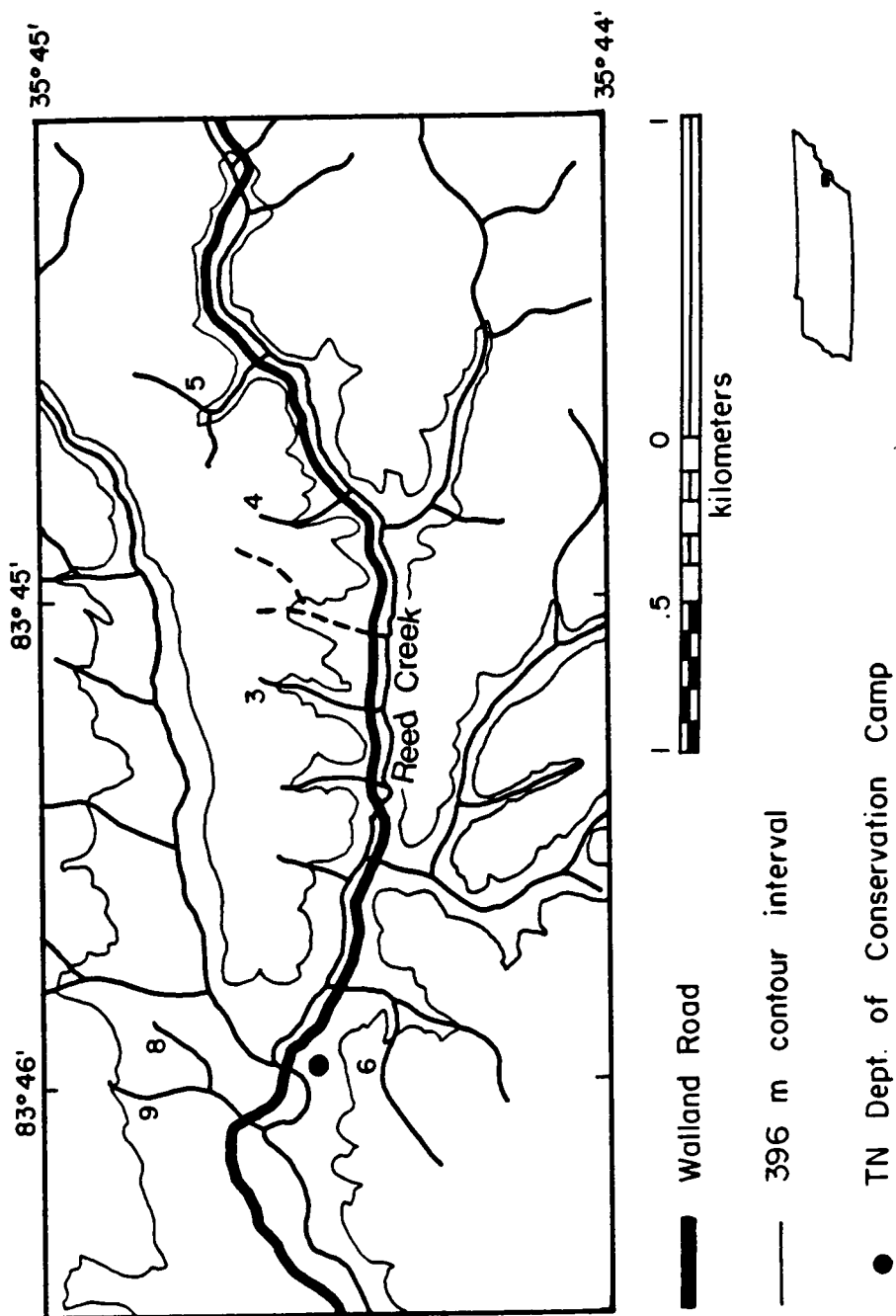


FIGURE 2. Map of the study area. The numbered streams were sampled for Desmognathus fuscus females and nests.

slightly more xeric and open. Desmognathus fuscus is the most abundant plethodontid in the streams, although D. quadramaculatus also is fairly common. Several other plethodontid salamanders occur with lower abundances. Adult Eurycea bislineata enter the streams only during their reproductive season in the early spring.

Desmognathus monticola, Pseudotriton montanus, and Gyrinophilus porphyriticus are present but rare. Desmognathus fuscus from Reed Creek were identified by Stephen G. Tilley; voucher specimens were deposited in The University of Tennessee, Knoxville, Vertebrate Zoology Collection (UTKVZC).

All streams were divided into segments 5 m long to provide reference points for recording the position of captured salamanders. Streams 4, 5, 8, and 9 were visited once every twelve to fourteen days from May through September 1983; in 1983, stream 3 was sampled at the same intervals, but only during August and September. In 1984, all streams were visited every ten to twelve days from May through September. In 1985, I sampled only streams 3, 4, and 5, at approximately ten day intervals from May through June. From July through September 1985, sampling was increased to once every four days to better monitor female reproductive success. At each visit, I searched the streams and streambanks for female D. fuscus by turning rocks, small logs, and mats of moss and leaf litter. Cover objects were replaced in their original position as much as possible to minimize habitat deterioration. Data on female wet weight, snout-vent length (SVL), body width at axilla, reproductive condition, and size

and location of the cover object were recorded. Female length and width were measured to the nearest millimeter with a metal ruler and wet weight to the nearest 0.1 g was obtained with a Pesola scale. A female was classified as non-gravid if her follicles viewed through the body wall appeared small and white-colored. Females with enlarged, yellowish follicles less than 1 mm in diameter were considered to be in early stages of oogenesis. Females with larger follicles were considered gravid. All determinations were made on unanaesthetized animals. In 1983, captured females were toe-clipped and released; in 1984 and 1985 females were photographed in the field for future identification (modified from Tilley 1977 and Forester 1977) and released at the point of capture.

For females attending a clutch of eggs, I also recorded clutch size and egg diameter. Embryonic development was estimated without visual magnification using a qualitative scale (Table 1). In 1984 and 1985 I also measured the distance from the nest to the stream, and to the nearest nests upstream and downstream. Nests were checked at each visit until all the eggs in the clutch had either hatched or disappeared.

If a clutch was in the undifferentiated egg stage or if oviposition actually was observed, then I used the date of discovery of the clutch as the date of oviposition. If a clutch was developmentally advanced, then date of oviposition was estimated. I used data from 1985, which were more precise than data from 1983 or 1984 because of the greater frequency of sampling, to obtain the

TABLE 1. Developmental stages of Desmognathus fuscus embryos found in the Reed Creek study area.

Stage	Description
ovipositing	female ovipositing eggs
egg undifferentiated	no noticeable embryological development
early differentiation	some features discernible but without dark, well-developed eye vesicles
eye vesicle	dark pigment in eye vesicles, with little or no dorsal pigment
pigmented embryo	embryo darkly pigmented with well-developed limbs
yolk plug	yolk sac reduced to a small region in abdomen
hatching	embryo beginning to emerge from egg membranes

median duration of each developmental stage. The age of a clutch in a given stage was estimated by summing the durations of the earlier stages. Subtracting this age from the date of discovery yielded an approximate date of oviposition.

I calculated two measures of reproductive success: nest success and hatching fraction. A nest was considered successful if at least one egg was observed hatching, or if a clutch that was missing at an observation contained eggs in the heavily pigmented or yolk plug stages at the previous observation. The latter assumes that the eggs were not taken by a predator and actually hatched in the interval between the two observations; eggs in the pigmented embryo or yolk plug stages probably were sufficiently well developed to have done so. Clutches in earlier developmental stages at the last observation were considered unsuccessful, with nest failure probably due to either predation or flooding caused by storms. If a nest was successful, then hatching fraction was calculated as the ratio of the number of eggs hatching (or at last observation) to the number of eggs initially. The date of first hatching observed or final observation was considered the hatching date for a successful clutch. This method would tend to overestimate hatching success and underestimate development time.

In 1985 I used the following scheme to differentiate between clutch failure due to predation and clutch failure due to storms. If a clutch was observed at any given time but was missing at the subsequent sampling, and if a storm had not occurred in the interim,

then I assumed that the mortality was due to predation. If a storm did occur in the intervening time, then two other criteria were used to attempt to determine whether or not the nest had been flooded. First, if the distance of the clutch from water, appearance of the vegetation, and degree of channelization of the stream bed had changed, then it was likely that the site had been flooded. Secondly, I placed a cork bead at the nest site adjacent to the clutch at the initial visit. This bead was tethered by ca. 5 cm of nylon monofilament line to a stake driven into the substrate. If a clutch disappeared and its cork was found downstream, then it was likely that mortality was due to inundation. If, however, a clutch was missing but the cork remained at the site, then mortality was attributed to predation.

Fecundity of Desmognathus fuscus was estimated in two ways. I used the initial size of clutches found in the study area as an index of the number of eggs a female actually might lay. This measure, however, would underestimate fecundity if some of the clutch were lost between oviposition and the time that I discovered the nest. Therefore, I also counted the ovarian follicles of female D. fuscus from the Blue Ridge and Ridge and Valley Physiographic Provinces within Tennessee in the UTKVZC as a second estimate of fecundity. Snout-vent lengths of museum specimens were measured to the nearest 0.1 mm with vernier calipers.

Statistical Methods

The relationship between fecundity and female size was examined by linear regression of initial clutch size and follicle number against female snout-vent length. Other regressors have been suggested, e.g., clutch volume vs. body volume (Tilley 1968), but the linear relationship is biologically reasonable since the reproductive tract of a female D. fuscus lies parallel to the long axis of her body so that fully developed eggs are arranged more or less linearly within the body cavity. For purposes of comparison with Tilley (1968), I also calculated regression equations for logarithmically transformed follicle number versus snout-vent length of museum specimens. Yearly differences in initial clutch size in the Reed Creek population was examined using analysis of variance.

Data on reproductive success, phenology, female size, and nest site characteristics were analyzed in three stages. Initially, I used discriminant analysis to determine whether or not some combination of phenological variables, female SVL and weight, or nest site characteristics could predict nest success (see Table 2 for a description of these variables). This analysis, performed on all variables, was used to indicate general patterns in the data. Secondly, I tested for effects of constraints (year and SVL) by examining the relationship between measures of reproductive success (clutch success and hatching fraction) and female snout-vent length nested within each year using analysis of variance. Finally, I used linear regression to remove any effects of female snout-vent length on

TABLE 2. Variables used in the discriminant function analysis of reproductive success in clutches of Desmognathus fuscus. The variables MINWAT, DISTUP, and DISTDN were not measured in 1983.

Variable name	Description
YEAR	year of study
OVIPDAY	month and day of oviposition
SVL1	female snout-vent length at initial observation
WT1	female weight at initial observation
D	logarithmically transformed depth of object covering the nest
MINWAT	logarithmically transformed minimum distance from nest to stream
DISTUP	logarithmically transformed distance from nest to nearest nest upstream
DISTDN	logarithmically transformed distance from nest to nearest nest downstream
NUMEGG1	clutch size at initial observation

hatching success, then used the residuals in an analysis of variance with female weight, fecundity, date of oviposition, and nest site characteristics as independent variables. This analysis was done on data pooled from 1983, 1984, and 1985.

Other statistical tests are described in the results. All analyses were done using SAS85 (SAS Institute Inc. 1985), with $\alpha = 0.05$.

Results

Mark-Recapture Studies

Over the three years of the study, I captured 686 non-brooding female Desmognathus fuscus, and observed 157 egg clutches. These data are summarized in Table 3. The recapture fraction of non-brooding females within years was low: 5.85 percent in 1983 (11 of 188 marked animals), 2.63 percent in 1984 (6 of 229 marked animals) and 5.00 percent in 1985 (10 of 200 marked animals). Because of the change in marking technique, comparisons of females between years is possible only for 1984 and 1985. Not surprisingly, recaptures between years were low also; 7.19 percent of the animals photographed in 1984 were recaptured in 1985. These recapture successes are in the range that has been obtained in other studies of female Desmognathus in comparable habitat (e.g., D. ochrophaeus: 5.88 percent in an open population (Forester 1977)) but are too low to permit reliable estimates of population size (Tanner 1978). Studies conducted on rock faces yield substantially higher recapture success (e.g., Tilley 1980),

TABLE 3. Numbers of non-brooding female Desmognathus fuscus captured and D. fuscus nests discovered in the Reed Creek population over the three years of the study. The stream numbering refers to Figure 2. Entries for each stream are the total number of non-brooding females, with the number successfully marked shown in parentheses.

Year	Stream	Non-brooding females	Nests
1983	3	2 (2)	5
	4	29 (28)	16
	5	76 (71)	29
	6	15 (13)	2
	8	29 (28)	9
	9	54 (46)	7
	total	205 (188)	68
1984	3	40 (32)	13
	4	62 (50)	17
	5	93 (81)	18
	6	17 (15)	0
	8	24 (14)	3
	9	45 (37)	1
	total	281 (229)	52
1985	3	53 (53)	18
	4	43 (43)	7
	5	104 (104)	12
	total	200 (200)	37

probably because populations in such habitats are more closed than those along stream margins (Tilley 1980).

Most of the females recaptured within years and between 1984 and 1985 were recaptured within 15 m of the stream segment in which they were found initially. Similar results have been observed for D. fuscus within a single year (Barbour et al. 1969, Barthalamus and Bellis 1972) and for female D. ochrophaeus between years (Forester 1977). These observations suggest that Desmognathus females exhibit fidelity for a relatively short section of stream at least during the spring and summer.

Fecundity Relationships

Analysis of variance of initial clutch size using year, SVL and the year x SVL interaction indicated statistically significant effects of all three variables (Table 4). This suggests that the number of eggs at first observation of a clutch varied among years, among females of different sizes, and for females of the same size in different years. The adjusted mean of initial clutch size (e.g., corrected for the effects of female SVL) was significantly higher in 1983 than in 1984 and 1985 (Student's *t*; 1983 vs. 1984: $P < 0.0001$; 1983 vs. 1985: $P < 0.0005$); the adjusted mean of initial clutch size did not differ between 1984 and 1985 (Student's *t*; $0.50 < P < 0.75$). Because of the significance of year and interaction effects on initial clutch size, I did univariate regression analyses of initial clutch size versus female SVL separately for each year.

TABLE 4. Analysis of variance for initial clutch size in Desmognathus fuscus from Reed Creek.

Source of variance	Df	SS	MS	F
Model	5	706.70	141.34	15.89***
Error	119	1058.31	8.89	
(Type III SS)				
Year	2	78.06	39.03	2.08*
SVL	1	484.59	484.59	54.49***
Year x SVL	2	94.52	47.26	5.31**

* $P < 0.05$

** $P < 0.01$

*** $P < 0.0001$

The number of eggs in a clutch at first observation was positively correlated with female SVL (Figure 3). Linear regression of initial clutch size against SVL yielded statistically significant relationships in all years (1983: $P < 0.0001$, $r^2 = 0.46$; 1984: $P < 0.005$, $r^2 = 0.22$; 1985: $P < 0.005$, $r^2 = 0.33$). Clutch size tended to be larger in 1983 than in 1984 and 1985.

Follicle number and SVL also were significantly correlated in preserved museum specimens (linear regression analysis: $P < 0.0001$, $r^2 = 0.4004$, $n = 60$, $y = -6.97 + 6.71 \text{ SVL}$; regression with logarithmic transformation: $P < 0.0001$, $r^2 = 0.4053$, $n = 60$, $\log(y) = 1.2150 + 1.2717 \log(\text{SVL})$). Follicle number yielded a higher estimate of fecundity than initial clutch size. For example, the linear regression equation for follicle number predicts 20 offspring for a female 40 mm long; the initial clutch size equation for 1983 nests predicts 16 offspring. It is likely that fecundity estimated from egg clutches was depressed by early egg mortality.

Reproductive Ecology

Every female captured in the spring was in some stage of yolking follicular eggs. By May, these eggs generally were well-developed and easily visible through the body wall. In all three years, I began to find ovipositing females and recently deposited egg clutches in early July, with estimated date of egg deposition ranging from late June to early July. Oviposition continued throughout July and August (Figure 4). The date of oviposition over the entire study population differed

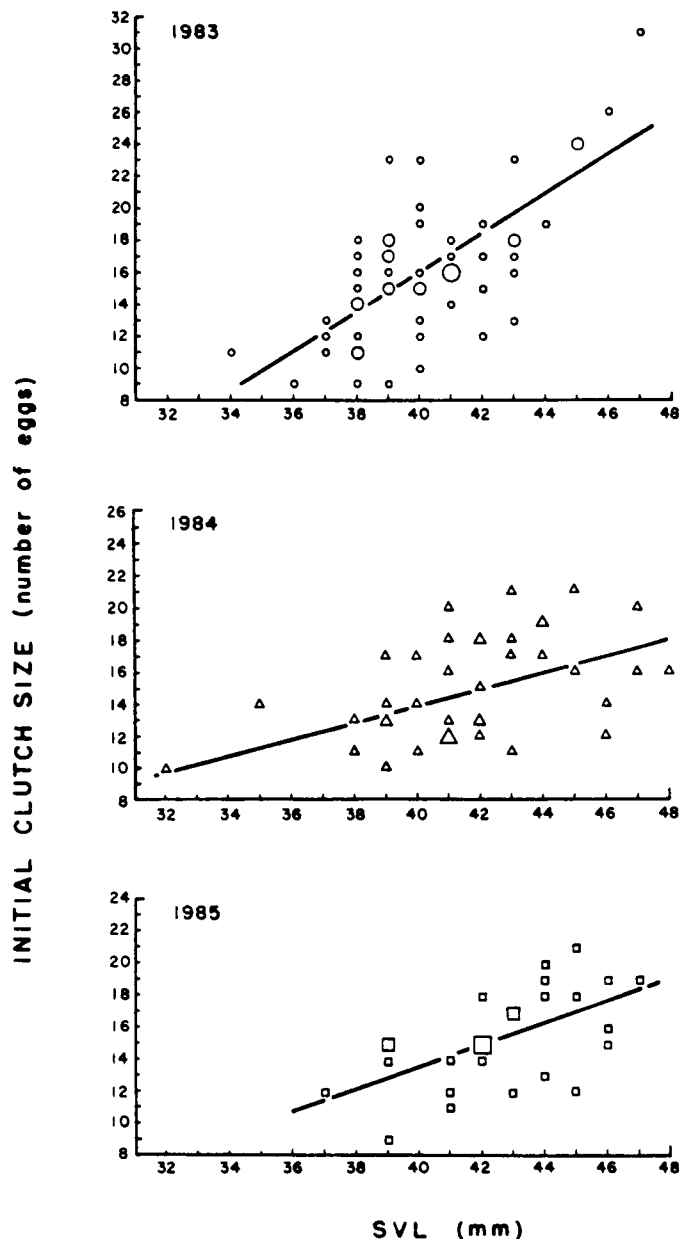


FIGURE 3. Relationship of initial clutch size to female SVL in Desmognathus fuscus. Small symbols represent one observation; medium sized symbols represent two observations; large symbols represent three to five observations. Data for females with unusually low clutch sizes were omitted from the regression analysis (listed as Cartesian coordinates: 1983: (43,5), (44,6); 1985: (41,8),(45,9)) but were included in other statistical tests. Regression equations were 1983: $y = -30.67 + 1.17 \text{ SVL}$; 1984: $y = -4.71 + 0.47 \text{ SVL}$; 1985: $y = -13.50 + 0.68 \text{ SVL}$.

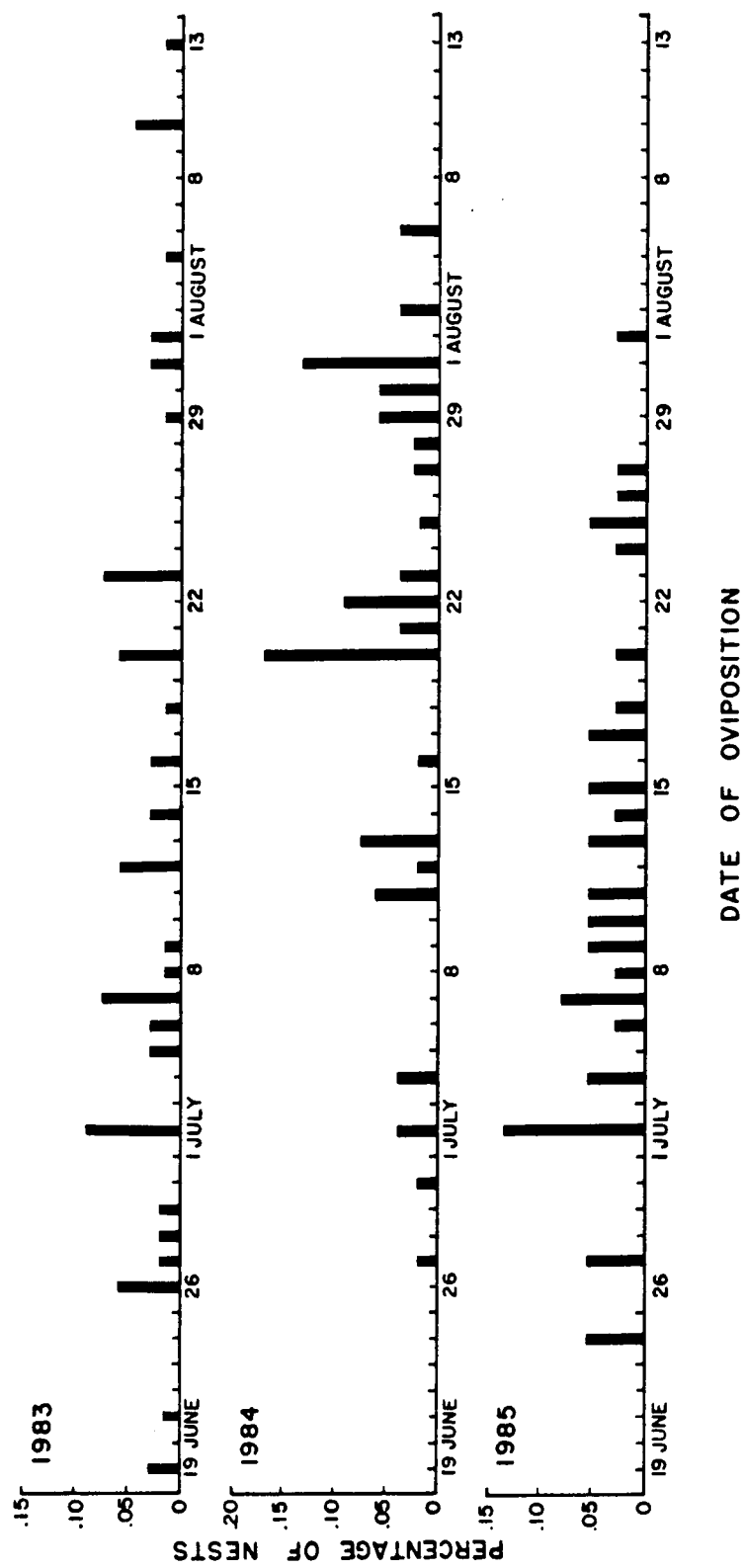


FIGURE 4. Estimated dates of oviposition for female Desmognathus fuscus.

among years; in 1984, oviposition tended to peak later in the year than in 1983 and 1985 ($F_{2, 154} = 9.55$, $P < 0.001$, one-way analysis of variance).

I was able to observe both oviposition and hatching in one female in 1983 and one female in 1984. The duration of brooding for these clutches was 46 and 54 days, respectively. I also observed hatching in 12 clutches that had been found while in the undifferentiated egg stage. The 90 percent confidence interval for length of brooding in these nests was 47.08 ± 14.42 days, with a maximum of 59, a minimum of 35, and a median of 47 days. Thus, most egg clutches in the Reed Creek population probably hatch in 40 to 60 days. Table 5 shows the median duration of stages of egg development in 1985 clutches.

Nests generally were located on the banks of streams in moist soil beneath moss (Atrichum undulatum, Thuidium delicatulum, Mnium affine), although liverworts with a finely divided thallus (Tricholia tomentola), small logs, or cobbles also were used as cover objects (Table 6). The nest chamber itself consisted of a smooth, shallow depression just large enough to fit the female and her clutch and usually did not have an opening visible from the exterior. Quantifiable nest site characteristics did not differ among years (Table 7) or vary with female size (Table 8).

Over the three years of the study, I found only 10 out of 157 egg clutches without an attending female present. In one case, the female was present at the next visit to the site. The other nine unattended clutches disappeared or became covered with fungal hyphae in

TABLE 5. Duration of stages of egg development in Desmognathus fuscus from Reed Creek in 1985. The duration shown for each stage is the median number of days from the previous to the current stage. N is the number of observations of a given stage.

Stage	N	Median duration in days	Range
egg undifferentiated	4	7	4 - 7
early differentiation	8	9	7 - 14
eye vesicle	6	8	4 - 9
pigmented embryo	9	10	4 - 12
yolk plug	6	8	3 - 9
yolk plug to hatching	7	8	3 - 9

TABLE 6. Types and frequency of cover objects used by brooding female Desmognathus fuscus. Numbers in each cell are the number of clutches found beneath the type of cover object. Liverworts and mosses were grouped together under the category 'moss' in 1983.

Cover object type	1983	1984	1985
moss	58	49	32
liverworts	--	1	2
rocks	6	1	1
small logs	4	1	2

TABLE 7. Analysis of variance for nest site characteristics in Desmognathus fuscus with YEAR as the independent variable. Only one nest site characteristic, depth of cover object (D), was measured in 1983. There were no statistically significant differences between groups.

Years	Dependent variable	F
1983-1985	D	F 2,144 = 0.60
1984-1985	D	F 1, 85 = 0.23
	MINWAT	F 1, 83 = 2.27
	DISTUP	F 1, 84 = 0.57
	DISTDN	F 1, 84 = 0.06

TABLE 8. Analysis of variance for nest site characteristics in Desmognathus fuscus, with female SVL nested within YEAR as the independent variable. Only one nest site characteristic, depth of cover object, was measured in 1983 so the analysis for 1983 - 1985 includes only D as a dependent variable. There were no statistically significant differences between groups.

Years	Dependent variable	F
1983-1985	D	F 3,138 = 0.26
1984-1985	D	F 2, 79 = 0.59
	MINWAT	F 2, 72 = 1.22
	DISTUP	F 2, 76 = 1.69
	DISTDN	F 2, 76 = 1.65

subsequent weeks. A female attending a clutch of eggs usually was oriented so that her head faced out toward the stream, with the eggs lying over the dorsal surface of her head, neck, and shoulders. Most females did not attempt to escape when the clutch was uncovered and could not be removed from the nest without being prodded gently in the tail or posterior portions of the trunk. For the most part, females remained with the clutch when returned to the nest. Thus, it appears that females rarely abandon nests, and also, that unattended eggs do not survive. Similar observations have been made for Desmognathus species by Organ (1961), Krzysik (1980a, 1980b), Forester (1979), and E. Juterbock (personal communication).

Reproductive success averaged over the Reed Creek population was variable but low. The proportion of successful nests (i.e., nests in which at least one egg hatched) within a year was 49.3 percent in 1983 (35 of 71 nests), 30.8 percent in 1984 (16 of 52 nests), and 24.3 percent in 1985 (9 of 37 nests). Within successful nests, however, hatching fraction (i.e., the ratio of the number of eggs that hatched to the number of eggs originally found in the nest) tended to be high; most successful clutches hatched over 70 percent of their eggs (Table 9). Hatching fraction was independent of the developmental stage of the clutch at first discovery ($F_{1,55} = 7.08$, $0.10 > P > 0.05$, one-way analysis of variance). This implies that the high hatching success probably is not a sampling artifact biased by clutches that were in advanced developmental stages when initially found.

TABLE 9. Hatching fraction for individual Desmognathus fuscus nests. The categories represent percentages of the original clutch that hatched; entries in each cell are the number of clutches with that hatching fraction.

Hatching fraction	1983	1984	1985
>0 - 24	0	0	0
25 - 49	5	1	0
50 - 69	4	0	1
70 - 79	10	0	1
80 - 89	8	1	0
90 - 99	4	1	0
100	4	13	7

Discriminant function analysis using the variables YEAR, OVIPDAY, SVL1, WT1, D, and NUMEGG1 indicated a statistically significant difference between successful and unsuccessful nests in the analysis for 1983, 1984, and 1985 combined (Table 10). The first canonical variable loaded heavily on YEAR, which suggests that the significant relationship may have been due to differences in nest success between years. Analysis with the constraints YEAR and SVL deleted yielded non-significant results ($F_{4,133} = 0.3029$, $0.90 > P > 0.75$). The analysis for only 1984 and 1985 included MINWAT, DISTUP, and DISTDN

TABLE 10. Discriminant function analysis of temporal variables, female size, and nest site characteristics between successful and unsuccessful Desmognathus fuscus nests in 1983 - 1985.

Canonical correlation	Eigenvalue	F	P
0.3120	0.1078	$F_{6,131} = 2.3546$	$P < 0.05$
Standardized canonical coefficients			
YEAR	1.1180		
OVIPDAY	0.2035		
SVL1	-0.5740		
WT1	0.6348		
D	0.0827		
NUMEGG1	0.1798		

with the set of variables above; discriminant function analysis for all variables yielded non-significant results ($F_{9,67} = 0.6139$, $0.090 > P > 0.75$). The analysis with constraints deleted also failed to detect statistically significant differences between groups ($F_{7,69} = 0.7622$, $0.75 > P > 0.50$).

Female snout-vent length did not affect whether or not a nest would be successful ($F_{1,145} = 0.024$, $0.90 > P > 0.75$, one-way analysis of variance). Analysis of only the successful clutches, however, indicated that large females were more likely to hatch a higher fraction of their clutch than smaller females ($F_{3,52} = 5.41$, $P < 0.005$).

Analysis of the residuals of hatching fraction, with the effects of female SVL removed, versus nest site characteristics yielded non-significant results for all years combined ($F_{4,49} = 0.22$, $0.095 > P > 0.90$). This suggests that site variables and phenology do not affect the hatching success of an individual female D. fuscus.

Only two of the 26 unsuccessful clutches in 1985 may have been flooded. The corks that had been tethered to the substrate at the first visit to the two nests were found outside the nest cavity at a check that followed a rainstorm. These nests also were located relatively near the stream (less than 15 cm from the stream, compared to a mean of 23.7 cm for all clutches), so it seems probable that flooding was the cause of clutch failure. Overall, however, rainfall levels in 1985 were substantially lower than the normal for East Tennessee (National Oceanographic and Atmospheric Administration 1985)

and most nest sites did not appear to have been inundated at any time during the year. Conversely, none of the clutches appeared to be dessicated in spite of the drier conditions. Thus, it is likely that the majority of clutch failures in 1985 were caused by predation rather than by either flooding of the nest site or dessication.

Discussion

Over the course of the study, I found fewer clutches in each successive year. In addition, annual nest success decreased over time, and initial clutch size was highest in 1983. It is possible that these declines were caused by investigator disturbance, particularly with regard to disruption of potential nest sites on the streambank. However, a permanent decline in population size due to sampling disturbance is unlikely; population manipulations in Desmognathus assemblages have required repeated removals over an extended period to reduce population size (e.g., Hairston 1986).

Given that I found 157 egg clutches and captured over 600 adult female D. fuscus, it seems likely that females nested in sites that I was unable to search. Sampling disturbance could have caused larger proportions of female D. fuscus in the streams I studied to shift nest sites to subterranean drainages. Because of their inaccessibility, little is known of the reproductive ecology of females that brood clutches in underground sites. Comparisons between surface and subterranean nests cannot be made with present knowledge.

Within any given year, climate may affect the availability of arthropod prey, the physical condition of D. fuscus after overwintering, and the activity levels of individuals. These variables, in turn, could affect reproductive phenology and thereby account for the differences in annual patterns of oviposition date that I observed. Determining the relationships between these variables, however, would require extensive physiological studies and meteorological data collected at the scale of microhabitats. It may be sufficient for most purposes to note that the timing of reproduction in Desmognathus fuscus can vary from year to year.

Fecundity in D. fuscus clearly is dependent upon body size, but the influence that ecological interactions or environmental variables may have on fecundity is not thoroughly understood. Tilley (1968) proposed that reproductive effort may be higher in Desmognathus subject to high mortality from intrageneric predation (e.g., D. fuscus from Virginia) than Desmognathus without that source of potential mortality (e.g., Ohio D. fuscus). However, the regression equations he obtained for both Ohio and Virginia populations of D. fuscus predict higher fecundity than the regression equation based on the logarithmically transformed follicle counts of this study (for a 40 mm female: Ohio predicts 21 follicles and Virginia predicts 23 follicles (based on Table 1 in Tilley 1968); Tennessee predicts 19 follicles). Thus, although natural selection may act to increase the fecundity of females subject to high predation risk, the relationship may not be straightforward and interactions with other life history parameters

that may affect fecundity and survivorship must be considered. For example, a female with high fecundity may be less able to escape predators when gravid than another female of the same size but lower fecundity. The fecundity of females that must regenerate appendages (possibly lost to predators) may be lower than the fecundity of females of equal size that lack this additional demand on growth (Maiorana 1976). In addition, high fecundity may be achieved at the expense of smaller adult size (e.g., Hirshfield 1980). It is conceivable that predation risk affects reproductive effort in several ways that may be difficult to quantify empirically.

Female D. fuscus in the Reed Creek population tended to brood egg clutches beneath moss. In comparison, Organ (1961) reported clutches in a variety of microhabitats for D. fuscus in Virginia. Desmognathus fuscus nests in Ohio and Pennsylvania populations have been found primarily beneath rocks (Dennis 1962, Davic 1977, Krzysik 1980a). Thus, there appears to be considerable variation in microhabitat use for nests both within a population and over the range of the species. Ultimately, the quality of a nest site can be indicated by the egg survivorship within a clutch brooded at that site. Since clutch success was independent of the nest site characteristics I measured, a female D. fuscus may be unable to affect her reproductive success through choice of a nest site over the range of nest sites that I found. If this were the case, then streambank microhabitats could be categorized as either suitable or totally unsuitable for brooding eggs (possibly because of insufficient cover), without subdivision of

suitable sites into those that are optimal and those that are suboptimal. Consequently, energy expended by a female in finding a potential nest site may have an inconsequential effect on her fitness.

Clutches in Reed Creek seemed to require no more than 60 days to hatch. In comparison, longer incubation times have been reported for D. fuscus in northern portions of its range (e.g., Dennis 1962, Danstedt 1975). If soil temperature within nest sites in Tennessee were higher than temperatures for northern populations, then this could have accelerated developmental rates in the Tennessee population. An alternative hypothesis is that D. fuscus in Reed Creek have been selected to reduce the amount of time spent in ontogenetic stages vulnerable to mortality from predation (Stearns 1982a).

Based on the Reed Creek population, the probability of nest failure in D. fuscus is fairly high and may vary between years. The hatching fraction within a successful nest may be greater for large females than for smaller females. Flooding of the nest site does not seem to be a major source of clutch failure; it is likely that egg mortality is caused by predation. Organisms known to prey on salamanders and their eggs include large plethodontids, such as D. quadramaculatus and Gyrinophilus porphyriticus, carabid beetles, and the ringneck snake, Diadophis punctatus. Forester (1978, 1979) found that D. ochrophaeus attending egg clutches generally were successful in defending their brood against intruding conspecifics and carabid beetles, but were less successful against snakes and larger plethodontids. Desmognathus fuscus females probably defend their

clutch in much the same way. Moreover, if large female D. fuscus were better able to effectively defend their clutch against intruders, then this could account for their higher hatching success in the Reed Creek population. Size-specific observations of clutch defense are needed to provide a rigorous test of this hypothesis.

Although this study provides no direct evidence to support or refute the hypothesis that predation affects the structure of Desmognathus assemblages, its results are provocative. They suggest that predation probably has an important influence on the reproductive success of individual female Desmognathus fuscus. Through its effects on reproductive success and female survivorship, predation may exert selective pressures on fecundity, size at sexual maturity, and other life history traits in ways that are not yet clearly understood. In addition, the high rates of nest failure that probably are attributable to predation may result in low juvenile recruitment and, therefore, could be an important mechanism for determining the abundance and distribution of species of Desmognathus.

CHAPTER 4

COVER OBJECT SELECTION

Introduction

Selection of cover objects and occupancy of the area beneath them have been shown to be important components of the ecology of plethodontid salamanders. Cover objects may provide a refuge from predators (Krzysik 1979), a source of prey items in dry weather when the animals are less able to forage in the open (Feder and Landos 1984, Jaeger et al. 1981, 1982), or a microhabitat with higher substrate moisture than ambient conditions for rehydration (Keen 1984, Spight 1967). Defense of home sites has been demonstrated in Plethodon cinereus (Jaeger et al. 1982) and in Desmognathus monticola (Keen and Sharp 1984). The subjects of these studies, however, have been males and non-gravid females. Several lines of evidence suggest that interactions between gravid females and conspecifics may be slightly different. In particular, interactions that affect nest site availability could influence the reproductive output of individual females in the population.

Forester (1977) found a positive correlation between density of nest sites and habitat quality, and observed tendencies for female D. ochrophaeus to seek nests far in advance of oviposition and to be philopatric. He concluded that nest sites may be a limiting resource for this species. Davic (1983) noted smaller body size in female D. fuscus when sympatric with D. monticola than when allopatric. He

hypothesized that the smaller size would enable female D. fuscus to exploit egg deposition sites that were unfavorable for the larger D. monticola. Other observers have found female D. ochrophaeus and D. fuscus that contained large, amorphous, follicular eggs during late summer (Hom, personal observation, Robert L. Jones, personal communication). These animals may have been females that were unable to find suitable nest sites and, therefore, were resorbing eggs.

This investigation examined cover object use by female Desmognathus fuscus within the context of the reproductive ecology of this species. In D. fuscus, courtship and oogenesis take place in the late fall prior to the onset of cold weather and again throughout the spring (Organ 1961). During the summer, each female deposits a single mass of eggs in a nest site along the banks of small, slow-moving stream (Danstedt 1975, Organ 1961). Nests usually consist of shallow depressions in moist soil covered by a rotting log, rock, mat of moss, or leaf litter (Krzysik 1980a, Organ 1961). It appears that a female broods her clutch from oviposition until hatching, which usually requires 45 to 60 days (Danstedt 1975, Organ 1961, Wilder 1917), and remains in the nest during this period (Krzysik 1980b, Organ 1961). Females leave the nest after their eggs hatch and remain active until surface waters become frozen. The study reported here was composed of five parts: field observations of brooding and non-brooding female D. fuscus and four laboratory experiments designed to test specific hypotheses about use of cover objects.

Materials and Methods

Field Methods

Observations of a natural population of Desmognathus fuscus were made in five permanent first-order streams in the Reed Creek watershed, Chilhowee Mountain, Tennessee (U.S.G.S. Kinzel Springs and Wear Cove Quadrangles). These streams were, on the average, 0.5 to 1.5 m wide and ranged in length from approximately 90 to 300 m. Each stream was visited once every ten days from May through September in 1983 and 1984, and May through June 1985. Sampling was increased to once every four days from July through mid-September 1985. At each visit, I searched the streams and streambanks for female D. fuscus by turning rocks, small logs, and mats of moss and leaf litter. Cover objects were replaced in their original position as much as possible to minimize habitat deterioration. Data on female size, reproductive condition (Chapter 3) and location were recorded. The female was then photographed in the field for future identification (modified from Tilley 1977 and Forester 1977) and released at the point of capture.

Laboratory Methods

All animals used in laboratory experiments were collected from Reed Creek within 2 km of the streams sampled in the field study. In each experiment, the animals were transported to the laboratory on ice and used in tests that started within six hours of the time of collection. I performed four separate tests: 1) female choice of natural versus surrogate manufactured cover objects; 2) multiple cover

objects and moisture levels with one female; 3) two cover objects with one female; and 4) one cover object with two females. Each experiment was conducted several times to provide statistically independent replicates. Each animal was used only once.

The experiments were conducted in enclosures that consisted of molded rubber tubs, 60 cm in diameter x 24 cm high, covered with window screen. Just prior to the start of the tests, potting soil was added to each tub to a depth of approximately 4 cm. Cover objects and water were added to the enclosures as described below for the different experiments. All observations were done in rooms that received reduced levels of natural indirect light. With one exception (see Discussion), room temperature was maintained at approximately 17-22 C.

Experiment I: surrogate versus natural cover objects. The cover objects used throughout this study correspond to rocks, small logs, and moss found in the field. Habitat variables that may influence selection of a cover object by female D. fuscus include substrate moisture and the type and size of a particular cover object. Consequently, tests of choice of cover object type should control moisture and use cover objects of uniform size. Natural rocks and logs tend to vary in size, whereas the size of manufactured items can be well regulated. This experiment compared female choice of natural to man-made cover objects. Two categories of cover objects were tested: flat rocks with clay tiles as surrogate flat rocks, and small pieces of weathered wood with rough sawn oak blocks as surrogate logs.

The surrogate objects were 10 x 10 cm and the natural objects were a similar size or slightly larger. Rock/tile tests were conducted separately from log/wood block tests. For each trial, a natural and a surrogate cover object were placed over shallow depressions in uniformly moist potting soil. One female D. fuscus was added to the enclosure. The position of the female was noted at 0800, 1200, 1600, 2000, and 2400 h for two days. The top layer of soil was removed and replaced with fresh soil between trials and all cover objects were rinsed thoroughly with tap water. Eleven females were tested for each category of cover object; at least three replicates of each category were run simultaneously. This experiment was done in March 1986 and all females were either in early stages of oogenesis or gravid (cf. Table 1, p. 1). Each female was used in only one trial.

Experiment II: multiple cover objects, one salamander. This experiment tested for differences in choice of cover objects within a moisture gradient in gravid and non-gravid female D. fuscus. The cover objects used were glazed clay tiles, pieces of rough sawn oak, and clumps of moss (Atrichum undulatum) removed from Reed Creek. All cover objects were 10 X 10 cm. The moisture gradient was formed by placing the enclosures on a 25° incline and saturating the soil at the lower end of the enclosure with tap water (Figure 5). This usually required 500 to 800 ml of water depending on initial soil moisture. Capillary action wicked enough water to the soil at the middle of the

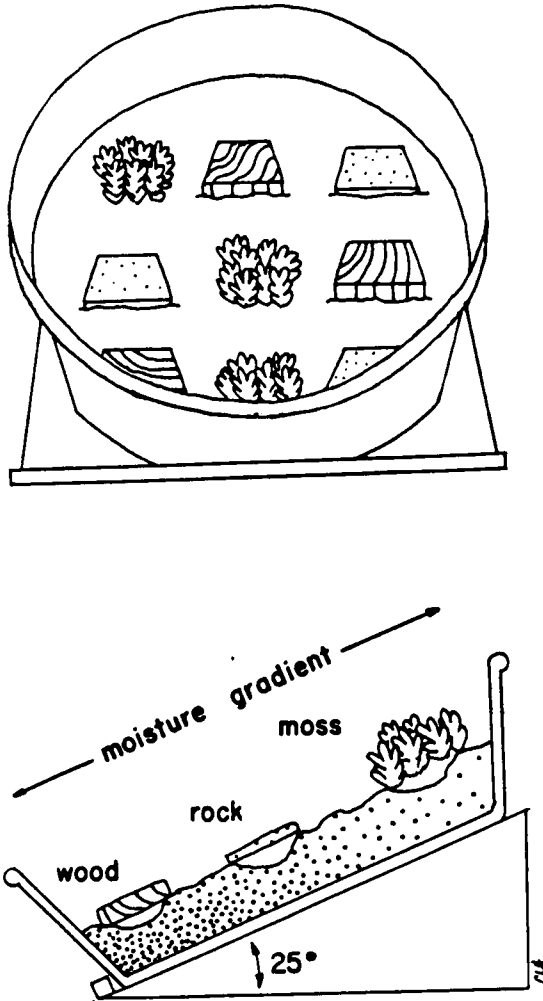


FIGURE 5. The enclosure used for testing cover object choice in Experiment II. The cover objects available were wood, rock, and moss over a gradient in soil moisture. Stippling in the lower drawing indicates relative moisture levels; higher moisture levels are more heavily stippled.

tub to create moist soil conditions there, whereas the soil at the top of the enclosure remained fairly dry.

Cover objects were arranged in a 3 x 3 array over shallow depressions in the soil. Positions of cover objects were assigned at random within each row. Each row, which represented dry, moist, and wet conditions from top to bottom, contained all cover object types. Thus, every cover object x moisture level combination was represented once within the array. To allow hydric conditions to equilibrate, I set up the enclosures 3 h prior to the introduction of the salamanders.

This experiment was run three separate times: in April 1984, when females were in early to intermediate stages of oogenesis, in June 1984, just prior to oviposition in field populations, and in September 1984, just after emergence of summer egg clutches. September females were non-gravid. Five test runs were performed consecutively during each month with two replicate enclosures in each run. The position of the females was noted at 0830, 1500, and 2130 over a period of 48 hours. At the end of each trial, the top layer of soil and the saturated soil were removed from each tub and replaced with fresh potting soil. The fresh and old soil were mixed thoroughly and allowed to air 6 h before addition of water to reestablish a moisture gradient. All animals were returned to the area from which they were collected after the last trial.

Experiment III: two cover objects, one female. This experiment, conducted in June 1985, examined whether or not gravid females exhibit a preference for fresh moss over moss that had been conditioned by the presence of other conspecific females (termed conditioned moss). Two clumps of moss (A. undulatum), one fresh and one conditioned, were placed over shallow depressions in uniformly moist potting soil. One female D. fuscus was added to each enclosure. The position of each female relative to moss type was noted at 2400, 0800, 1200, 1600, and 2000 for two days. The top layer of soil and both clumps of moss were replaced between test runs. A total of 8 females were tested and each female was used in only one trial.

Experiment IV: one cover object, two females. The goal of this experiment was to examine whether size asymmetries in female D. fuscus affected the positions of the two females relative to a single clump of moss. The experiment was carried out in June 1985. In each test, I placed a single clump of moss (A. undulatum) over a shallow depression in uniformly moist soil within an enclosure and added two gravid female D. fuscus. The position of each female (beneath moss, no cover, or in the soil) was noted at 2400, 0800, 1200, 1600, and 2000 h for 48 hours. The top layer of soil was replaced between trials. I ran two versions of this experiment: one using fresh moss in each trial, with 14 pairs of females, and one in which the moss had been conditioned by the presence of other female conspecifics, with a total of 12 pairs of females. Each female was used only once.

Statistical Analysis

Because the data consist of multiple observations of each animal (position at different times of the day over two days), any set of observations taken on a given animal probably violates the assumption of statistical independence. Inclusion of time of day and day of sampling as additional variables in the analysis alleviates this problem by creating a single observation for every combination of time, day, and location. Each experiment was analyzed using contingency table methods (Fienberg 1977).

Experiment I was analyzed in a three-way table (time (T) x day (D) x cover object type (C)). I tested the main effects of each variable, independence of each variable conditioned upon fixed values of all other variables, and all two-way interactions.

For Experiment II, females were analyzed using a 5-way table (time (T) x day (D) x month (M) x cover object (C) x moisture level, referred to as "water" (W)). In a five dimensional table, there are many ways in which combinations of two or more variables can be formed. Rather than testing all possible combinations of variables for lack of interaction, I decided a priori to test only main effects; two-way interactions with the other three variables fixed; M x W x C, the three-way interaction of greatest interest, with T and D included as simple effects; and independence of each variable conditioned upon fixed values of all other variables. Thus, for example, the model for the test of two-way interaction between C and W would include all main effects and all two-way interactions other than

C x W. The test of the conditional independence of C, with all other variables fixed, would be a test of the model that included the main effects of all variables and all interaction terms excluding those that involved C.

Experiment III was analyzed in a 3-way table (T x D x C). As in Experiment I, all possible interactions were tested.

Experiment IV was analyzed two ways: in a four-way table, with variables T, D, C, and female (F), and as a three-way table with variables T, D, and occupant of the moss (O), with categories large, small, both, and neither. The four-way table was analyzed in a manner analogous to the five-way table of Experiment II; all interactions were analyzed in the three-way table. Data for trials that used fresh moss were analyzed separately from trials that used conditioned moss.

Results

Field Results

I observed 216 non-brooding females D. fuscus in 1983, 244 non-brooding females in 1984, and 205 non-brooding females in 1985, with 11, 6, and 10 recaptures in each year, respectively. Only data for the initial captures are reported. All females captured in the spring and early summer were gravid; non-brooding females captured after August were non-gravid or in early stages of oogenesis.

Both non-gravid and gravid D. fuscus exhibited strong seasonal differences in utilization of cover objects (Figure 6). The animals

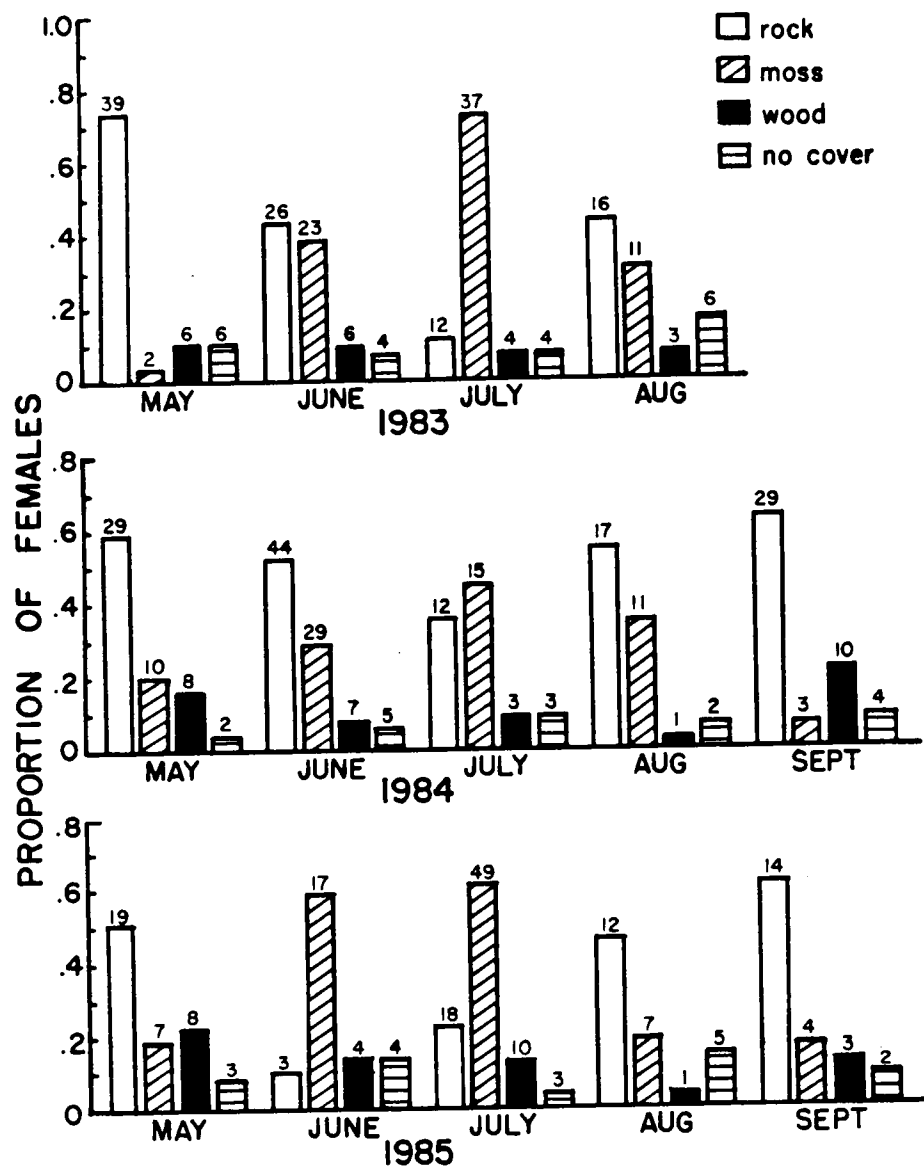


FIGURE 6. Seasonal differences in cover object choice in non-brooding female *Desmognathus fuscus* in a natural population. Numbers above each bar represent sample sizes for that month.

tended to use rocks as cover objects in late spring and late summer and moss growing on the soil of streambanks during July of all three years. In 1985, use of moss also predominated in June. The month x cover object interaction was statistically significant in all three years (1983: $G = 68.17$, $df = 12$, $P < 0.0001$; 1984: $G = 28.88$, $df = 12$, $P < 0.005$; 1985: $G = 28.88$, $df = 12$, $P < .005$).

Over the three years of the study, I found a total of 164 egg clutches. Most females brooded clutches under moss, although rocks and logs also were utilized by a few females (Table 11). In most cases, I was not able to observe deposition of the clutch, but I estimate that most females oviposited between early July and mid August.

TABLE 11. Cover objects used for nest sites in a natural population of Desmognathus fuscus. Entries in each cell denote the total number of egg clutches found beneath the given cover object type in each year.

Cover object type				
Year	Rock	Moss	Log/leaf litter	Total
1983	6	58	4	68
1984	1	50	2	52
1985	1	34	1	36

Laboratory Results

The initial response of almost every animal after introduction to the enclosures was to move around the enclosure until stopped by the side of the tub, then to walk or run around the perimeter. The animals did not attempt to climb the sides of the enclosures and no animal escaped during the course of a trial.

Experiment I. Females did not appear to differentiate between natural and surrogate cover objects, although some females burrowed slightly beneath the surface of the soil rather than choosing one of the two cover objects (Table 12). Most animals were observed under the same cover object throughout. The moisture-holding capacity of the rocks and tiles seems comparable, since I often observed condensation on the lower surface of both rocks and tiles at 0800. None of the effects tested was statistically significant (Table 13).

Experiment II. Females exhibited a preference for moss as a cover object over wood or rock regardless of the month of the test (Table 14). Only the main effect for cover object type was statistically significant; all interactions between two or more variables were not significant (Table 15). This suggests that under the conditions of the experiment, cover object type was the only variable that affected female choice of cover.

Experiment III. The initial observation of females in this experiment occurred only 3 to 4 hours after release of the females into the enclosures. Six out of the eight animals were active and not

TABLE 12. Cover object choice with regard to natural versus surrogate cover objects in female Desmognathus fuscus. Cover objects tested were natural rock versus a clay tile surrogate, and a natural log versus a wood block surrogate. Some animals used the soil substrate itself, creating a third type of cover object. Entries in each cell are the number of observations of separate individuals for the given day-time-cover object combination.

Day	Time	Rock/Tile			Log/Wood Block		
		Natural	Surrogate	Soil	Natural	Surrogate	Soil
Day 1	0800	3	3	5	5	1	5
	1200	4	3	4	5	1	5
	1600	4	3	4	4	3	4
	2000	4	3	4	5	3	3
	2400	3	3	5	5	4	2
Day 2	0800	4	3	4	5	4	2
	1200	4	3	3	5	4	2
	1600	2	4	5	5	4	2
	2000	4	3	4	4	5	2
	2400	4	3	4	4	6	1

TABLE 13. Results of three-way contingency table analyses for choice of natural versus surrogate cover objects in female Desmognathus fuscus. Variables in the analysis were time of day (T), day of trial (D), and cover object type (C). Cover objects were natural or surrogate objects. See statistical methods section for further explanation. None of the tests were statistically significant.

Hypothesis tested	df	Rock/Tile: G	Log/Wood Block: G
T main effect	26	2.49	13.98
D main effect	23	2.46	13.98
C main effect	24	4.12	18.96
T, other variables fixed	20	2.33	6.79
D, other variables fixed	14	1.58	9.12
C, other variables fixed	18	2.41	13.98
T x D interaction	12	1.46	1.93
T x C interaction	16	2.30	6.79
C x D interaction	10	1.54	9.12

TABLE 14. Selection of cover objects and soil moisture by female *Desmognathus fuscus*. Types of cover objects were moss (M), wood (W), rock (R), and no cover (NC). Levels of soil moisture were dry, moist, and wet. Entries in each cell were the number of observations of separate individuals for the given cove object-soil moisture-time combination.

Month	Day	Time	Dry						Moist						Wet					
			M			W			R			NC			M			W		
			M	W	R	M	W	R	M	W	R	M	W	R	M	W	R	M	W	R
April	Day 1	0830	3	0	0	0	0	0	1	1	1	1	1	0	3	0	0	0	0	0
		1500	4	0	0	0	0	0	1	1	0	0	0	0	2	0	1	1	1	1
		2130	4	0	0	0	0	0	2	1	0	0	0	0	2	0	1	0	1	0
	Day 2	0830	3	0	0	0	0	0	1	1	0	0	0	0	5	0	0	0	0	0
		1500	3	1	0	0	0	0	0	0	0	0	0	0	5	0	1	0	1	0
		2130	3	1	0	0	0	0	0	0	0	0	0	0	5	0	1	0	1	0
	Day 1	0830	1	0	0	0	0	0	0	0	0	0	0	0	6	1	1	0	1	0
		1500	1	0	0	0	0	0	0	1	1	0	0	0	4	1	1	0	1	0
		2130	1	0	0	0	0	0	0	1	1	0	0	0	3	1	1	1	1	1
June	Day 2	0830	1	0	0	0	0	0	1	0	0	0	0	0	4	1	2	0	0	0
		1500	2	0	0	0	0	0	3	3	0	1	0	1	0	1	0	0	0	0
		2130	1	1	0	0	0	0	0	0	0	1	1	0	5	0	1	0	1	0

TABLE 14, continued.

Month	Day	Time	Dry				Moist				Wet			
			M	W	R	NC	M	W	R	NC	M	W	R	NC
September	Day 1	0830	2	0	0	0	3	4	0	0	1	0	0	0
		1500	2	0	0	0	3	3	0	1	0	1	0	0
		2130	3	0	0	0	3	3	0	0	0	1	0	0
	Day 2	0830	3	0	0	0	1	2	0	0	1	2	1	0
		1500	3	0	0	0	1	3	0	0	0	1	2	0
		2130	3	0	0	0	1	1	0	1	3	0	1	0

TABLE 15. Results of five-way contingency table analyses for choice of cover object and soil moisture in Desmognathus fuscus. Variables tested were time (T), day (D), and month (M), cover object (C), and soil moisture (W).

Hypothesis tested	df	G
T main effect	207	206.45
D main effect	206	206.43
M main effect	207	207.16
C main effect	208	351.76**
W main effect	207	217.11
T, other variables fixed	165	104.68
D, other variables fixed	158	111.65
M, other variables fixed	165	143.06
C, other variables fixed	187	159.25
W, other variables fixed	182	184.42

TABLE 15, continued.

Hypothesis tested	df	G
T x D interaction	151	99.88
T x M interaction	153	99.88
T x C interaction	155	103.61
T x W interaction	153	101.44
D x M interaction	151	101.97
D x C interaction	152	100.32
D x W interaction	151	109.49
M x C interaction	155	106.01
M x W interaction	153	125.92
C x W interaction	172	137.47
M x C x W interaction	172	116.49

** $P < 0.001$

under cover at the time of this observation (Table 16). I believe that this represented exploratory behavior rather than a manifestation of the activities of acclimatized animals. I therefore omitted the first observation data from the analyses.

In subsequent observations, females were found beneath moss much more often than beneath soil or without cover. They also tended to choose fresh moss more often than moss that had been conditioned by conspecific females. The results of contingency table analyses suggest that females choose their position based on cover object type alone, regardless of the time and day of observation (Table 17). Although females chose soil more frequently during day 1 than day 2, the time x cover and day x cover interactions were not significantly different from zero. This suggests that the apparent preference for fresh moss was not due to temporal changes such as, for example, deterioration caused by drying in either type of moss.

Experiment IV. As in Experiment III, female position during the first observation appeared to be an artifact of the female's lack of acclimatization within the three hours since introduction to the enclosure. Consequently, I omitted these data from the analyses.

The trials that used conditioned moss yielded different results from trials that used fresh moss (Table 18). In trials with fresh moss, the female x cover object interaction was statistically significant, as were the tests of significance of female with time, day, and cover object as fixed variables, and cover object main effect (Table 19). The larger female almost always was found beneath the moss, whereas the

TABLE 16. Cover object choice with regard to moss condition by female Desmognathus fuscus. Possible cover objects were moss conditioned by the presence of a conspecific female, fresh moss, no cover object, and soil. Entries in each cell are the number of observations of separate individuals for the given day-time-cover object combination.

Day	Time	Cover object			
		Conditioned	Fresh	No cover	Soil
Day 1	2400	0	1	6	1
	0800	1	3	0	4
	1200	2	2	0	4
	1600	2	4	0	2
	2000	2	3	0	3
Day 2	2400	2	3	2	1
	0800	2	5	0	1
	1200	1	1	0	1
	1600	2	5	0	1
	2000	4	3	1	0

TABLE 17. Contingency table analyses for cover object choice in female Desmognathus fuscus. Variables in the analysis were time of day (T), day of trial (D), and cover object type (C). Cover objects were fresh moss, marked moss, no cover object, and soil. See Statistical Methods section for further explanation.

Hypothesis tested	df	G
T main effect	31	26.32
D main effect	28	25.21
C main effect	30	49.40*
T, other variables fixed	21	13.98
D, other variables fixed	12	11.63
C, other variables fixed	24	22.85
T x D interaction	9	3.53
T x C interaction	18	12.05
D x C interaction	9	9.70
T x D x C interaction	6	2.42

* $P < 0.05$

TABLE 18. Position of pairs of female *Desmognathus fuscus* relative to a single cover object in separate trials using fresh and conditioned moss. The category "large" refers to the larger of the pair tested in each trial, "small" refers to the smaller. Categories of cover objects were moss, no cover, and soil.

Day	Time	Fresh						Conditioned					
		Large			Small			Large			Small		
		Moss	No cover	Soil	Moss	No Cover	Soil	Moss	No cover	Soil	Moss	No cover	Soil
Day 1	2400	2	12	0	1	13	0	2	10	1	1	10	1
	0800	14	0	0	8	4	2	5	1	6	7	1	4
	1200	14	0	0	10	3	1	5	0	7	7	1	4
	1600	14	0	0	10	3	1	5	0	7	6	0	6
	2000	14	0	0	10	2	2	5	1	6	8	1	3
Day 2	2400	11	3	0	11	2	1	6	4	2	5	3	4
	0800	12	2	0	11	1	2	7	1	4	4	3	5
	1200	14	0	0	10	2	2	5	1	6	4	0	8
	1600	13	1	0	10	2	2	6	1	5	3	1	8
	2000	10	2	0	8	4	2	5	2	5	3	4	5

TABLE 19. Results of contingency table analyses for position of two female Desmognathus fuscus relative to a single cover object. Variables considered were time (T), day (D), cover object (C), and female (F). See Statistical Methods for additional information.

Hypothesis tested	Fresh moss		Conditioned moss	
	df	G	df	G
T main effect	49	42.37	49	37.93
D main effect	46	32.33	46	37.93
C main effect	47	260.18**	47	92.94**
F main effect	46	32.33	46	37.93
T, other variables fixed	32	20.04	40	29.87
D, other variables fixed	23	17.63	31	20.65
C, other variables fixed	37	52.23*	37	37.93
F, other variables fixed	32	47.88	32	16.91
T x D interaction	20	16.71	28	15.69
T x C interaction	25	19.92	33	29.96
T x F interaction	21	16.70	29	15.62
D x C interaction	19	17.56	27	20.61
D x F interaction	18	16.64	26	15.60
C x F interaction	27	47.80*	27	16.05

TABLE 19, continued.

Hypothesis tested	Fresh moss		Conditioned moss	
	df	G	df	G
T x D x C interaction	13	3.78	13	6.31
T x D x F interaction	10	1.54	9	3.53
T x C x F interaction	16	3.13	15	5.35
D x C x F interaction	10	4.72	9	10.36
T x C x D x F interaction	8	1.45	7	3.51

* P < 0.05

** P < 0.0001

smaller female of the pair often remained on the surface without cover or was buried beneath the surface of the soil. In contrast, large females did not appear to behave differently from the smaller females in trials with conditioned moss. Only the main effect of cover object was statistically significant (Table 19).

The results of the second set of analyses are revealing. On the first day of trials that used fresh moss, either the larger female or both females occupied the moss for all observations save the first (Table 20). On the second day, there were fewer large females in the moss, and for all observation times, there was at least one enclosure in which neither female was beneath the moss. The interaction of day x occupant was statistically significant (Table 21).

In comparison, I did not observe these patterns in the trials that used conditioned moss (Table 20). Neither the main effects nor the interactions between variables were statistically significant (Table 21). This suggests that moss occupancy was random relative to the variables tested.

Discussion

Cover object use by the females in the natural population of D. fuscus observed in this study varied seasonally. Gravid females shifted their utilization of microhabitat from rocks to moss just prior to the time when most females in the population oviposited. Almost all of the egg clutches I found were located beneath moss. At the end of the summer, when the majority of the clutches had hatched,

TABLE 20. Position of pairs of female *Desmognathus fuscus* relative to a single cover object in trials using fresh or conditioned moss. The variable "occupant" refers to whether the larger, smaller, both, or neither of the pair tested were beneath the moss at the time of observation.

Day	Time	Fresh: Occupant			Neither	Large	Conditioned: Occupant		
		Small	Both	Neither			Small	Both	Neither
Day 1	2400	2	1	0	11	2	1	0	9
	0800	6	0	8	0	2	4	3	3
	1200	3	0	11	0	2	4	3	3
	1600	4	0	10	0	1	3	3	5
	2000	4	0	10	0	1	4	4	3
Day 2	2400	1	1	10	2	3	2	3	4
	0800	1	0	12	1	4	1	3	4
	1200	4	0	9	1	3	1	2	6
	1600	3	0	10	1	3	0	3	6
	2000	2	0	8	4	3	1	2	6

TABLE 21. Results of contingency table analyses for position of two female Desmognathus fuscus relative to a single cover object. Variables considered were time of observation (T), day of observation (D), and occupant of the moss (O). See Statistical Methods section for additional information.

Hypothesis tested	Fresh moss		Conditioned moss	
	df	G	df	G
T main effect	31	26.82	31	18.61
D main effect	28	26.82	28	18.61
O main effect	30	171.58**	30	27.08
T, other variables fixed	18	12.08	24	6.34
D, other variables fixed	11	15.86	15	16.07
O, other variables fixed	24	26.82	24	18.61
T x D interaction	6	4.32	12	2.51
T x O interaction	15	12.08	21	6.34
D x O interaction	8	15.86*	12	16.07
T x D x O interaction	3	3.99	9	2.42

* $P < 0.05$

** $P < 0.0001$

the shift was reversed and most females again used rocks as cover. These observations suggest that seasonal differences in female cover object use may be related to the reproductive cycle.

The cause-effect relationship between cover object selection and reproduction is not clear, however. It is possible that the reproductive success, in terms of the number of eggs laid that survive to hatch, of females that nest under moss is higher than the reproductive success of females that nest beneath other types of cover. This difference in reproductive success could be caused by abiotic factors, such as dessication, or by biotic interactions, such as predation. If differential egg survivorship occurred, and if nest site choice were a heritable trait, then natural selection should favor females that choose moss. This hypothesis could be tested by comparing the hatching success of clutches brooded beneath different types of cover objects. Such a test may not be possible for the Reed Creek population because of the low frequency with which clutches beneath non-moss cover objects may be found.

This predominance of moss as a cover for nest sites in D. fuscus has not been observed previously. Most studies of D. fuscus reproductive ecology have reported egg clutches primarily under rocks, with moss infrequently or never used (Davic 1977, Dennis 1962, Krzysik 1980a). These investigations, however, have been on populations of D. fuscus located within the range of fewer plethodontids relative to populations in the southern Appalachians. The presence of large Desmognathus can cause shifts in cover object choice in smaller

species (Keen 1982, Southerland 1986a, 1986b, 1986c). Furthermore, intrageneric predation can influence the structure of Desmognathus assemblages (Hairston 1986). Thus, it is possible that the differences in microhabitat use between this and other studies may reflect geographic variation in predation pressure.

Exogenous factors, such as temperature, precipitation, or photoperiod, could cause females to prefer moss as a cover object during the summer independent of their effects on reproductive success. In this case, females would nest beneath moss in response to these other variables rather than as a result of differential clutch survivorship. Testing this hypothesis would require fine control of the environmental variables and a precise knowledge of the physiological ecology of D. fuscus. In addition, if seasonal shifts in microhabitat were unrelated to the female reproductive cycle, then similar shifts should be observed in males and juveniles.

In comparison to the seasonal differences in female preference for cover objects in natural populations, females in laboratory Experiment II preferred moss regardless of the the month of the test. Two possible reasons for this discrepancy are as follows. 1) The patterns observed in both the field and the laboratory could have been artifacts of sampling, but this seems unlikely given the distinct preferences that I observed. 2) The females in the laboratory may have responded to some other variable. Three variables, in particular, may have affected female choice. Firstly, air temperatures in the June and September trials were maintained at 17 -

22 C throughout the day and night, whereas a series of failures in the air conditioning system caused the temperature of the room in which the April trials were conducted to rise to approximately 25 C on several occasions. The animals did not seem to have been physiologically stressed by the higher temperatures; if this had occurred, then more animals should have chosen wet and fewer dry sites. If females in natural populations use ambient air or soil temperatures as cues for determining choice of cover object, then either the erratic and slightly high temperatures in April or the constancy of temperatures in June and September could have altered the females' behavior. Secondly, the area beneath the screened enclosures was fairly dark. Overall light levels may have been closer to summer conditions, when relatively little light penetrates the canopy, than to early spring or autumn conditions. Ignoring photoperiod, if females use light levels as proximal cues for determining microhabitat choice, then the reduced insolation also could have caused the behavioral differences. Finally, the types of cover objects available were not rocks and rotting logs but clay tiles and wood blocks as surrogates for the natural objects. It is possible that the females based their choice of location on avoidance of these cover objects rather than on a preference for moss. The lack of distinction between natural and surrogate objects observed in Experiment I, however, argues against this. Using natural cover objects and field enclosures (Keen 1982) in future studies would eliminate what may have been confounding effects in this investigation.

The results of the third and fourth experiments suggest that female D. fuscus can recognize substrates conditioned by the presence of other conspecifics and tend to avoid such substrates. Similar behavior has been reported for Plethodon cinereus: Jaeger and Gergits (1979) found that P. cinereus avoided areas marked by a conspecific salamander even in the absence of the marking animal. Also, in encounters staged between territory-holding and intruder P. cinereus, intruders often left marked sites prior to threat displays or aggressive defense by the resident (Jaeger et al. 1982). Conflicts that involved physical contact between participants usually were won by the resident. The injuries that typically occur in such encounters are bites on the head and tail. Bites on the head impair a salamander's chemosensory ability (Jaeger 1981). Injury to a female's tail often causes autotomy and subsequent loss of caudal fat reserves, which may reduce the female's reproductive potential (Maiorana 1976). If D. fuscus residents are more likely to be successful in site defense than conspecific intruders, then avoidance of marked cover objects would be adaptive if it reduced the number of behavioral encounters with site-holders, and thus the possibility of injury resulting from such encounters, experienced by an individual female.

Size asymmetries appeared to affect occupation of the substrate beneath cover objects in this study. In Experiment IV, either the larger female or both females occupied fresh moss, which suggests that the larger female may have determined whether only one or both animals would use the moss. The mechanism by which this may have

occurred cannot be ascertained from these data. It is possible that the larger female excluded the smaller from the moss, or the smaller female avoided moss occupied by a larger conspecific, perhaps through a greater tendency to avoid cover objects conditioned by conspecifics. The latter would not be surprising since small animals are more likely than larger animals to be attacked and injured in direct behavioral interactions (Keen and Sharp 1984).

Overall, these results suggest that interactions between gravid females may influence occupancy of potential nest sites, specifically moss, in natural populations of D. fuscus. This, in turn, has several implications for female reproductive success. If a female is unable to find and occupy a nest site, then her reproductive success for the year will be zero. Conflicts over cover objects may result in injuries that could reduce a female's ability to reproduce successfully (Jaeger 1981, Maiorana 1976). Direct observations of the sequence of events that occur during female-female interactions incorporating asymmetries in size and occupancy are necessary to gaining a better understanding of the role of cover object choice in the reproductive ecology of Desmognathus fuscus.

CHAPTER 5

CLUTCH DEFENSE

Introduction

In the southern Appalachians, female Desmognathus fuscus inhabit the banks of small streams at mid to lower elevations. This microhabitat also is used by larger plethodontid salamanders (e.g., D. quadramaculatus, D. monticola, Pseudotriton ruber, Gyrinophilus porphyriticus) and small snakes (e.g., Diadophis punctatus, Carphophis amoenus). Because of their size and general food habits, these salamanders and snakes present a potential cause of mortality to D. fuscus adults, juveniles, and eggs. Thus, it is reasonable to expect that the behavior of individual D. fuscus in the presence of these species might reflect selection to minimize risk of mortality and losses in reproductive potential caused by predation.

Defense of an egg clutch against potential predators by the female parent has been described in several of the evolutionary lineages within the Plethodontidae (Salthe and Meacham 1974, Nussbaum 1985). Egg clutches deprived of the female succumb to fungal infestation or are eaten by predators (Snyder 1971, Forester 1979, Bachmann 1984), thus defense appears to be essential to the survivorship of the clutch. Its adaptive value, however, is unclear in situations in which continued care may cause injury to the attending female or a reduction in her future reproductive value.

To examine the responses of female Desmognathus fuscus to different predator types and the intensity of clutch defense during different developmental stages of egg clutches, I staged laboratory encounters between female D. fuscus attending egg clutches ("attendants", sensu Forester 1979) and potential egg predators ("intruders"). I tested females and clutches in early and advanced developmental stages and used three types of potential predators: conspecific males; a larger congener, D. quadramaculatus; and a ringneck snake, Diadophis punctatus.

Materials and Methods

The brooding female Desmognathus fuscus and egg clutches used in this study were collected from a 1.5 km section of Reed Creek in the Chilhowee Mountain Wildlife Management Area (U.S.G.S. Kinzel Springs and Wear Cove Quadrangles) during July and August of 1984 and 1985. Each female and her clutch were transported to the laboratory on ice with the eggs wrapped in damp paper toweling to prevent oophagy by the female, and held in individual clear plastic boxes (9.5 x 19 x 9.5 cm) with 1 to 2 cm moist soil as a substrate. A plastic 9 cm petri dish containing 1 cm moist soil also was placed within each box to facilitate moving the female and eggs. Every female was used in defense observations within ten days of capture. The day before a scheduled observation, the female was fed apterous Drosophila ad libitum and the relative development of the egg clutch was determined. The developmental stage of clutches classified as "early" ranged from

eggs that had not undergone differentiation perceptible to the unaided eye to embryos with well-developed optic capsules but undifferentiated limbs. Clutches classified as "advanced" contained heavily pigmented embryos with differentiated toe buds or eggs at or very near hatching. Each female and clutch were used in only one defense encounter.

Male D. fuscus and D. quadramaculatus were collected from Reed Creek in mid-July; the ringneck snake was captured in May 1985 in the Catoosa Wildlife Management Area, approximately 80 air km from Reed Creek. All intruders were used for no more than two encounters per day, with at least 30 minutes between observations.

Clutches within each developmental stage were assigned to intruder type on a random basis. Encounters between females and a single conspecific males or D. quadramaculatus were staged in an enclosed opaque plexiglass arena with dimensions 15 x 20 x 15 cm. For these encounters, the female and her clutch were transferred to the arena in the petri dish and allowed 15 to 30 minutes to acclimate. I did not proceed with the trial unless the female was quiescent and contacting her eggs. The potential predator was introduced into a smaller adjoining chamber and was allowed to enter at will the portion containing the female and eggs. The interactions between the female and intruder were observed until an approach by the intruder resulted in either its withdrawal or abandonment of the clutch by the attendant. An encounter was terminated if such an approach did not occur within 30 minutes.

Observations of encounters between attending females and the ringneck snake were made in a 40 l glass aquarium. The female was allowed time to acclimate as described above, then the snake was released into the aquarium at a point distant from the female.

All encounters were staged between 1930 and 0100 h in a darkened room under dim incandescent red light. I recorded observations of each encounter on magnetic tape and scored female behavior following Forester 1979. Each occurrence of these behaviors received 1 point: attendant appeared attentive to intruder; attendant advanced toward intruder; attendant faced intruder with an open mouth; attendant nudged intruder with her snout; while beneath the intruder, the attendant elevated her thorax so as to lift the intruder; attendant pursued the intruder for up to 2 cm. Each occurrence of the following actions received 2 points: attendant advanced toward and made physical contact with intruder; attendant pursued intruder for more than 2 cm. Bites or snaps directed toward the intruder by the attendant often occurred in rapid succession; these were awarded $n+1$ points, where n was the number of bites or snaps. The female's cumulative point total was used for the score of a particular trial. A total of 21 females were used in defense observations. Table 22 shows the size of each female (measured as snout-vent length) and number of eggs in the clutch used in encounters with a specific predator type. Because of the limited number of observations, I report my results without statistical analyses.

TABLE 22. Combinations of intruder type, clutch development, female body size (SVL) and clutch size of Desmognathus fuscus used in defense observations.

Intruder	Early		Advanced	
	Female SVL	Clutch size	Female SVL	Clutch size
	5.1	20	5.0	12
<u>Desmognathus</u>	4.6	16	4.8	17
<u>fucus</u> male	4.4	17	4.6	28
	4.4	19	4.5	11
	4.1	16	4.4	16
	5.8	34	4.7	25
<u>Desmognathus</u>	5.0	28	4.5	15
<u>quadramaculatus</u>	4.5	19	4.4	16
			4.3	10
	4.7	25	4.2	17
<u>Diadophis</u>	4.6	8	3.9	12

Results and Discussion

Most of the females appeared to acclimate to the encounter arena fairly rapidly; the majority did not leave the petri dish following the transfer to the arena. Those that did leave the dish generally returned to their eggs in a few minutes; only 2 failed to return and reestablish contact with their eggs within 30 minutes. These were not used for defense observations. The scores for the 21 actual encounters are shown in Figures 7 and 8, plotted against female snout-vent length and clutch size, respectively.

Encounters between attendants and an intruding D. fuscus male or D. quadramaculatus tended to follow similar patterns. The intruder generally entered the encounter arena within a few minutes of release into the smaller chamber. In most cases, it appeared to be exploring the chamber rather than foraging for food. In all encounters, the attendant remained with the clutch throughout this exploratory period. Many attendants appeared to watch the intruder: they oriented their head or body to face the intruder as it moved around the arena. When the intruder approached the clutch, the attendant often responded by elevating the anterior portion of her thorax from the substrate. Continued approach of the intruder generally elicited nudges, lifts, snaps, bites, or a combination of these from the female. The level of aggression of the attendant appeared to reflect the level of aggression and persistence of the intruder. Intruders that actually attacked the eggs or female, or touched the eggs with their snout tended to generate the highest level of response from the attendant.

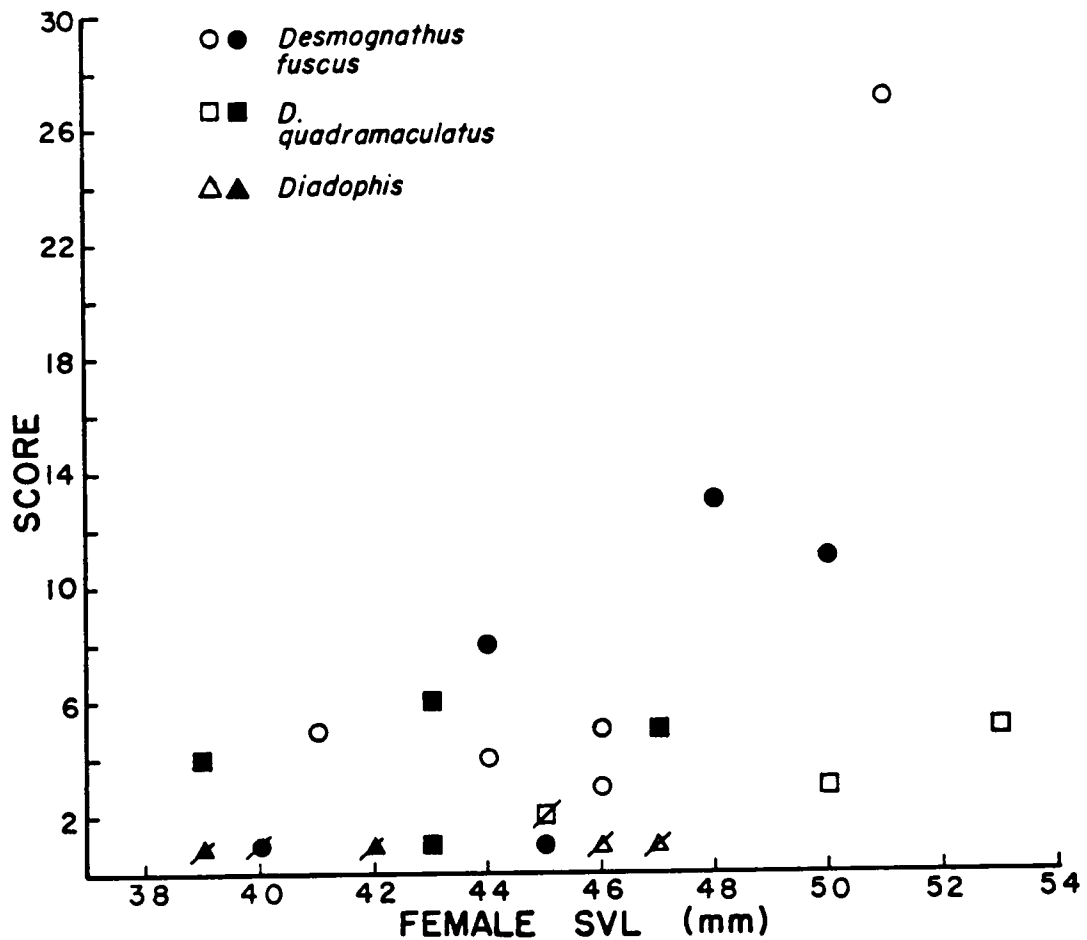


FIGURE 7. Results of encounters between attending female *Desmognathus fuscus* and intruders, with female body size as the abscissa. Females whose clutches were in early development are indicated by hollow symbols; solid symbols represent clutches in late stages of development. Plain symbols indicate that the female was successful in driving off the intruder. Encounters in which the female was unable to drive off the intruder are denoted by a slash through the symbol.

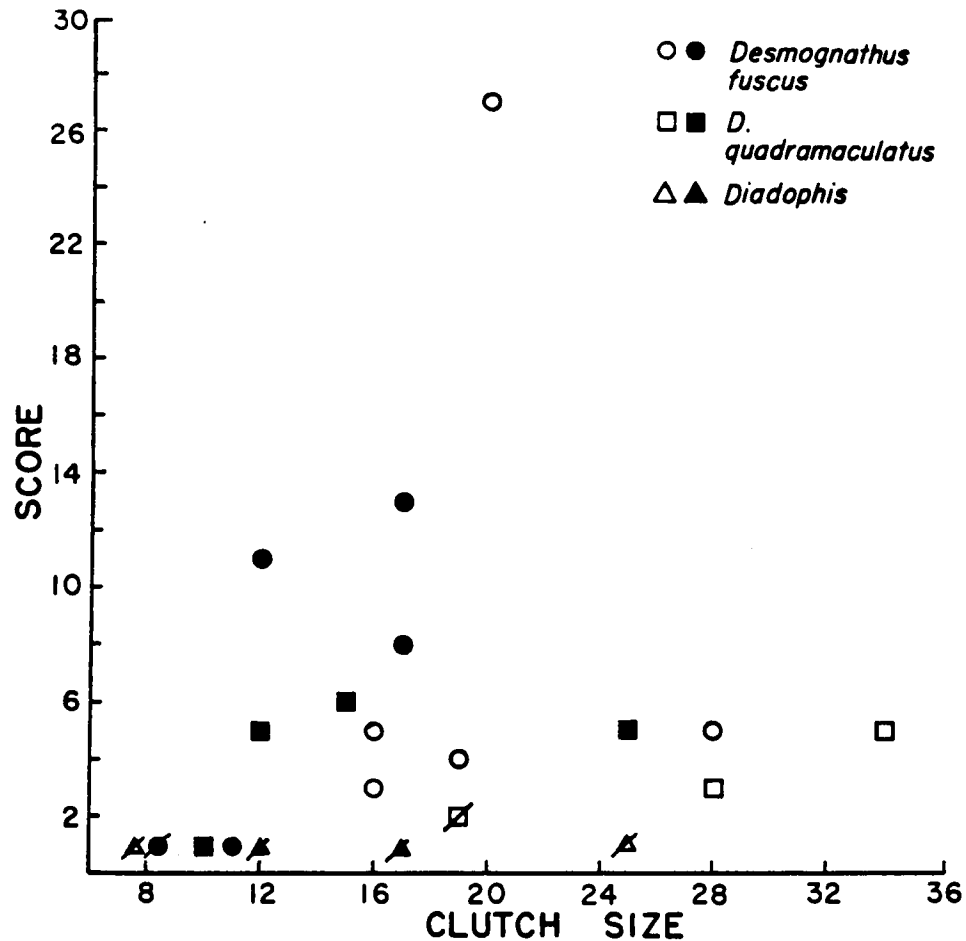


FIGURE 8. Results of encounters between attending female Desmognathus fuscus and intruders, with clutch size as the abscissa. Females whose clutches were in early development are indicated by hollow symbols; solid symbols represent clutches in late stages of development. Plain symbols indicate that the female was successful in driving off the intruder. Encounters in which the female was unable to drive off the intruder are denoted by a slash through the symbol.

Attendants usually were successful in causing the intruding salamander to retreat and leave the vicinity of the clutch. In several cases, the female pursued the intruder for up to 10 cm.

Encounters with the ringneck snake were very different from encounters with other Desmognathus. In all four cases, the attendant remained motionless and with her clutch as the snake approached. One of the attendants abandoned her clutch and ran to the opposite side of the aquarium when the head of the snake was within 1 cm of the female. The other three attendants did not abandon the clutch until tongue-flicked by the snake.

In general, these data appear to suggest the lack of a relationship between intensity of defense and egg developmental period, and between intensity of defense and clutch size (Figure 8). They may indicate a positive correlation between female size and intensity of defense (Figure 7). The data, however, are insufficient to determine whether or not these relationships actually exist and I am reluctant to draw conclusions based on the limited sample used here. Other studies on clutch defense in plethodontid salamanders offer contradictory results. Forester (1983) observed more intense defense in female D. ochrophaeus attending clutches in advanced stages of development relative to females attending eggs in younger developmental stages. Bachmann (1984), however, did not find this temporal correlation in Plethodon cinereus. Future studies of clutch defense in plethodontid taxa should re-examine whether a) there is a positive correlation between age of a clutch and intensity of defense

by the female; b) intensity of defense varies with intruder type or level of aggression of the intruder; c) intensity and efficacy of defense vary with female size.

CHAPTER 6

GROWTH AND LIPID CYCLING

Introduction

The ultimate indicator of the evolutionary success of a genotype is its fitness, which in turn, can be measured by reproductive output. In many taxa, however, the ability of an organism to reproduce successfully is closely linked to body size and accumulation of storage products (e.g., herbs: Bierzychudek 1984, Cochran 1986; cladocerans: Tessier and Goulden 1984; salamanders: Maiorana 1976; lizards: Vitt and Cooper 1985). Thus, in some circumstances, it may be advantageous for an organism to allocate resources to growth or storage rather than directly to reproduction. To understand more fully the influence that these variables may have on the reproductive ecology of a particular species, laboratory studies and investigations that monitor growth and seasonal levels of storage products in individuals in a natural population often are useful.

There is considerable evidence indicating that adult female salamanders in the genus Desmognathus do not grow after attaining sexual maturity, whereas male Desmognathus appear to exhibit indeterminate growth (Organ 1961, Fitzpatrick 1973, Tilley 1973, 1980). This suggests that either growth in females is determinate, or the reproductive activities of the female restrict increases in adult body size. Both of these observations are consistent with the data,

but have different implications for life history allocation in females.

Lipids appear to have several functions in the reproductive ecology of Desmognathus. In D. ochrophaeus, carcass lipids are used as energy reserves for physiological maintenance during brumation (i.e., overwintering) and egg brooding (Fitzpatrick 1973). Lipids stored in the abdomen (the corpora adiposa) seem to be important in follicular development (Fitzpatrick 1973). In other plethodontid species, however, lipid deposits in the tail appear to have this function (Maiorana 1976).

The purposes of this study were to: 1) investigate growth of females in a natural population of Desmognathus fuscus; 2) examine the relationship between food availability and growth in brooding female D. fuscus; 3) quantify growth in female D. fuscus during the interval between nesting seasons; and 4) examine lipid cycling in the lean tissues, caudal fat deposits, and ova and corpora adiposa of female D. fuscus over an annual cycle.

Materials and Methods

Growth of female Desmognathus fuscus in a natural population was studied in three first-order tributaries of Reed Creek in the Chilhowee Mountain Wildlife Management Area, Tennessee (U.S.G.S. Kinzel Springs and Wear Cove Quadrangles). Animals used in laboratory investigations of growth and for lipid analyses were collected from a

1.5 km section of Reed Creek near its confluence with the tributaries used in the mark-recapture study.

Growth in field populations was monitored in 1984 and 1985 by searching the banks and bed of the streams at least every ten days from May through September. All females captured were measured with a metal ruler to determine snout-vent length to the nearest millimeter and weighed to the nearest gram with a Pesola scale. After photographing the dorsal pattern for future identification (modified from Tilley 1977 and Forester 1977), the animal was released.

Growth in brooding female D. fuscus was examined in the laboratory. I collected brooding females whose eggs were in early developmental stages from Reed Creek in August 1984. The females and their clutches were housed in the laboratory in individual plastic containers with dimensions 9.5 x 19 x 9.5 cm and 1 - 2 cm moist soil as a substrate. Damp paper toweling was provided as a cover object. Females were assigned randomly to treatment groups. One group of females (n = 6) was fed apterous Drosophila melanogaster and small oligochaetes ad libitum every three days; females in the second group (n = 7) were not fed for the duration of egg brooding. The experiment was terminated when all clutches had hatched (approximately 30 days after collection). The snout-vent length and weight of each female was measured at the beginning and end of the experiment and clutch size was monitored throughout.

Growth of non-brooding D. fuscus females over a longer time period was followed in the laboratory using two levels of food. To

increase the probability that all females used in the growth study were at similar stages of vitellogenesis, I collected 18 egg clutches and attending female D. fuscus during July and August of 1985, held them in the laboratory, and began growth observations on the female as soon as the clutch had hatched. At that time, the female was weighed and measured, and then transferred to an individual plastic box with 1 to 2 cm moist soil as a substrate and damp sphagnum moss for cover. Females were assigned randomly to high and low feeding groups; each group initially consisted to 9 animals. Salamanders in the high food group initially received 3 to 4 small crickets three times per week; those in the low food group received 3 to 4 crickets once a week. All females were reweighed and remeasured once each month from September 1985 to 7 April 1986, when the experiment was terminated.

Females were collected for lipid analyses once each month from January to December 1985, except during July, when most females in the natural population were brooding egg clutches. All salamanders collected were transported to the laboratory on ice. At the laboratory, they were measured and weighed, and their reproductive condition noted, then frozen and stored at -80 C until further analysis.

In each female, reproductive structures (consisting of follicles and the associated corpora adiposa), caudal fat bodies, and the remainder of the carcass were analyzed separately for lipid content. Lipids were extracted from each of these tissues using the hot chloroform-methanol procedure of Fitzpatrick (1973). Raw data on

lipid mass were converted to percentages for comparison between compartments; the percentages were normalized using the inverse sine transformation prior to calculation of monthly sample means (Sokal and Rohlf 1981). In both the growth and the lipid studies, nonparametric statistical tests were used for comparison of group differences.

Results and Discussion

Growth in Natural and Laboratory Populations

I captured and successfully marked 229 female Desmognathus fuscus in 1984 and 200 females in 1985. Eleven of the animals photographed in 1984 were recaptured in 1985. Most of these females either did not grow from 1984 to 1985, or else grew only 1 or 2 mm (Table 23). Several individuals did increase their snout-vent length by as much as 4 mm, however. In addition, size records for females measured more than once during egg brooding indicated that individual females usually do not increase in SVL during the 50 day brooding period (Table 24).

In contrast with these data, the females maintained in the laboratory grew by appreciable amounts if given food during egg brooding, or high levels of food during the non-brooding interval. The snout-vent length of females that were fed while brooding increased relative to females that were not fed (Table 25; Mann-Whitney U test, $P < 0.001$). Females that were fed during brooding tended to lose less weight than those that were not fed, but the difference

TABLE 23. Size and growth in millimeters of female Desmognathus fuscus in natural populations from 1984 to 1985. The reproductive condition of the female (J: juvenile; NG: non-gravid adult; G: gravid adult; B: brooding egg clutch) is shown in parentheses.

1984 SVL	1985 SVL	Growth
31 (J)	32 (B)	1
39 (B)	41 (G)	2
40 (G)	40 (G)	0
41 (G)	41 (G)	0
41 (G)	41 (G)	0
41 (G)	45 (G)	4
42 (G)	42 (G)	0
42 (G)	45 (G)	3
42 (G)	46 (B)	4
47 (G)	47 (G)	0
47 (G)	34 (NG)	measurement error

TABLE 24. Growth in female Desmognathus fuscus in natural populations during egg brooding. Entries are numbers in each category.

Year	Grew	Did Not Grow
1983	11	20
1984	4	14
1985	3	13

TABLE 25. Weight (g), SVL (cm), and hatching success (percent) of female Desmognathus fuscus at two feeding levels during egg brooding.

Treatment	Initial SVL	Growth	Initial wt	Wt gain	Success
Food	4.0	0.1	1.1	0.2	100.0
	4.1	0.0	1.3	-0.1	86.7
	4.2	0.1	1.7	0.0	90.0
	4.3	0.1	na	na	82.4
	4.8	0.1	2.0	0.0	82.5
	5.2	0.1	2.3	0.2	100.0
No food	4.1	0.0	1.2	0.0	84.6
	4.2	0.0	1.2	0.0	100.0
	4.2	0.1	1.8	-0.4	95.2
	4.6	0.0	1.7	0.0	95.2
	4.6	0.1	2.4	-0.4	100.0
	4.9	0.0	na	na	92.3
	5.1	0.0	2.5	-0.2	66.7

(na) initial weight data not available

between groups was not statistically significant (Table 25; Mann-Whitney U test, $0.10 < P < 0.05$). This suggests that females may increase body length at the expense of weight loss during brooding. In addition, there was no significant difference in hatching success between the clutches of females in the two treatment groups (Table 25; Mann-Whitney U test). This is evidence that females deprived of food during brooding are not more prone to oophagy than females with a food source.

There were three main results in the study of female D. fuscus at two feeding levels over an 8 month period. First, I observed qualitative differences in feeding behavior between groups. Initially, salamanders in both treatments ate all the crickets added to the containers soon after addition of the prey. By late autumn, however, most females in the high food treatment consumed only 5 to 8 crickets per week. Consequently, the ration of the females in the high food group was reduced to 3 to 4 crickets twice per week from December until the end of the experiment.

Growth in females between autumn and spring appeared to depend on food levels (Table 26). Females in the high feeding treatment tended to grow more than females in the low feeding treatment, but this difference was not statistically significant (Mann-Whitney U test). Not surprisingly, within the high treatment, small females tended to grow more than larger females. Females gained significantly more weight when given high levels of food (Mann-Whitney U test, $P < 0.01$).

The follicles of females in the high food treatment also seemed

TABLE 26. Weight (g) and SVL (cm) of female Desmognathus fuscus at two feeding levels from September 1985 to April 1986.

Treatment	Initial SVL	Growth	Initial wt	Wt gain
High food	3.6	0.7	0.8	1.1
	4.2	0.6	1.2	1.4
	4.5	0.7	1.9	1.4*
	4.6	0.7	1.7	2.3*
	4.7	0.4	2.1	1.2
	5.1	0.1	2.6	0.2
	5.3	0.2	3.1	0.8*
Low food	4.2	0.3	1.3	0.1
	4.2	0.4	1.8	0.4
	4.4	0.1	1.4	0.1
	4.7	0.2	2.2	0.2
	5.0	0.1	2.4	0.4
	5.0	0.3	2.5	0.4

* follicles appeared to be fully developed in April

to develop more rapidly than the follicles of females given less food: throughout the experiment, the ova, viewed through the body wall, of females in the high food group appeared larger than the follicles of females in the low food group. Furthermore, three of the seven females in the high treatment seemed to have fully developed ova by the beginning of April. The ova of the remaining four females in the high treatment, although not fully developed, were larger than those of the females in the low food treatment. In comparison, females in natural populations do not appear to be fully gravid until June. Thus, the difference in weight gain between animals in the two treatments may have been due to increased body mass (fat and lean tissue) and increased mass of reproductive tissue. If enlarged follicles take up space in the coelomic cavity that otherwise would be occupied by materials passing through the salamander's gut, then advanced ovarian development could have been a factor in causing the decrease in food consumption observed in females at the high food level.

These observations of growth and reproduction suggest that while the availability of invertebrate prey may not be a proximate factor determining the species composition of Desmognathus assemblages (Hairston 1980), restriction of adult body size, and therefore of fecundity, via food intake ultimately may have an effect on salamander abundance.

Lipid Cycling

Because of snow cover and environmental temperatures near or below freezing, I was unable to find more than two female D. fuscus on any one day in January 1985. The field collections, therefore, do not include that month. Salamanders collected in March did not contain visible deposits of tail fat, hence caudal lipids were recorded as zero for March. The sample collected in September 1985 was lost in processing.

The total amount and location of body lipids in natural populations of female D. fuscus varied seasonally. These data are summarized in Table 27 and in Figures 9 - 11. Total lipids were lowest in the late winter and early spring, reached a maximum in June, then decreased through the autumn months (Figure 9). The distribution of lipids in lean carcass, reproductive structures, and caudal fat bodies through time is shown in Figure 10. On the average, lipids comprised less than 15 percent of carcass mass, with minima below 5 percent in some individuals in February and March. There were statistically significant differences in the percentage of carcass tissue composed of lipids between the months of April and May, August and October, and December and February (two-sided Wilcoxon rank-sum test, $P < 0.05$; Figure 10). The lipid fraction of follicles and corpora adiposa showed considerable variation between individuals, but in most females, it tended to be approximately 50 percent of the total mass of reproductive structures throughout the year. Differences from month to month were not statistically significant over May, June, and

TABLE 27. Dry weight of female Desmognathus fuscus by compartments. Values shown are monthly means $\pm$ 1 standard deviation (g).

Month	N	Carcass		Follicles		Caudal fat	
		Lipid-free	Lipids	Lipid-free	Lipids	Lipid-free	Lipids
Feb	4	.2688 $\pm$.0658	.0151 $\pm$.0110	.0018 $\pm$.0017	.0044 $\pm$.0043	.0013 $\pm$.0009	.0005 $\pm$.0002
Mar	5	.2809 $\pm$.0928	.0201 $\pm$.0205	.0063 $\pm$.0112	.0053 $\pm$.0098	—	—
Apr	6	.3861 $\pm$.1508	.0492 $\pm$.0314	.0283 $\pm$.0283	.0227 $\pm$.0209	.0025 $\pm$.0025	.0024 $\pm$.0016
May	5	.3993 $\pm$.0952	.0622 $\pm$.0261	.0671 $\pm$.0294	.0425 $\pm$.0156	.0021 $\pm$.0012	.0020 $\pm$.0012
June	5	.4133 $\pm$.0434	.0579 $\pm$.0076	.0683 $\pm$.0404	.0452 $\pm$.0206	.0048 $\pm$.0011	.0030 $\pm$.0012
Aug	3	.3154 $\pm$.1084	.0563 $\pm$.0461	.0069 $\pm$.0070	.0164 $\pm$.0070	.0037 $\pm$.0008	.0029 $\pm$.0024
Oct	5	.3967 $\pm$.1465	.0324 $\pm$.0148	.0073 $\pm$.0081	.0078 $\pm$.0090	.0036 $\pm$.0016	.0020 $\pm$.0009
Nov	4	.3057 $\pm$.0872	.0316 $\pm$.0231	.0056 $\pm$.0052	.0057 $\pm$.0056	.0036 $\pm$.0016	.0014 $\pm$.0006
Dec	4	.3509 $\pm$.1312	.0682 $\pm$.0245	.0200 $\pm$.0127	.0213 $\pm$.0156	.0032 $\pm$.0013	.0045 $\pm$.0051

— Caudal fat not dissected in March

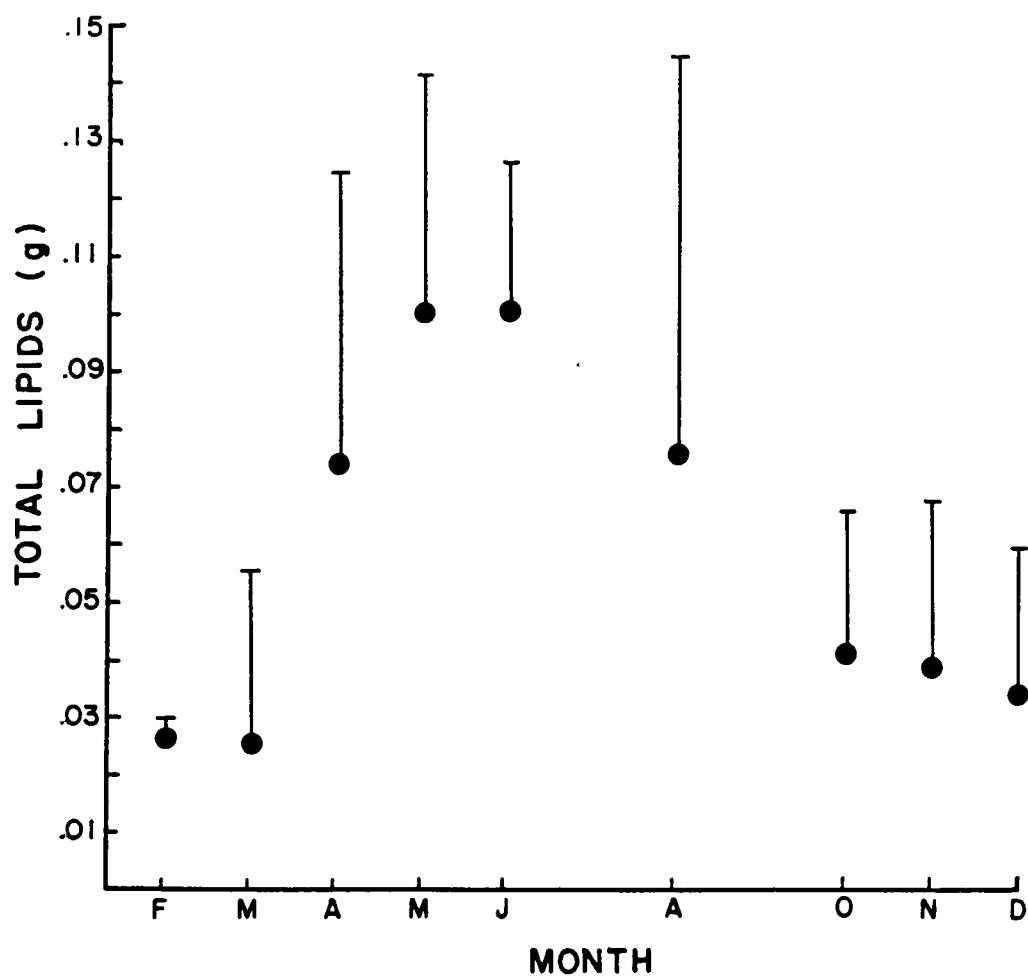


FIGURE 9. Total lipids in female *Desmognathus fuscus*. Symbols represent monthly sample means, with standard deviation indicated by the bar.

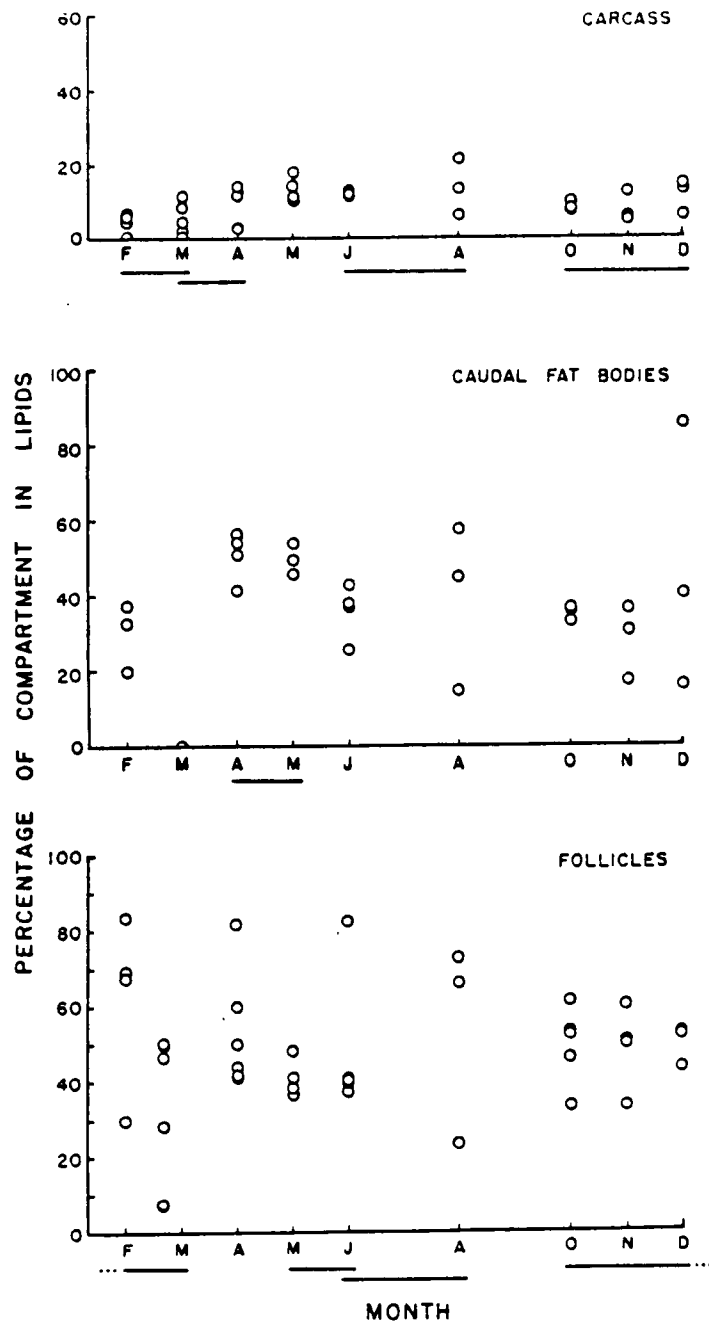


FIGURE 10. Percentage of carcass, caudal fat bodies, and reproductive structures composed of extractable lipids. Each symbol represents an observation on a single animal. Months joined by a bar did not differ significantly in lipid fraction for that compartment (Wilcoxon rank-sum test, $P < 0.05$).

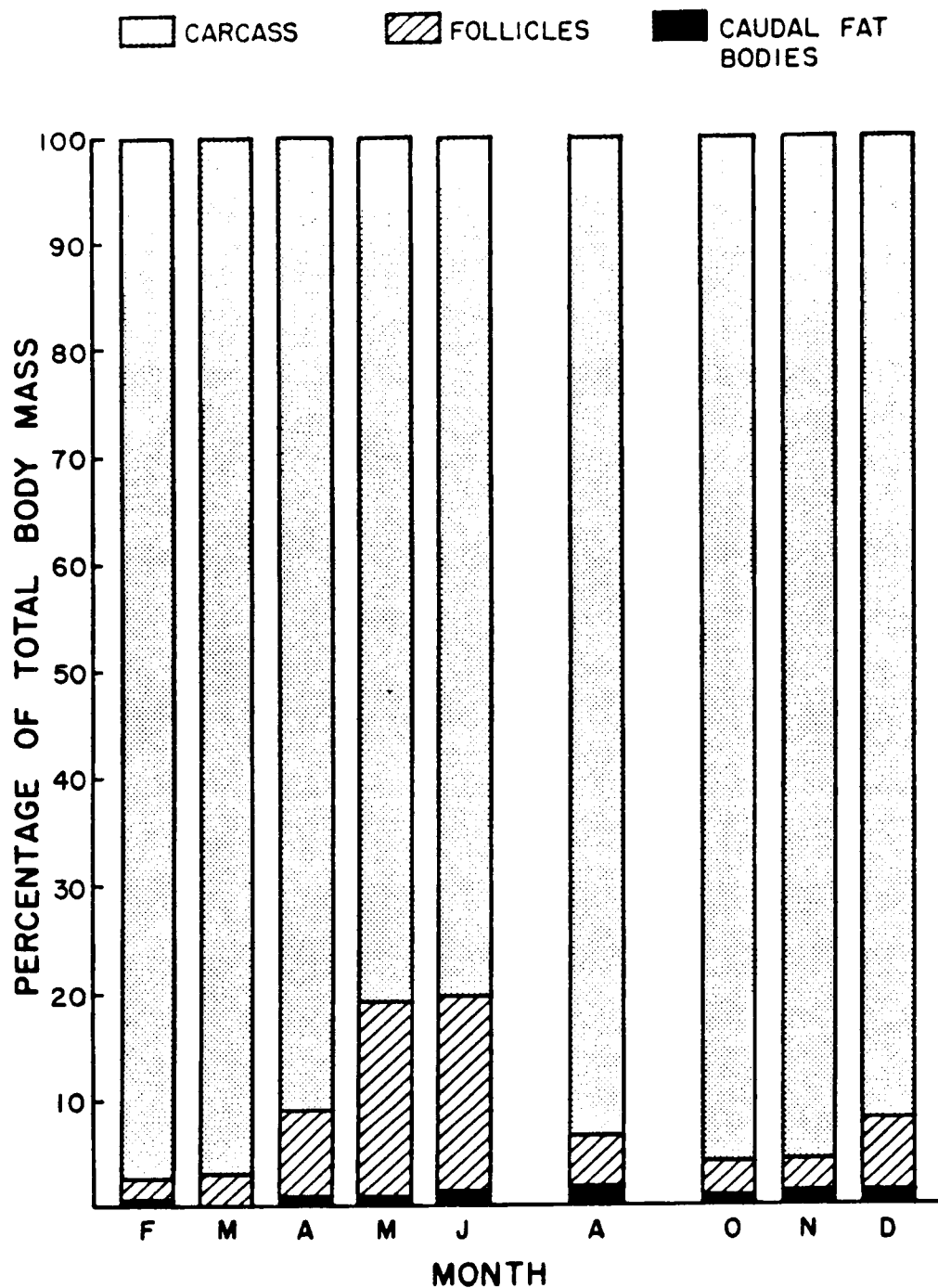


FIGURE 11. Mean percentage of total body mass in carcass, caudal fat bodies, and reproductive structures (follicles and corpora adiposa).

August; and October, November, December, and February (two-sided Wilcoxon rank-sum test, $P < 0.05$; Figure 10). The percentage of lipids in caudal fat bodies fluctuated with a pattern similar to that of total lipid mass, however, the maximum lipid fraction in caudal fat bodies occurred in April. All monthly differences except for April-May were statistically significant (two-sided Wilcoxon rank-sum test, $P < 0.05$). Lean carcass tissue constituted most of dry body mass regardless of month (Figure 11); caudal fat bodies comprised no more than 1.8 percent of total dry body mass during any month. Both relative and absolute amounts of reproductive tissue increased throughout the spring and reached a maximum of approximately 18 percent of dry body mass in May and June.

These patterns of lipid deposition suggest that the peak in total lipids that occurred in late spring probably was attributable to increase in the mass of ova and fat bodies associated with follicles. Decreases in total lipids in spring and autumn may have been due to mobilization of carcass and tail lipids for energy during brumation and egg brooding, respectively. Because the lipid fraction in reproductive structures remained relatively high throughout the year, lipids associated with follicles did not appear to be used for seasonal physiological maintenance. In addition, although lipids composed only a small fraction of carcass tissue, the mass of carcass relative to the mass of reproductive structures and tail fat was so large that approximately 55 to 90 percent of total lipids occurred in the carcass. These results are in general agreement with those of

Fitzpatrick (1973) on D. ochrophaeus, and Kleeberger (1984) on D. monticola.

CHAPTER 7

A MATHEMATICAL MODEL OF DESMOGNATHUS REPRODUCTION:

I. FORMULATION AND ANALYSIS

Introduction

Understanding the life history attributes of organisms -- that is, processes involving reproduction and mortality -- is central to understanding ecology at the population level. Phenomena that influence the timing of life history events may be especially critical to determining the fitness of an individual. Qualitative empirical tests of theories that seek to explain total reproductive effort, age at first reproduction, frequency of reproduction, and number and quality of offspring have been conducted using a variety of organisms (Stearns 1976, 1977). In turn, observations of natural history have stimulated modification of this theory.

For several reasons, salamanders in the genus Desmognathus, the dusky salamanders, are well suited to the study of life histories. They are large and fairly conspicuous, their reproductive characteristics are quantifiable, and they exemplify some of the potential compromises implicit in the evolution of life history attributes. These compromises include allocation of energy to growth versus allocation to reproduction; the timing of reproduction; and investment in maternal care.

Desmognathus are are long lived, delay sexual maturity, usually reproduce on an annual cycle, and care for their offspring. Specifically, female dusky salamanders become sexually mature at 4 to 5 years of age and appear to reproduce annually for 3 to 4 years thereafter (Tilley 1968). Fecundity is positively correlated with body size (Tilley 1968, Chapter 3). During the summer, each female lays a clutch of eggs beneath moss on streambanks and then broods her eggs until they hatch 45 to 60 days later (Organ 1961, Chapter 3). During this period, the females remain concealed and do not appear to leave the nest to forage for food. Opportunistic feeding on organisms that intrude into the nest does occur (Montague and Poinski 1978, Krzysik 1980b). Females provide maternal care in the form of removal of eggs infected with pathogenic fungi and defense against potential egg predators (Forester 1978, 1979, Chapter 5).

This study is a theoretical exploration of the timing and patterns of resource allocation in the life history of female salamanders. The purpose of the analysis is to determine a scheme for energy allocation that maximizes the reproductive output of a female salamander. The problem -- optimization under a set of constraints based on organismal biology -- suggests application of techniques of control theory.

Optimal control theory is a mathematical approach to problem solving that allows balancing of costs and benefits subject to a set of constraints determined by the nature of the system under study. The first application of control theory to the study of reproductive

allocation was by Cohen (1971). His model for an annual plant included state variables for vegetative growth and for reproduction, with investment in either compartment determined by a control variable. Seed set at the end of a fixed growing season was the quantity to be maximized. Other investigators subsequently have modified this basic formulation in a number of ways, such as including loss terms to account for mortality or a separate state variable for storage. These models generally yield the following predictions.

1. Reproduction is maximized by allocation schemes that incorporate investment in reproduction at the end of the growing season (Cohen 1971, Paltridge and Denholm 1974, Macevicz and Oster 1978, Oster and Wilson 1978, King and Roughgarden 1982a, 1982b, Schaffer et al. 1982, Chiarello and Roughgarden 1984).

2. If the state equations are linear in the control variables, then reproduction is maximized by initially allocating all resources to non-reproductive biomass and later switching to reproduction. This switch in allocation occurs only once if the parameters of the model are constant (Cohen 1971, Paltridge and Denholm 1974, Macevicz and Oster 1978, Oster and Wilson 1978, Schaffer et al. 1982, Chiarello and Roughgarden 1984). Multiple switches may occur if model parameters vary over time (King and Roughgarden 1982a). Graded allocation, in which the organism allocates to reproductive and non-reproductive biomass simultaneously, can occur if the model is stochastic (Cohen 1971), if the state equations are non-linear in the controls (King and Roughgarden 1982b), or if allocation is terminated by organismal

senescence rather than by the end of the growing season (Paltridge et al. 1984).

3. Allocation to a separate storage compartment may maximize future reproduction if the effects of seasonality on growth rate and the efficiency of reproduction are partially decoupled and if storage products can be mobilized to increase reproductive biomass (Schaffer et al. 1982, Chiarello and Roughgarden 1984).

4. The inclusion of mortality in model formulation may decrease the size of reproductive and non-reproductive biomass, but does not alter the qualitative nature of the optimal allocation scheme predicted by models that assume no mortality (Kozlowski and Wiegert 1986).

With few exceptions (e.g., Ziolkowski and Kozlowski 1983, Kozlowski and Wiegert 1986, Schaffer 1983, Werner and Gilliam 1984) these results have been obtained for models based on plants or on eusocial insects. The insect and plant models, however, do not include features, such as maternal care, that may be important variables in the life history of vertebrates. The insights gained in a study of a vertebrate may be useful in elucidating trade-offs in the general theory of life history evolution. Werner and Gilliam (1984) have initiated comparative studies of vertebrates within higher taxonomic categories. In contrast, this study focuses on a single species and examines reproductive allocation in female salamanders in detail.

Model Assumptions

The characteristics of dusky salamander reproductive ecology discussed above can be stated as a set of assumptions from which I formulated an appropriate model of salamander reproduction. I assumed that:

A1. The year can be divided into two intervals, which I define as an initial non-brooding interval, and a brooding interval, which commences upon egg-laying and ends when the eggs hatch.

A2. A female salamander can be described as three state variables: ova and reproductive structures, fat, and all other somatic tissues (referred to as "body" for convenience). These are measured in terms of calories.

A3. The average mortality rate of females in the population is constant.

During the non-brooding period, I made the additional assumptions that:

A4. Food intake is constant and always exceeds maintenance costs.

A5. Increases in any of the state variables depends on food intake and body size.

A6. The food consumed by a female can be allocated to any state variable.

A7. Stored fat can be mobilized and used for reproduction or for growth. For simplicity, I assumed perfect transfer efficiency.

During the brooding interval I assumed that:

A8. Food intake equals maintenance costs. Because female dusky salamanders do not forage while attending eggs (Krzysik 1980b), the food intake of some individuals may be barely sufficient or even inadequate for physiological maintenance. On the other hand, some females grow while brooding (Chapter 6), which indicates that they opportunistically obtain food in excess of maintenance needs. Thus, the assumption that food intake equals maintenance probably is a reasonable approximation for an average female.

A9. Total egg mortality occurs in the absence of maternal care.

A10. Reductions in egg mortality are proportional to female investment in maternal care.

A11. Energy for maternal care is derived from the body (e.g., from glycogen) and has some maximum rate of allocation.

The model assumptions can be depicted in a box-and-arrow diagram (Figure 12) and the variables and parameters used in the model are defined in Table 28. In particular, the variables u_i , $i = 1, 2, 3$, v_j , $j = 1, 2$, and z_1 are control variables. Their values determine the compartment to which the female allocates food resources and fat reserves. Constraints on the control variables are 1) $\sum_{i=1}^3 u_i = 1$, i.e., all food not used in physiological maintenance must be allocated to some state variable; 2) $\sum_{j=1}^2 v_j \leq 1$, i.e., some proportion of fat may be allocated to growth or to reproduction; 3) $\phi_0 - \kappa z_1 B \geq 0$, i.e., the reduction in egg mortality due to maternal care cannot exceed the mortality that would occur in the absence of maternal care.

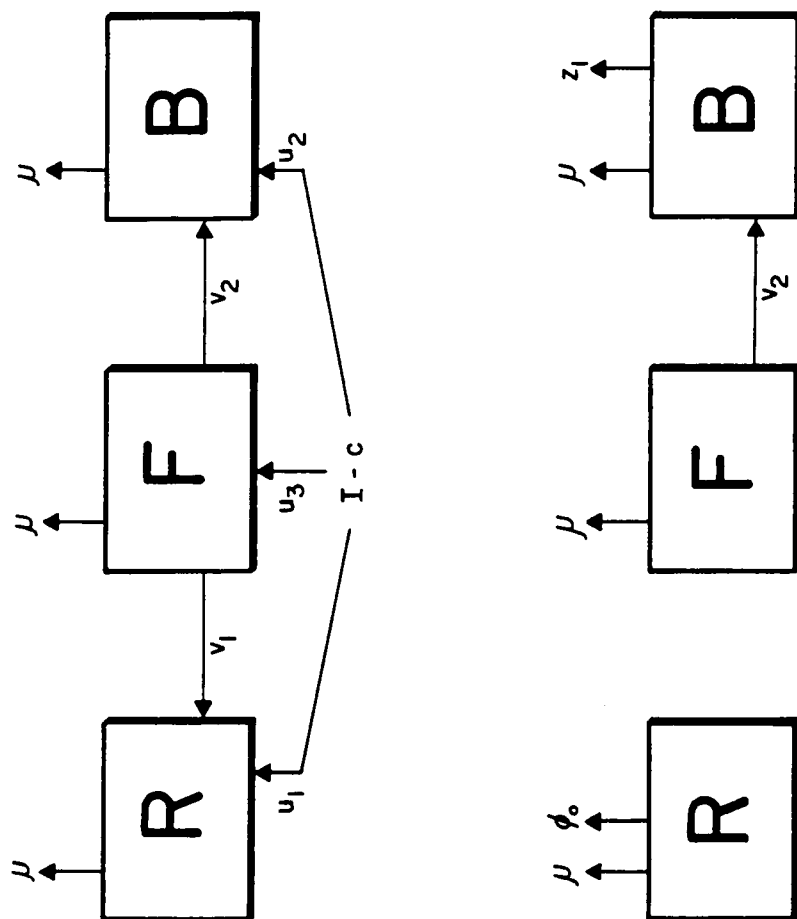


FIGURE 12. Schematic representation of reproductive allocation in female *Desmognathus fuscus*. Variables and parameters are described in Table 28 and in the text. Top: reproductive allocation during the non-brooding period; bottom: reproductive allocation during egg brooding.

TABLE 28. Definitions of the variables and parameters used in the control model.

Variable		Units	Interpretation
Temporal variables	T_0	time	beginning of annual reproductive season
	T_1	time	the time at which eggs are laid
	T_2	time	end of the annual reproductive season
State variables	R	cal	ova and reproductive structures
	B	cal	non-lipid somatic tissues
	F	cal	fat
Parameters	I	cal/t	rate of food intake
	c	cal/t	rate of maintenance requirements
	β	1/cal	growth per cal
	μ	1/t	predation mortality per time
	δ	1/cal	fat conversion per cal
	ϕ_0	1/t	egg mortality in the absence of maternal care
	κ	1/cal t	survivorship per investment in maternal care
	u_1	---	percent of net food intake converted to ova
Control variables	u_2	---	percent of net food intake converted to body
	u_3	---	percent of net food intake converted to fat
	v_1	---	percent of fat converted to ova
	v_2	---	percent of fat converted to body
	z_1	---	percent allocation to maternal care

Note that if $F = 0$ at some time, then the system is reduced to two state variables so necessarily $v_1 = v_2 = u_3 = 0$.

The model assumptions yield the following system of equations for the non-brooding interval (T_0, T_1) :

$$\frac{dR}{dT} = u_1(I - c)\beta B + v_1\delta f - \mu R$$

$$\frac{dB}{dT} = u_2(I - c)\beta B + v_2\delta F - \mu B \quad (1.1)$$

$$\frac{dF}{dT} = u_3(I - c)\beta B - (v_1 + v_2)\delta F - \mu F ,$$

and equations (2.1) for the brooding interval (T_1, T_2) :

$$\frac{dR}{dT} = -\mu R - (\phi_0 - \kappa z_1 B)R$$

$$\frac{dB}{dT} = v_2\delta F - (\mu + z_1)B \quad (2.1)$$

$$\frac{dF}{dT} = -v_2\delta F - \mu F .$$

The objective of the analysis is to find values for the control variables that result in maximization of the female's total reproductive output at the end of the season by using optimal control

theory methods (Leitmann 1981). Namely, the objective is to find controls that achieve

$$\begin{aligned}
 J = \max R &= \min \int_{T_0}^{T_1} -dR \\
 &= - \int_{T_0}^{T_1} [u_1(I - c)\beta B + v_1\delta F - \mu R]dT
 \end{aligned}
 \tag{1.2}$$

for the non-brooding interval and

$$\begin{aligned}
 J = \max R &= R(T_1) - \min \int_{T_1}^{T_2} dR \\
 &= R(T_1) - \min \int_{T_1}^{T_2} [-\mu R - (\phi_0 - \kappa z_1 B)R]dT
 \end{aligned}
 \tag{2.2}$$

for the brooding interval (Leitmann 1981). For ease of analysis without loss of generality, these models can be non-dimensionalized by rescaling time (Table 29). The equivalent non-dimensionalized equations are

$$\frac{dr}{dt} = u_1 b + v_1 \delta' f - \mu' r$$

TABLE 29. Conversions used to non-dimensionalize the model.

$t = T(I - c)\beta$	
$r = \frac{\kappa R}{(I - c)\beta}$	$\mu' = \frac{\mu}{I - c}$
$b = \frac{\kappa B}{(I - c)\beta}$	$\phi'_0 = \frac{\phi_0}{I - c}$
$f = \frac{\kappa F}{(I - c)\beta}$	$\delta' = \frac{\delta}{I - c}$

$$\frac{db}{dt} = u_2 b + v_2 \delta' f - \mu' b \quad (1.1')$$

$$\frac{df}{dt} = u_3 b - (v_1 + v_2) \delta' f - \mu' f$$

with objective functional

$$J = \max r = - \int_{t_0}^{t_1} [u_1 b + v_1 \delta' f - \mu' r] dt \quad (1.2')$$

for the non-brooding interval, and

$$\frac{dr}{dt} = -\mu' r - (\phi_0' - z_1 b) r$$

$$\frac{db}{dt} = v_2 \delta' f - (\mu' + z_1) b \quad (2.1')$$

$$\frac{df}{dt} = -v_2 \delta' f - \mu' f$$

and

$$J = \max r = r(t_1) - \min \int_{t_1}^{t_2} [\mu' r + (\phi_0 - z_1 b) r] dt \quad (2.2')$$

for the brooding interval.

The models corresponding to the two periods are analyzed separately; note, however, that the terminal values for the state variables at the end of the non-brooding interval become the initial values for the state variables at the beginning of brooding. Thus, $r(t_1)$ represents initial clutch size and the quantity to be minimized in (2.2') is the female's loss in investment in reproduction.

The Non-Brooding Model

Formulation

Finding the controls to satisfy (1.2) is equivalent to maximizing the Hamiltonian

$$\begin{aligned}
 H = & (-\lambda_0 + \lambda_1)[u_1 b + v_1 \delta' f - \mu' r] \\
 & + \lambda_2[u_2 b + v_2 \delta' f - \mu' b] + \lambda_3[u_3 b - (v_1 + v_2)\delta' f - \mu' f] \quad .
 \end{aligned}
 \tag{1.3}$$

The equations for the adjoint variables associated with (1.3) are

$$\begin{aligned}
 \dot{\lambda}_0 &= \frac{-\partial H}{\partial J} = 0 \\
 \dot{\lambda}_1 &= \frac{-\partial H}{\partial r} = -\{(-\lambda_0 + \lambda_1)(-\mu')\} \\
 \dot{\lambda}_2 &= \frac{-\partial H}{\partial b} = -\{(-\lambda_0 + \lambda_1)u_1 + \lambda_2 u_2 - \lambda_3 u_3\} \\
 \dot{\lambda}_3 &= \frac{-\partial H}{\partial f} = -\{(-\lambda_0 + \lambda_1)v_1 \delta' + \lambda_2 v_2 \delta' - \lambda_3[(v_1 + v_2)\delta' - \mu']\}
 \end{aligned}
 \tag{1.4}$$

The maximum principle requires that $\lambda_0 = \text{constant} < 0$. For

convenience, choose $\lambda_0 = -1$. The terminal transversality condition, which is a boundary condition on the adjoint variables, requires that $\lambda_i(t_1) = 0$, $i = 1, 2, 3$. Using these two conditions to solve for λ_1 , the Hamiltonian and adjoint equations can be rewritten as

$$\begin{aligned}
 H = & \exp[\mu'(t - t_1)][u_1 b + v_1 \delta' f - \mu' r] + \lambda_2 [u_2 b + v_2 \delta' f - \mu' b] \\
 & + \lambda_3 [u_3 b - (v_1 + v_2) \delta' f - \mu' f] ,
 \end{aligned}
 \tag{1.5}$$

and

$$\begin{aligned}
 \lambda_0 &= -1 \\
 \lambda_1 &= \exp[\mu'(t - t_1)] \\
 \dot{\lambda}_2 &= -\exp[\mu'(t - t_1)]u_1 - \lambda_2 u_2 + \mu' \lambda_2 - \lambda_3 u_3 \\
 \dot{\lambda}_3 &= -\delta' \exp[\mu'(t - t_1)]v_1 - \delta' \lambda_2 v_2 + \delta' \lambda_3 (v_1 + v_2) + \mu' \lambda_3 .
 \end{aligned}
 \tag{1.6}$$

For convenience, define $\sigma_{u_i} = \partial H_i / \partial u_i$, i.e., the coefficient of u_i . These are termed switching functions (Leitmann 1981). From (1.5), the switching functions for the non-brooding model are

$$\begin{aligned}
 \sigma_{u_1} &= \exp[\mu'(t - t_1)]b \\
 \sigma_{u_2} &= \lambda_2 b \\
 \sigma_{u_3} &= \lambda_3 b
 \end{aligned}
 \tag{1.7}$$

$$\sigma_{u_1} = \exp[\mu'(t - t_1)]\delta'f - \lambda_3\delta'f$$

$$\sigma_{v_2} = \lambda_2\delta'f - \lambda_3\delta'f.$$

Since the Hamiltonian (1.5) is linear in the control variables, if the switching functions are zero only at isolated points, then the Hamiltonian is maximized by setting the u_i with the largest non-zero switching function equal to one and setting the other u_i 's equal to zero (Leitmann 1981). An analagous procedure is used for the v_j 's. If a switching function σ_{u_i} or σ_{v_j} equals zero on an interval, then singular control in which the u_i or v_j , respectively, take intermediate values, may occur.

Results

Analysis of (1.1') with methods of optimal control yield the following propositions:

Proposition 1.1: Singular control does not occur in (t_0, t_1) .

Proposition 1.2: So long as $F > 0$, the combinations of controls possible on (t_0, t_1) are

$$a) \quad u_1 = 1, u_2 = u_3 = 0,$$

$$v_1 = 1, v_2 = 0;$$

$$b) \quad u_2 = 1, u_1 = u_3 = 0,$$

$$v_2 = 1, v_1 = 0;$$

$$c) \quad u_3 = 1, u_1 = u_2 = 0,$$

$$v_1 = 1, v_2 = 0.$$

Proposition 1.3: At the terminal time, t_1 , $u_1 = 1$ and $v_1 = 1$.

Proposition 1.4: None of the following switches in control variables can occur as part of the optimal control over (t_0, t_1) :

- a) from $u_3 = 1$ to $u_2 = 1$;
- b) from $u_3 = 1$ to $u_1 = 1$;
- c) from $u_2 = 1$ to $u_1 = 1$.

The proofs of Propositions 1.1 - 1.4 are given in Appendix 1.

Propositions 1.2 - 1.4 imply firstly that $u_3 = 1$ cannot occur, so $u_3 = 0$ throughout (t_0, t_1) . Secondly, if $u_1 = 1$ at any time then $u_1 = 1$ thereafter until t_1 . It follows that the control schemes that maximize r must take one of the following two forms:

(1.i) Allocate all food and convert all fat reserves to reproduction for all t in (t_0, t_1) , i.e.,

$$\begin{aligned}u_1 &= 1, u_2 = u_3 = 0, \\v_1 &= 1, v_2 = 0.\end{aligned}$$

(1.ii) Allocate all food and convert all fat to the body compartment, that is, invest in growth, up to some time t_r . For $t > t_r$, allocate food and fat to reproduction, i.e.,

$$\begin{aligned}u_2 &= 1, u_1 = u_3 = 0, \\v_2 &= 1, v_1 = 0\end{aligned}\quad \text{for } t \in (t_0, t_r)$$

and

$$\begin{aligned}u_1 &= 1, u_2 = u_3 = 0, \\v_1 &= 1, v_2 = 0\end{aligned}\quad \text{for } t \in (t_r, t_1).$$

The switching time, t_r , can be found by examining the adjoint equation for λ_2 and the switching functions σ_{u_1} and σ_{u_2} . The adjoint equation for λ_2 on $t > t_r$ is

$$\dot{\lambda}_2 = -\exp[\mu'(t - t_1)] + \mu'\lambda_3.$$

Integrating this back from t_1 yields

$$\lambda_2(t) = -\exp[\mu'(t - t_1)](t - t_1). \quad (1.8)$$

At t_r , the time of the switch in u , $\sigma_{u_1}(t_r) = \sigma_{u_2}(t_r)$ so that substituting the expressions from (1.7) and cancelling common factors yields

$$\lambda_2(t_r) = \exp[\mu'(t_r - t_1)].$$

Combining this expression with (1.8),

$$\exp[\mu'(t_r - t_1)] = -\exp[\mu'(t_r - t_1)](t_r - t_1),$$

or after simplification,

$$t_r = t_1 - 1.$$

In terms of unscaled time T , this result is

$$T_R = T_1 - \frac{1}{(I - c)\beta} .$$

Thus, the switching time depends on food intake and the efficiency with which the female metabolizes food resources. In particular, if her food intake is high, then the female should grow for a longer period of time prior to egg formation than she would if food levels were lower.

The Brooding Model

Formulation

As in the non-brooding model, the maximum principle requires that $\lambda_0 = \text{constant} < 0$, with the terminal transversality condition requiring $\lambda_i(t_2) = 0$, $i = 1, 2, 3$. Setting $\lambda_0 = -1$, the Hamiltonian for the nondimensionalized equations that correspond to the brooding period is

$$\begin{aligned} H = & (-1 - \lambda_1)[\mu'r + (\phi'_0 - z_1b)r] \\ & + \lambda_2[v_2\delta'f - \mu'b - z_1b] \\ & + \lambda_3[-v_2\delta'f - \mu'f] , \end{aligned} \tag{2.3}$$

with adjoint equations

$$\begin{aligned}
 \lambda_0 &= -1 \\
 \dot{\lambda}_2 &= (1 + \lambda_1)(\mu' + \phi_0 - z_1 b) \\
 \dot{\lambda}_3 &= -(1 + \lambda_1)z_1 r + \lambda_2(\mu' + z_1) \\
 \dot{\lambda}_4 &= -\lambda_2 v_2 \delta' + \lambda_3(v_2 \delta' + \mu')
 \end{aligned} \tag{2.4}$$

and switching functions

$$\sigma_{v_2} = \lambda_2 \delta' f - \lambda_3 \delta' f \tag{2.5}$$

$$\sigma_{z_1} = (1 + \lambda_1)br - \lambda_2 b$$

As in the non-brooding model, the controls that maximize (2.2) are found using these switching functions in conjunction with the terminal transversality condition and the state and adjoint equations.

Results

Analysis of the brooding season model with optimal control theory yields the following propositions:

Proposition 2.1: Both v_2 and z_1 take their maximum value at the terminal time.

Proposition 2.2: If the control variables switch to $v_2 = 1$ and $z_1 = 1$ at some time in (t_1, t_2) , then a switch in v_2 occurs before a switch in z_1 .

Proposition 2.3: The control variables v_2 and z_1 only take their extreme values, i.e., singular control does not occur.

The proofs of these propositions are given in Appendix 2.

The terminal transversality condition and Proposition (2.1) - (2.3) now can be used to deduce that there is only one control scheme that maximizes the reproductive output of a female salamander at the end of egg brooding:

(2.i) convert fat to the body compartment and provide maternal care throughout brooding, i.e.,

$$\begin{aligned} v_2 &= 1, \\ z_1 &= 1 \end{aligned} \quad t \in (t_1, t_2).$$

To prove (2.i): from Proposition 2.1, v_2 and z_1 take their maximum values at the terminal time. If no switch in either control variable occurs in (t_1, t_2) , then it follows that (2.i) is an optimal control scheme that maximizes reproductive output.

To show that any other control schemes are not optimal, suppose that switches occur in the control variables. Firstly, by Proposition 2.3, any switches must be from $v_2 = 0$ to $v_2 = 1$ and $z_1 = 0$ to $z_2 = 1$. Secondly, Proposition (2.1) and (2.2) imply that if only one switch occurs, then it must be in z_1 at a time t_2 within (t_1, t_2) , with $v_2 = 1$ on (t_1, t_2) .

Now at $t = t_z$, $\sigma_{z_1} = 0$ from eqn. (2.7). This is equivalent to $\lambda_2 = (1 + \lambda_1)$ at $t = t_z$. Consequently, the switching time t_z may be found by integrating the differential equations for λ_1 , λ_2 and r forward from t_z and substituting into the expression for λ_2 above. This yields

$$\lambda_2(t_1) \exp[\mu'(t - t_1)] = [1 + \lambda_1(t)] \exp[\mu' + \phi_0'](t_z - t_1)] r(t_1) \cdot \exp[-(\mu' + \phi_0')(t_z - t_1)] ,$$

or, after cancellation of common factors, $t_z = t_1$. Thus, the time at which the female should switch to defense is exactly the same as when the brooding interval starts, which yields (2.i).

Now suppose that two switches occur, with a switch in z_1 at t_z and in v_2 at $t_v < t_z$. From the switching function, this will happen if $\sigma_{v_2}(t_z) = 0$, which requires that $\lambda_2(t_v) = \lambda_3(t_v)$. But if this happens, equations (2.4) can be solved explicitly first for λ_2 and then for λ_3 on (t_v, t_z) . This yields the result that $\lambda_2(t) = \lambda_3(t)$ for all t in (t_v, t_z) and control is singular. This contradicts Proposition 2.3. Thus, switches in both v_2 and z_1 cannot occur so that (2.i) is the only scheme that maximizes the reproductive output of a female.

Discussion

The non-brooding model analyzed here leads to the intuitively reasonable prediction that a female salamander's reproductive output will be maximized by delaying reproduction under conditions of high

food intake. Given fixed levels of food and a constant rate of egg formation, the time necessary for a female to produce a clutch of eggs is fixed. The number of eggs in this clutch is determined by body size. Now suppose that food levels were high. If the female were to begin producing eggs immediately after hatching a clutch, then her eggs might be fully formed relatively early in the reproductive season and she could either lay her eggs during mid-winter, with probable low hatching success due to low environmental temperatures, or carry the fully-developed eggs internally for several months prior to egg-laying (i.e., probably through winter and spring). The latter conceivably might decrease her survivorship by making her more susceptible to predators by reducing her ability to escape. It also could reduce her ability to feed (by reducing her ability to fill her gut) and, therefore, could result in increased physiological stress. If, however, the female were to postpone reproduction and grow first, then she would be larger at the time of reproduction and thus could carry more eggs, and also, would carry the internal egg mass for a shorter period of time. Under conditions of low food intake, egg production would require more time, and the female should risk the possible increased vulnerability to predation and ensure that her eggs will be fully formed in time for summer laying by beginning egg formation earlier in the year.

The prediction that females should care for their egg clutches throughout brooding also is not unexpected. A female can increase the survivorship of her eggs through maternal care by reducing the

magnitude of the loss term $\phi_0 - \kappa z_1 B$ in equation (2.1). Note, though, that defense reduces the number of calories in the body compartment so that the model also predicts that a female may be less able to defend effectively later in the season.

Female salamanders in natural populations produce fat if enough food is available. The models analyzed here, however, predict that females should never allocate food resources to fat production. In the non-brooding model, fat eventually is converted to reproductive tissues. The female would not gain anything by producing fat first then later converting fat to reproduction. Thus, in some sense, fat is of secondary importance prior to egg-laying. In the brooding model, fat reserves can act to maintain the number of calories in a female's body compartment and thus to aid the female in reducing clutch mortality. Intuitively, females should allocate some resources to fat production in order to have this reserve available while brooding eggs. The current analysis does not provide for this coupling of reproductive intervals because the non-brooding and brooding models are treated separately. Consequently, although the prediction that females should not produce fat is a model artifact, it also is appropriate for this formulation.

Equations (1.1) and (2.1) do not include terms for the costs of switching allocation from compartment to compartment. It has been suggested to me that these costs, although probably small, may affect the timing of switches (Yosef Cohen, personal communication).

The formulation analyzed here is a short term model in that it does not account for future reproduction. It is conceivable that clutch defense could reduce the energy in the body compartment to the extent that the female would be less able to reproduce during the subsequent year. In this event, an allocation scheme that postponed defense or even predicted abandonment of the clutch might be optimal under some conditions. These considerations suggest the need for several extensions of this analysis: 1) coupling the brooding and non-brooding periods; 2) incorporating the costs of switching; 3) considering reproduction in future years. The present analysis remains useful, however, because the structure of the model and analytical methods are generalizable to a wide variety of vertebrates and therefore can be used to formulate testable quantitative predictions of timing and patterns of reproductive allocation.

CHAPTER 8

A MATHEMATICAL MODEL OF DESMOGNATHUS REPRODUCTION:

II. NUMERICAL RESULTS

Introduction

The analytical results of the model developed in Chapter 7 yield qualitative predictions of how a female Desmognathus fuscus should allocate her resources so that her reproductive output in a given year is maximized. These general results are valuable and allow insights into the reproductive ecology of Desmognathus or other organisms with similar life history traits, but it also would be informative to consider specific predictions generated by using a given set of model parameters. In particular, several questions that arise from the analysis can easily be addressed by numerical computation.

1. In the non-brooding model, the switching time, t_r , depends upon the magnitude of $(I - c)\beta$. In the brooding model, hatching success depends on the rate of egg mortality and the amount of energy allocated to maternal care. Consequently, the sensitivity of modelling predictions to small changes in the values of I , c , β , ϕ_0 , and κ should be examined.

2. Reductions in clutch mortality during egg brooding depend on female body size. Thus, predicted reproductive success for large and small females can be compared.

3. According to the model, neither allocation to fat, nor

simultaneous allocation to growth and reproduction will maximize reproductive output. These results, however, may be artifacts of the model structure. If the formulation had included nonlinear dependence on the controls, uncertainty in the terminal time, or less than perfect transfer efficiency in the fat state variable, then other optimal control schemes could have obtained (cf. Cohen 1971, King and Roughgarden 1982b, Chiarello and Roughgarden 1984). Numerical studies can be used to explore how allocation to fat, or simultaneous allocation to growth and reproduction, affect reproductive output.

5. Finally, in order to have any predictive power, the quantitative modelling predictions should match empirical observations of the reproductive ecology of D. fuscus.

Numerical Methods and Parameter Estimates

Exact analytical solutions for the state equations in each of the time periods (T_0, T_R) , (T_R, T_1) , and (T_1, T_2) were obtained using the optimal allocation schemes from Chapter 7. These expressions, and not numerical approximations, were used in the numerical studies. In addition, the exact solutions of the state equations during the brooding period are functions of time and the amounts of energy in fat, lean tissue (i.e., body size), and reproduction at the onset of egg brooding. Since these initial values are the terminal values for the variables in the non-brooding period, this effectively links the two reproductive intervals.

In carrying out the numerical computations, two additional

assumptions were necessary. Firstly, I assumed that winter has no effect upon female salamanders. That is, female salamanders neither grow, produce eggs, nor expend lipid reserves in maintenance during the winter so that the sizes of the state variables do not change from the end of autumn to the beginning of spring. Assuming that winter brumation (i.e., reduced activity during overwintering) lasts 90 days, this implies that the activity season for a female salamander is equivalent to 275 days. Based on data from D. fuscus at Chilhowee Mountain, I used 50 days for the duration of egg brooding. This left 225 days for growth and egg production.

Secondly, I assumed that overall female mortality was zero, so that the quantitative results could be interpreted as predictions for females that had survived, rather than as predictions of a population average. Inclusion of a non-zero mortality term will not alter the qualitative behavior of solutions; however, it would reduce the size of the state variables at the terminal time.

I obtained estimates for the remaining model parameters from my field and laboratory data, and from values from the literature. Ranges for the parameters, the values used in the numerical analyses, and the sources of the estimates are shown in Table 30. Miscellaneous conversion factors are given in Table 31. All estimates from the literature were based on studies of D. ochrophaeus; comparable data for D. fuscus were not available.

TABLE 30. Estimates for model parameters.

Parameter	Units	Range	Standard*	Source
I	cal day	30 - 45	38	Chapter 6, this thesis
c	cal day	5.4	5.4	Fitzpatrick 1973
β	cal	0.0001 - 0.0003	0.00015	Chapter 6, this thesis
μ	day	0	0	see text
δ	day	0.0135 - 0.0270	0.0135	Fitzpatrick 1973
ϕ_0	day	0.08 - 0.12	0.1	Chapter 3, this thesis; Forester 1979
κ	cal day	0.00005 - 0.00006	0.00005	Forester 1979

* this value used in simulations unless otherwise indicated

TABLE 31. Conversion factors used in numerical computations.

Conversion factor	Source
5310 cal/g of cricket	S. E. Riechert, personal comm
0.0114 g/cricket	Chapter 6, this thesis
4600 cal/g lean <u>Desmognathus</u> tissue	Fitzpatrick 1973
9000 cal/g lipid	White et al. 1964
50 cal/mm <u>Desmognathus</u>	Fitzpatrick 1973; Chapter 6, this thesis
60 cal/fully developed egg*	Tilley 1968; Fitzpatrick 1973
115 cal fat/small <u>D. fuscus</u>	Chapter 6, this thesis
1700 cal lean tissue/small <u>D. fuscus</u>	Chapter 6, this thesis
1300 cal fat/large <u>D. fuscus</u>	Chapter 6, this thesis
2200 cal lean tissue/large <u>D. fuscus</u>	Chapter 6, this thesis

* based on 45 cal per fully developed egg in D. ochrophaeus, a smaller species

Numerical computations were performed for sensitivity of the model to the parameters I and β using the standard parameter values listed in Table 30 for all other parameters. I examined the effects of initial body size on hatching success using standard values for parameters and initial body size ranging from 1000 to 2700 calories, with initial fat levels set at 20 percent of initial body size. In subsequent simulations, data on lipid levels from August field collections (Chapter 6) were used for initial conditions for the lipid and body size variables (Table 31). Small female D. fuscus were modelled by 115 cal fat and 1700 cal lean tissue; large females were modelled by 1300 cal fat and 2200 cal lean tissue. I examined the effects of a) different food levels on growth and reproductive success in small and large female D. fuscus using five different values for food intake; b) variations in the hatching success of small females for different values of ϕ_0 and κ ; c) growth of small females with different metabolization efficiencies or a longer activity season. In addition, I investigated numerically whether or not allocation to growth or lipid storage during vitellogenesis can affect reproductive success in small females by adding a small constant term to the body or lipid state variables at each time step. All numerical computations were done on a VAX-11 computer using extended precision arithmetic.

Numerical Results and Discussion

Overall, the numerical results appear to be in accordance with empirical observations of Desmognathus reproductive ecology.

Estimates for the fecundity of model salamanders (based on 60 cal per egg) tended to be 25 to 50 percent higher than observed fecundity obtained from follicle counts of D. fuscus from Tennessee (Chapter 3), but comparisons of fecundities obtained for different parameter values are relative so the conclusions discussed below should remain valid.

In the non-brooding model, reasonable estimates for food intake (I), respiration (c), and rate of conversion from food to salamander biomass (β), yield estimates for $1/((I - c)\beta)$ approximately equal to $T_1 = 225$, the time of oviposition. Since the formula for switching time is

$$T_R = T_1 - 1/((I - c)\beta)$$

this yields T near the beginning of the reproductive cycle. In particular, for food intake from 30 to 40 calories per day (roughly equivalent to 3 to 5 small crickets per week), and any conversion rate less than 0.0002 per day, the predicted switching time is within 30 days of the beginning of the activity season (Figure 13). This suggests that female Desmognathus in natural populations may have virtually no opportunity to grow after they reach sexual maturity. Fitzpatrick (1973) also reached this conclusion based on energetic analyses of D. ochropaeus. The results of my mark-recapture studies (Chapter 3) and

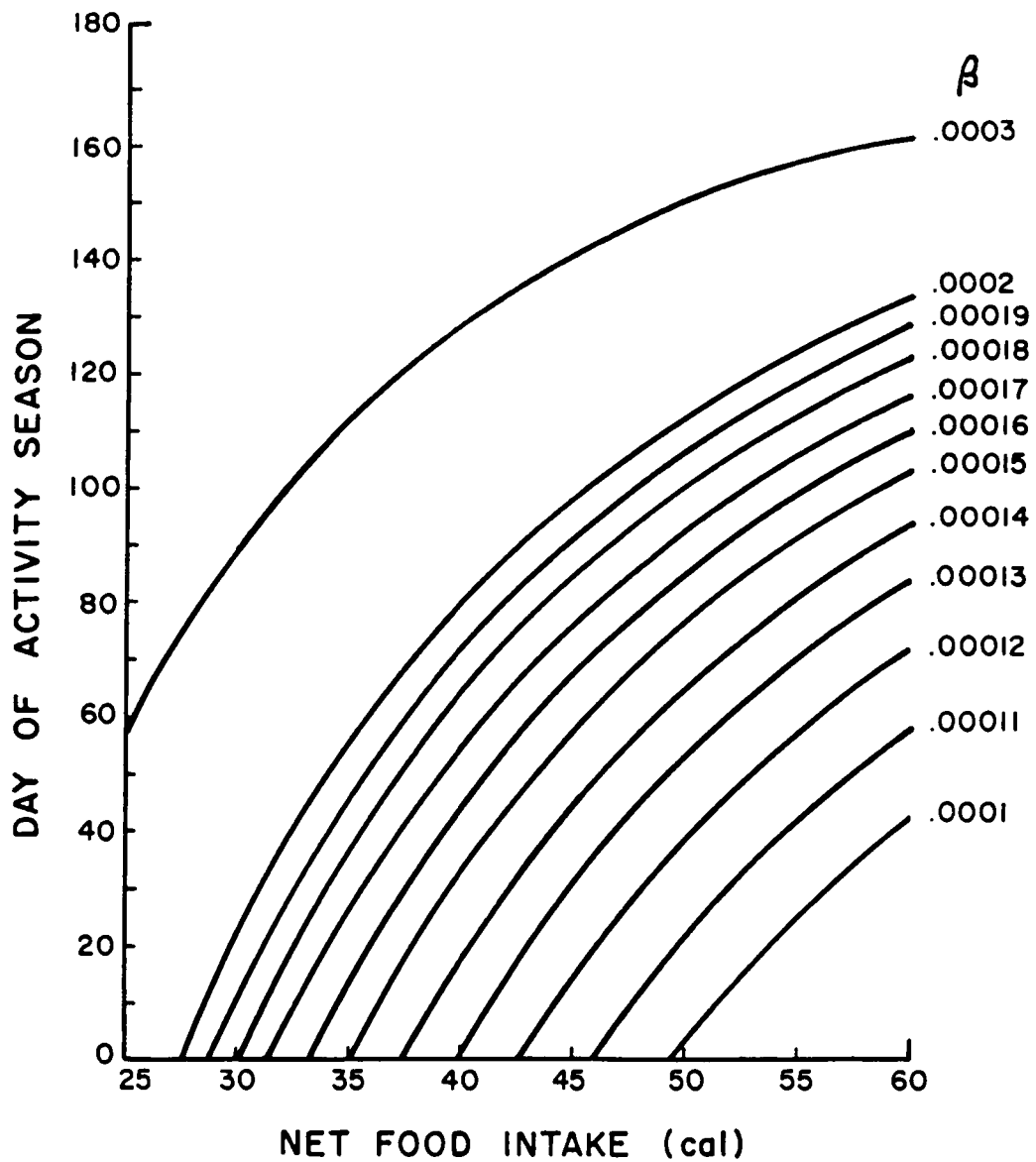


FIGURE 13. Predicted date that female salamanders should begin egg production at different levels of conversion efficiency (β) and net food intake.

those of Tilley (1980) and Forester (1977) corroborate the implication that adult females grow relatively little from year to year.

Female size, however, has a drastic effect on the hatching success predicted by the model (Figure 14). Females larger than 2000 cal (equivalent to 40 mm SVL) are able to hatch all or almost all their eggs, whereas smaller females experience considerably lower hatching success. In addition, the rate of change of hatching success increases with body size. This means that an 1800 cal (36 mm SVL) female that grows 100 cal (2 mm SVL) by the next reproductive season will increase her hatching fraction by 17 percent, whereas a 1600 cal female (34 mm SVL) that undergoes the same amount of growth will improve her hatching fraction by only 10 percent.

Switching time, body size and fecundity of model salamanders are all monotone increasing functions of food intake (Table 32). For small females, however, hatching success actually may decrease with food intake if food levels are so low that the female is unable to grow before yolking eggs. This implies that although the female is able to produce more eggs, her ability to care for them during brooding is unchanged so that proportionally more eggs are lost. Decreases in body size during brooding may be interpreted as loss of appendages to potential egg predators or reduction in glycogen stores or muscle tissue. These reductions mean that the salamander will start the next reproductive season at a smaller body size, so that unless she grows to compensate for these losses, her fecundity and reproductive success in the subsequent year will be lower.

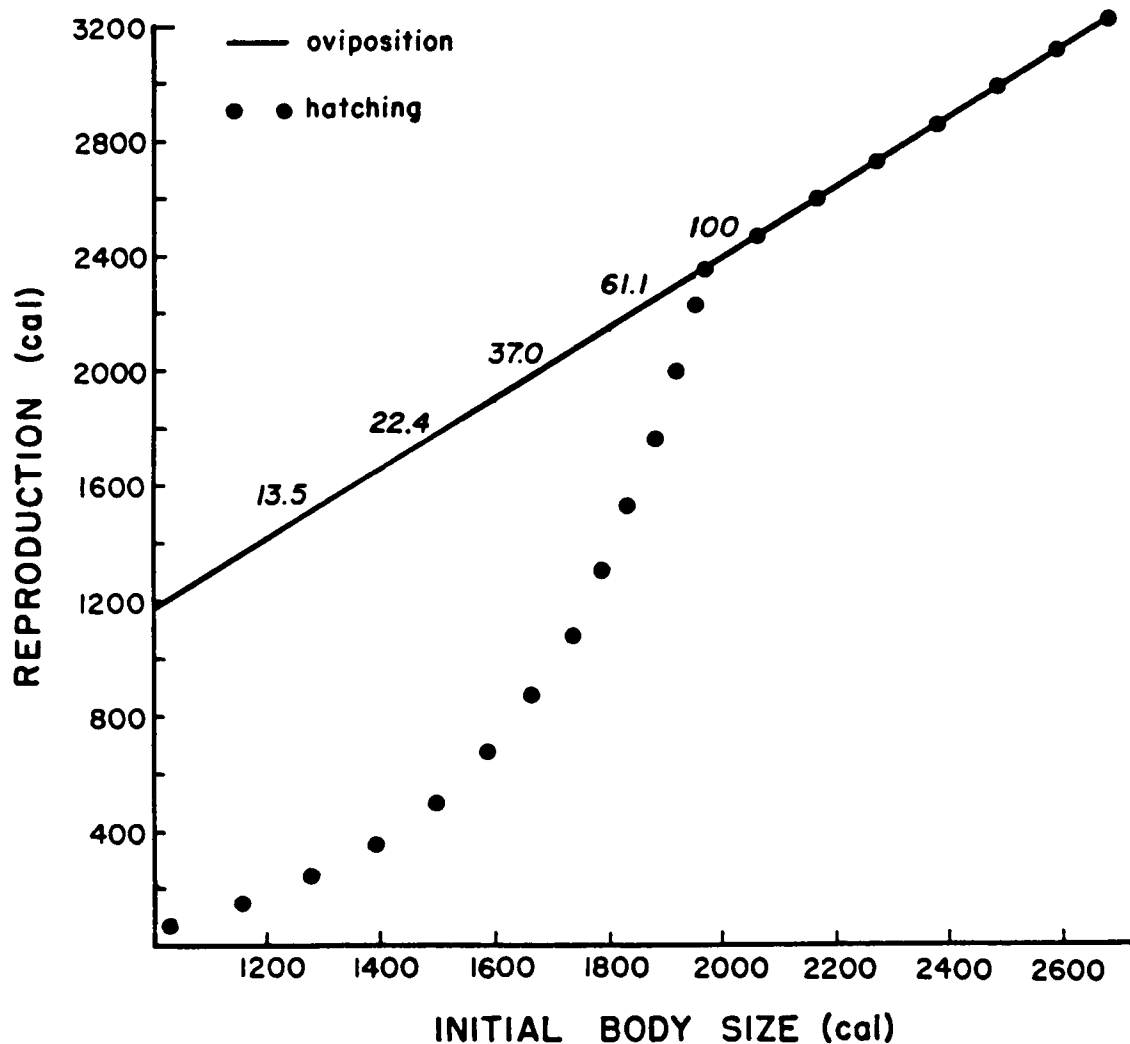


FIGURE 14. Reproductive output of model salamanders at oviposition and egg hatching. Numbers above the solid curve are hatching success (hatching/oviposition, in %) for a female of that body size at oviposition.

TABLE 32. Calories at the time of oviposition and hatching in fat (F), carcass tissue (B), and reproduction (R) for small and large *Desmognathus fuscus* at different food levels (I, in cal). The number of ova are based on 50 cal/follicle; reproductive success is the ratio of calories in reproduction at hatching to the number of calories in reproduction at oviposition.

I	Time	Small					Large				
		F	B	R	Ova	%	F	B	R	Ova	%
30	Ovip	6	1700	1515	25	31.8	63	2200	3055	51	100
	Hatch	3	1411	482	8		32	1850	3055	51	
35	Ovip	6	1700	1800	30	29.6	63	2200	3424	57	100
	Hatch	3	1358	533	9		32	1782	3424	57	
38	Ovip	6	1903	1980	33	46.1	63	2750	3672	61	100
	Hatch	3	1494	912	15		32	2180	3672	61	
40	Ovip	6	2040	2113	35	60.3	63	3069	3866	64	100
	Hatch	3	1576	1275	21		32	2395	3866	64	

Given a fixed body size, hatching fraction depends on the rate of egg mortality in the absence of maternal care (ϕ_0) and the efficacy of maternal care (κ). Figure 15 shows this relationship for a female with body size of 1700 cal. For example, with K fixed at 0.00005 per cal per day, a female could improve her hatching success from 45 to 75 percent by nesting in a habitat where ϕ_0 was 0.09 per day, rather than 0.105 per day. Similarly, for $\kappa = 0.10$ per day, a female could achieve the same 30 percent increment in success by increasing κ from 0.00005 to 0.000056 per cal per day. Thus, for at least some fixed values of ϕ_0 , small model salamanders may achieve large gains in reproductive success with a very small change in the amount of maternal care per calorie per unit time provided by the female. The term that determines the rate of egg mortality, however, is $\phi_0 - \kappa z_1 B$, i.e., the difference between the mortality rate for unattended eggs and improved survivorship accruing from maternal care. In natural situations, ϕ_0 probably varies both spatially and temporally. Fluctuations in ϕ_0 might be caused, for example, by changes in the microclimate of a nest site or by transitory activities of potential egg predators. Real female salamanders probably are unable to predict the clutch mortality rate associated with a particular nest site with the precision achievable by model salamanders. As a result, enhanced survivorship of Desmognathus clutches effected via larger κ could be offset by an increase in ϕ_0 . The higher survivorship has at least three interpretations consistent with current evolutionary theories. First, natural selection may have acted on maternal behavior in such a

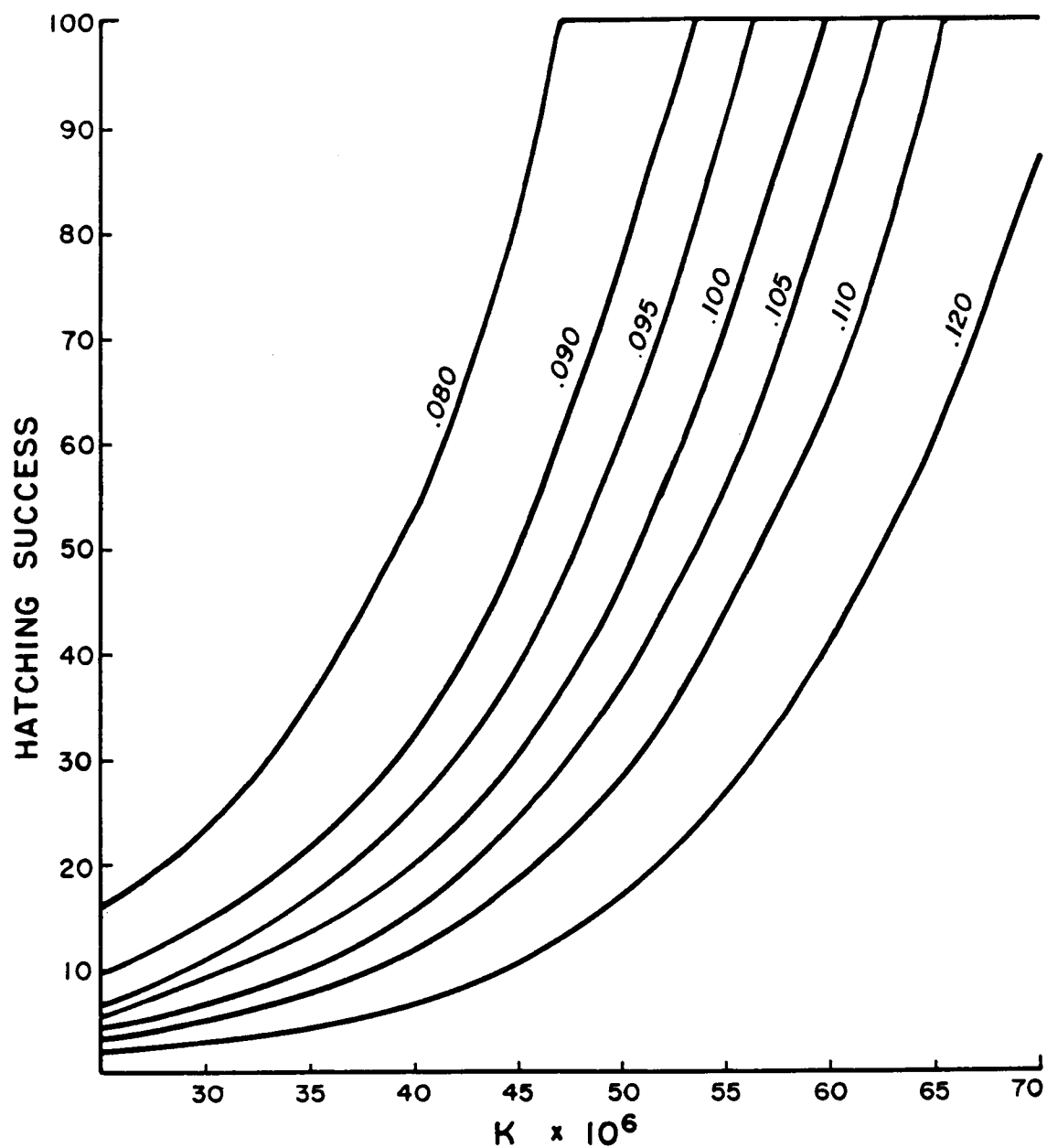


FIGURE 15. Hatching success for a small female salamander (1700 cal body size, 115 cal fat) at different levels of clutch defense (κ). Each curve represents fixed values for the rate of egg mortality in the absence of maternal care.

way that female allocation to clutch defense already is at its maximum value subject to constraints in organismal design. That is, further improvements in the effectiveness of clutch defense are not possible because of morphological or physiological limitations, regardless of environmental circumstances. The other two interpretations, however, are based on the unpredictability of environmental fluctuations. It is conceivable that the overall selective advantage gained by a female through modifying the effectiveness of clutch defense could be small relative to the selective benefits of changes in other life history attributes. Alternatively, female salamanders may have been selected to provide a rate of maternal care that allows them to achieve reproductive success under "average" environmental and ecological circumstances, rather than selecting for absolute maxima in the effectiveness of clutch defense. This would imply fitness in the sense of persistence of a genotype (i.e., satisficing; sensu Stearns 1982b, Wake et al. 1983), versus the maximization of the rate of its propagation in the population, which would be consistent with the first two hypotheses. The present state of the knowledge about the evolutionary biology of plethodontid salamanders does not lend conclusive support to any of these hypotheses. Available evidence indicates that plethodontids are an old lineage (dating back to the Mesozoic (Wake 1966)) characterized by morphological stasis, behavioral and physiological plasticity (Wake et al. 1983), and potentially rapid genetic and biochemical diversification (Tilley 1973, Larson 1984).

The importance of large body size to high reproductive success suggests that growth should be a high priority for small female salamanders. Under the hypothesis that allocation follows the control scheme of Chapter 7, this growth may be accomplished by increasing: a) food intake by substantial amounts (e.g., from 30 cal per day to 40 calories per day, cf. Table 32; b) the rate of conversion of food into salamander biomass; or c) the length of the activity season. Alternatively, if other control schemes are considered, growth can occur if the female simultaneously allocates food resources to both reproduction and non-reproductive structures.

Increasing the rate of conversion from 0.00015 to 0.00018 yields 100 percent clutch success for a small female (Table 33). The increase in clutch success is achieved through larger body size: a small female with a conversion rate of 0.00018 per day grows 14 mm (equivalent to a 41 percent increase) in the 225 days of the non-brooding period. This growth rate seems unrealistically high. Furthermore, substantial changes in conversion may be constrained by biochemical or physiological considerations, and therefore could be unlikely. It may be possible, however, for the female to forage during the winter as long as environmental temperatures do not become so low that her physiological processes are impaired. Extending the activity season (during the non-brooding period) by 14 days improves hatching success by 20 percent (Table 34).

TABLE 33. Calories at the time of oviposition and hatching in fat (F), lean tissue (B), and reproduction (R) for a small female Desmognathus fuscus at different metabolism rates (β). Reproductive success for each category is the ratio of calories in reproduction at hatching to the calories in reproduction at oviposition.

	Time	F	B	R	Success
0.00015	Ovip	5	1903	1980	
	Hatch	3	1493	912	46.1
0.00016	Ovip	5	2053	2125	
	Hatch	3	1584	1317	61.9
0.00017	Ovip	5	2228	2281	
	Hatch	3	1691	2002	87.8
0.00018	Ovip	5	2405	2449	
	Hatch	3	1796	2449	100.0

TABLE 34. Calories at the time of oviposition and hatching in fat (F), lean tissue (B), and reproduction (R) for a small female Desmognathus fuscus at with an activity season of 239 days. Reproductive success is the ratio of calories in reproduction at hatching to the calories in reproduction at oviposition.

	Time	F	B	R	Success
	Ovip	5	2067	2117	
	Hatch	2	1621	1401	66.2

Allocation of some of the food resources of a small female either to growth (Table 35) or to lipid storage (Table 36) during the latter stages of vitellogenesis does not have a substantial effect on fecundity. Model salamanders that allocate to fat have fewer calories in reproduction at hatching, and therefore lower reproductive success, than females that do not grow (cf. Table 32). In contrast, females that allocate to the body state variable have higher reproductive success than females that do not grow. In spite of the increment in body size, however, the small females still are unable replace lean tissue lost during egg brooding by the time of oviposition in a subsequent year of reproduction. Consequently, female body size during brooding in the second year is almost as small as or even smaller than the initial body size during the first year of reproduction. This again emphasizes the need for females to attain relatively large body size prior to sexual maturity.

TABLE 35. Calories at the time of oviposition and hatching in fat (F), carcass tissue (B), and reproduction (R) in small Desmognathus fuscus, with partial allocation to growth. Food levels are denoted by (I). The number of ova are based on 50 cal/follicle; reproductive success is the ratio of calories in reproduction at oviposition to the calories in reproduction at hatching. Results are shown for two consecutive years.

I	Time	Year 1				Year 2			
		F	B	R	Ova %	F	B	R	Ova %
35.2	Ovip	6	1713	1818	30 31.4	0	1386	1377	22 15.0
	Hatch	3	1373	569	9	0	1108	207	3
38	Ovip	6	1916	1981	33 47.4	0	1672	1656	27 27.4
	Hatch	3	1503	838	15	0	1309	455	7
40	Ovip	6	2053	2113	35 62.1	0	1877	1869	31 42.0
	Hatch	3	1586	1313	21	0	1448	1786	13

TABLE 36. Calories at the time of oviposition and hatching in fat (F), carcass tissue (B), and reproduction (R) in small Desmognathus fuscus, with partial allocation to fat. Food levels are denoted by (I). The number of ova are based on 50 cal/follicle; reproductive success is the ratio of calories in reproduction at oviposition to the calories in reproduction at hatching. Results are shown for two consecutive years.

I	Time	Year 1					Year 2				
		F	B	R	Ova	%	F	B	R	Ova	%
35.2	Ovip	19	1700	1785	29	30.7	14	1368	1352	22	14.5
	Hatch	10	1368	549	9		7	1100	197	3	
38	Ovip	19	1903	1955	32	46.4	14	1655	1631	27	26.7
	Hatch	10	1498	908	15		7	1302	435	7	
40	Ovip	19	2040	2040	34	60.8	14	1861	1844	30	40.9
	Hatch	10	1581	1269	21		7	1442	755	12	

CHAPTER 9

SYNTHESIS

The life history of female Desmognathus fuscus appears to be characterized by delayed maturity, annual reproduction, high investment in reproduction, maternal care, and low annual reproductive success. These features may be best understood within the context of the ecology of the species as a whole, rather than as a set of isolated traits.

There is little doubt that fecundity in female Desmognathus is positively correlated with snout-vent length (Tilley 1968; Chapter 3). Intuitively then, it seems reasonable to hypothesize that a female ultimately may benefit by being large. The model for reproductive allocation analyzed in Chapter 7 predicts that a female salamander can maximize her reproductive output by using an allocation scheme that includes growth. Specifically, if her net food intake is high, then she should grow first and yolk eggs later in the activity season. If her net food intake is low, then she should skip the growth period altogether and invest in reproduction throughout the activity season. Many of the female D. fuscus in the Reed Creek population appeared to begin vitellogenesis soon after ending egg incubation; some females seemed to initiate vitellogenesis while brooding eggs. The contrast between model predictions and these empirical observations can be interpreted in several ways. First, the model could be inappropriate. The formulation analyzed in Chapter 7 is relatively simple: it

ignores demographic and environmental stochasticity, and assumes linearity in the control variables, temporal homogeneity, and constant model parameters. Models that incorporate some of these assumptions, however, (e.g., Cohen 1971, King and Roughgarden 1982a) still predict an initial period of growth. Thus, regardless of model structure, this analytical result may be robust. Secondly, it is possible that larger body size confers a disadvantage caused by phenomena not included in the model. This could occur, for example, if interspecific competition with larger Desmognathus restricted the maximum size of D. fuscus. This seems unlikely, however, since competition for resources other than nest sites should act on both sexes, yet male D. fuscus often become considerably larger than conspecific females (Organ 1961, Tilley 1977). Interspecific competition for nest sites probably did not occur in this study, since D. monticola and D. quadramaculatus do not nest in streambank habitats in Reed Creek. Thirdly, perhaps females in natural populations do not obtain sufficient food to allow a prolonged interval of growth. Based on the numerical calculations of Chapter 8, females that consume the caloric equivalent of 3 to 5 crickets per week will grow for only 30 days before beginning vitellogenesis. Females with lower food intake will grow for even shorter periods of time. Finally, it is possible that salamanders are conservative: if females are unable to predict their food supply, then the safest allocation scheme would be to begin vitellogenesis early in the year so that they would be ensured of having fully developed ova by the subsequent summer.

Observations of growth in a natural population and at two levels of food in the laboratory (Chapter 6) lend credence to the latter two hypotheses. Female D. fuscus given 6 to 12 small crickets per week grew as much as 7 mm in 8 months (Chapter 6). Females given 3 or 4 crickets per week grew less than 4 mm; females from Reed Creek tended to grow even less. All females in the laboratory appeared to begin vitellogenesis early in the autumn; in April, females at the low food level and females in the natural population seemed to be at similar stages of vitellogenesis. The similarity in patterns of growth and yolk deposition between the females given the lower levels of food and females in Reed Creek suggests that, on the average, salamanders in nature may not get much more food than the equivalent of 3 or 4 crickets per week. Also, the apparent occurrence of simultaneous allocation to growth and egg production in females given high levels of food suggests that female D. fuscus indeed may be conservative in reproductive allocation. Further, it implies that models that incorporate uncertainty in the terminal time or in parameter values may be more appropriate representations of reproductive allocation in Desmognathus.

Evidence from several sources indicates that sexually mature female Desmognathus in natural populations are unlikely to grow by substantial amounts after beginning reproduction. Egg production and brooding are energetically expensive and can require almost half of the annual energy budget of a female D. ochrophaeus (Fitzpatrick 1973). Regeneration of lost appendages appears to have high priority

in morphogenesis within plethodontids (Maiorana 1976, Hom personal observation), and probably supersedes allocation of energy to increasing body length. Females in some species of Desmognathus occasionally may skip opportunities for reproduction (Tilley 1977), presumably because of the high energetic demands of reproduction and regeneration. These animals might have opportunities for growth not available to females that reproduce annually, yet most studies of Desmognathus demography have reported annual reproductive cycles as the norm for the population (e.g., Forester 1977, Tilley 1980, Chapter 3). Finally, mark-recapture data verify that females in natural populations usually do not grow more than a millimeter or two from year to year (Forester 1977, Tilley 1980, Chapter 6).

The optimal reproductive scheme predicted in Chapter 7 did not include allocation to storage, yet females given high levels of food in the laboratory synthesized fat (Chapter 6). Female Desmognathus observed in natural populations at the end of the summer (presumably after emergence of the year's clutch) often appear thin and emaciated (Tilley 1972, Forester 1977), although lipid levels in the corpora adiposa seem to remain high throughout the year (Fitzpatrick 1973, Chapter 6). This suggests that females undergo an energy deficit during egg brooding and subsequently mobilize lipids in the carcass for physiological maintenance. The structure of the model examined in Chapters 7 and 8 does not incorporate use of storage products for maintenance. Analysis of a revised formulation that includes periods

of possible energy deficit may help to better understand the function of lipids in reproductive allocation in Desmognathus.

Female body size can affect reproductive success in several ways that are independent of its relationship with fecundity. The results of cover object selection experiments in Chapter 4 suggest that larger females may be at an advantage relative to smaller conspecifics in gaining access to cover objects. Microhabitat variables may not affect reproductive success directly (Chapter 3), however, a female that is injured in a conflict over occupancy of a particular nest site may have lower fecundity (Maiorana 1976), or impaired sensory ability (Jaeger 1981) and therefore may be more susceptible to gettin' munched on by something hungry. Keen and Sharp (1984) note that smaller females are more likely to be injured in such conflicts than larger individuals. Furthermore, observations from Reed Creek indicate that large females were able to hatch a larger fraction of their egg clutch than smaller females (Chapter 3). The higher hatching success of large females may have been due to improved ability of the female to defend the clutch against potential egg predators (Chapter 5), although additional observations of clutch defense are necessary to confirm this phenomenon. Numerical results discussed in Chapter 8, however, suggest that the size dependence is substantial and that hatching success as a function of female body size is concave up.

If reproductive success is at least partially determined by body size, and if female salamanders are unlikely to increase their body size after beginning reproduction, then the optimal life history for

Desmognathus should include delayed maturity. This appears to be the case; all species of Desmognathus have a prolonged juvenile period. For example, Desmognathus fuscus female do not become sexually mature until about four or five years of age (Tilley 1968). Using size as a surrogate for age, delayed maturity is an implication of the numerical results (Chapter 8). Interestingly, size at which hatching success for model salamanders begins to increase rapidly (e.g., an increment in hatching success of over 20 percent with a 2 mm increase in body length) is approximately 36 mm SVL. This is roughly the size at which real female D. fuscus become sexually mature; it is relatively rare for smaller females in Reed Creek to be gravid.

Other ecological factors, however, may present opposing selective forces in Desmognathus life history. For example, the nature of interspecific interactions may depend on age structure (Southerland 1986c): small or juvenile D. quadramaculatus may act as competitors with D. fuscus, whereas larger D. quadramaculatus might be predators on the smaller species. Elevation and habitat also may interact to affect growth rate (Tilley 1974). Consequently, restricting life history investigations to adult members of a population may lead to naive conclusions.

Overall, the results of the allocation model suggest that the fitness of a female D. fuscus ultimately is determined by events that occur after oviposition. Models, together with the corresponding empirical studies, that examine lipid metabolism in periods of energy deficit during brooding, and specific aspects of maternal care and

clutch defense may yield additional insights into the biology of Desmognathus. Furthermore, the predictions of the model analyzed in this dissertation are not restricted to plethodontid salamanders; they may be applicable to any species in which size affects the ability of the organism to reproduce successfully. Phenomena that may elicit such an effect include mate choice and offspring quality, as well as parental care. Incorporating these considerations into theories of life history allocation can only help to improve the understanding of the reproductive ecology of specific taxa.

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APPENDIXES

APPENDIX 1

PROOF OF PROPOSITION 1.1 - 1.4

For biological realism, assume $b > 0$ and $f \geq 0$ for all t .

Proposition 1.1: Singular control does not occur in (t_0, t_1) .

Proof. Consider singular control in the u_i 's. If singular control in u_i occurs on an interval in (t_0, t_1) , then $0 < u_i < 1$ and $\sigma_{u_i} = 0$ on that interval. For u_i , $\sigma_{u_i} = 0$ if and only if

$$\exp[\mu'(t - t_1)]b = 0$$

Since $b > 0$ by hypothesis, this cannot be true. Hence, singular control in u_i cannot occur. For singular control in u_2 and u_3 , first recall that from (1.7), the Hamiltonian, control variables, and switching functions are related by

$$H = \sigma_{u_1} u_1 + \sigma_{u_2} u_2 + \sigma_{u_3} u_3 + \sigma_{v_1} v_1 + \sigma_{v_2} v_2$$

Suppose $\sigma_{u_2} = 0$. If $\sigma_{u_1} > 0$ or $\sigma_{u_3} > 0$ then $u_2 = 0$ and $u_1 + u_3 = 1$ when the Hamiltonian is maximal. Thus, for $\sigma_{u_2} = 0$, it will be sufficient to examine the cases in which $\sigma_{u_1} < 0$ and $\sigma_{u_3} < 0$. The proofs for σ_{u_3} and for σ_{v_1} are analogous. Clearly,

for u_2 and u_3 , the requirement that $\sigma_{u_1} < 0$ cannot be satisfied. Thus, singular control does not occur for any u_i .

For v_1 , the existence of singular control requires that $\sigma_{v_1} = 0$ and $\sigma_{v_2} < 0$. Substituting from the switching functions yields

$$\lambda_2 < \lambda_3 = \exp[\mu'(t - t_1)]$$

over the interval of singular control. This immediately implies that $\sigma_{u_2} < \sigma_{u_3} = \sigma_{u_1}$ on that interval. The proof is completed by assuming that singular control occurs and obtaining a contradiction.

If control in v_1 is singular, then $\sigma_{u_3} = \sigma_{u_1}$ so that $u_1 + u_3 = 1$ with $u_2 = 0$. It follows from the switching function σ_{v_1} that

$$\lambda_3 = \exp[\mu'(t - t_1)] .$$

However, the terminal transversality condition requires $\lambda_3(t_1) = 0$, which cannot be satisfied if control is singular. Hence, control must switch to either (i) $v_1 = 1, v_2 = 0$; or (ii) $v_1 = 0, v_2 = 1$ at some $t^* < t_1$.

For (i), integrating the adjoint equation for λ_3 forward from t^* and simplifying yields

$$\lambda_3(t) = \exp[\mu'(t - t_1)] .$$

But this is the condition for singular control. Thus, control remains singular for $t > t^*$, which implies that the terminal transversality condition will not be satisfied. Consequently, singular control

cannot switch to $v_1 = 1$ and $v_2 = 0$ at $t = t^*$.

For (ii), if control switches to $v_2 = 1$ and $v_1 = 0$ at $t = t^*$, then $\dot{\lambda}_2(t^*) > \dot{\lambda}_3(t^*)$ and $\lambda_2(t^*) = \lambda_3(t^*)$. Substituting the adjoint equations (1.6) into the preceding inequality and simplifying,

$$-\exp[\mu'(t^* - t_1)] > 0$$

This cannot occur, hence singular control cannot switch to $v_2 = 1$ at $t = t^*$. It follows that control cannot be singular in v_1 .

For v : singular control in v_2 requires that $\sigma_{u_2} = 0$, that $\lambda_2 = \lambda_3$, and that $\dot{\lambda}_2 = \dot{\lambda}_3$ on an interval. It follows that $\sigma_{u_1} < \sigma_{u_2} = \sigma_{u_3}$ so that $u_1 = 0$ and $u_2 + u_3 = 1$. The adjoint equations become

$$\dot{\lambda}_2 = -\lambda_2 + \mu' \lambda_3$$

$$\dot{\lambda}_3 = \mu' \lambda_3$$

This contradicts $\lambda_2 = \lambda_3$. Thus, control in v_2 cannot be singular.

Proposition 1.2: So long as $f > 0$, the combinations of controls possible on (t_0, t_1) are

$$\begin{aligned} \text{a) } u_1 &= 1, u_2 = u_3 = 0, \\ v_1 &= 1, v_2 = 0; \end{aligned}$$

$$b) \quad u_2 = 1, u_1 = u_3 = 0,$$

$$v_2 = 1, v_1 = 0;$$

$$c) \quad u_3 = 1, u_1 = u_2 = 0,$$

$$v_1 = 0, v_2 = 0.$$

Proof. It is sufficient to show that the values of u_i uniquely determine the values of v_i on (t_0, t_1) .

Suppose that $u_3 = 1$. Then $\sigma_{u_3} > \sigma_{u_1}$ and hence

$$\lambda_3 > \exp[\mu'(t - t_1)],$$

from which it follows that $\sigma_{v_1} < 0$ and hence $v_1 = 0$. Also, $\sigma_{u_3} > \sigma_{u_2}$, which similarly implies that $\sigma_{v_2} < 0$ and hence $v_2 = 0$. Therefore, case (c) is the only possible combination of controls with $u_3 = 1$.

Now suppose that $u_1 = 1$. Then $\sigma_{u_1} > \sigma_{u_2}$, hence

$$\exp[\mu'(t - t_1)] > \lambda_2$$

and so $\sigma_{v_1} > \sigma_{v_2}$. If $v_1 = v_2 = 0$, which occurs if and only if both $\sigma_{v_i} < 0$, a reversal of the argument for case (c) shows that $u_3 = 1$, a contradiction. Hence $v_1 = 1$ and $v_2 = 0$, proving that (a) is the only possible control with $u_1 = 1$. The argument for the case $u_2 = 1$ is nearly identical and is left to the reader.

Proposition 1.3: At the terminal time, t_1 , $u_1 = 1$ and $v_1 = 1$.

Proof. The terminal transversality condition requires that $\lambda_i(t_1) = 0$. Then $\sigma_{u_2}(t_1) = \sigma_{u_3}(t_1) = 0$ and $\sigma_{u_1}(t_1) = b(t_1)$, which is greater than 0 by hypothesis. Thus, $u_1 = 1$ at $t = t_1$. For v_1 , note that $\sigma_{v_1}(t_1) = \delta'f(t_1)$ and $\sigma_{v_2}(t_1) = 0$. It follows that $v_1 = 1$ at $t = t_1$.

Proposition 1.4: None of the following switches in control variables can occur as part of the optimal control over (t_0, t_1) :

- a) from $u_3 = 1$ to $u_2 = 1$;
- b) from $u_3 = 1$ to $u_1 = 1$;
- c) from $u_1 = 1$ to $u_2 = 1$.

Proof. Proposition 1.4 is proven by contradiction, using the adjoint equations (1.6), switching functions (1.7), and Proposition (1.2).

Consider a switch from $u_3 = 1$ to $u_2 = 1$ at $t = t^*$. For $t < t^*$, $\sigma_{u_3} > \sigma_{u_2}$ and $\lambda_3 > \lambda_2$. This implies that

$$\lambda_3 > \exp[\mu'(t - t_1)] , \quad t < t^* .$$

Similarly, for $t > t^*$, $\sigma_{u_2} > \sigma_{u_3}$ so that $\lambda_2 > \lambda_3$ and $\sigma_{u_2} > \sigma_{u_1}$, hence

$$\lambda_2 > \exp[\mu'(t - t_1)] , \quad t > t^*$$

At $t = t^*$, $\lambda_2(t^*) = \lambda_3(t^*)$ and $\dot{\lambda}_2(t^*) > \dot{\lambda}_3(t^*)$. Substituting the

expressions from the adjoint equations (1.6) into the latter inequality and simplifying,

$$-\lambda_2(t^*)(u_2 + u_3) > 0$$

Then $\lambda_2(t^*) < 0$, which contradicts $\lambda_2 > \exp[\mu'(t-t_1)]$. Therefore, control cannot switch from $u_3 = 1$ to $u_2 = 1$ at $t = t^*$.

Now assume that control switches from $u_3 = 1$ to $u_1 = 1$ at $t = t^*$. In order for this to occur, it is necessary that $\sigma_{u_1} > \sigma_{u_3}$ at $t = t^*$. Substituting from the switching functions (1.7) evaluated at $t = t^*$,

$$\mu' \exp[\mu'(t - t_1)]b + \exp[\mu'(t - t_1)]\dot{b} > \dot{\lambda}_3 b + \lambda_3 \dot{b}.$$

Cancelling common terms yields the contradictory inequality

$$\mu'b + \dot{b} > \mu'b + \dot{b}.$$

Thus, a switch from $u_3 = 1$ to $u_1 = 1$ cannot occur in (t_0, t_1) .

Finally, assume that a switch from $u_1 = 1$ to $u_2 = 1$ occurs at $t = t^*$. This requires that for $t > t^*$, $\sigma_{u_2} > \sigma_{u_1}$, which is true if and only if

$$\lambda_2(t) > \exp[\mu'(t - t_1)].$$

Integrating the adjoint equation for λ_2 forward from t^* ,

$$\lambda_2(t) = \lambda_2(t^*) \exp[(\mu' - 1)(t - t^*)],$$

so that if

$$\lambda_2(t^*) \exp[(\mu' - 1)(t - t^*)] > \exp[\mu'(t - t_1)] ,$$

then

$$\exp[-(t - t^*)] > 1$$

This cannot be true since $t > t^*$.

APPENDIX 2

PROOF OF PROPOSITIONS 2.1 - 2.3

The following lemmas will be useful for the proofs of Proposition 2.1 - 2.3.

Lemma 2.1: $-1 < \lambda_1(t) < 0$ for all $t \in (t_1, t_2)$.

Proof. Because $\phi_0 - z_1 b > 0$ by definition, the sign of the slope of λ_1 in equation (2.4) is determined by the sign of $(1 + \lambda_1)$. Let t^* be a point in (t_1, t_2) . Now if $\lambda_1(t^*) < -1$ for some t^* , then $\lambda_1(t^*)$ is decreasing on $[t^*, t_2)$ and the terminal transversality condition, $\lambda_1(t_2) = 0$, cannot be satisfied. If $\lambda_1(t^*) > 0$ for some t^* then $\lambda_1(t)$ is increasing on $[t^*, t_2)$. In this case, the terminal transversality condition also cannot be satisfied. If $\lambda_1(t^*) = -1$ then $\lambda_1 \equiv -1$ so that again, the terminal value cannot be reached. Therefore, $-1 < \lambda_1(t) < 0$ for all t in (t_1, t_2) .

Lemma 2.2: $\lambda_2(t) > 0$ for all $t \in (t_1, t_2)$.

Proof. Assume for purposes of contradiction that $\lambda_2(t^*) < 0$ at some t^* in (t_1, t_2) . Then (2.4) shows that λ_2 is decreasing on $[t^*, t_2)$, which implies that the terminal transversality condition cannot be satisfied.

Lemma 2.3: $\lambda_2(t) > \lambda_3(t)$ in an interval $[t^*, t_2)$ for some $t^* \geq t_1$.

Proof. By Lemma 2.2, $\lambda_2 > 0$ for all t in (t_1, t_2) , so it will be sufficient to prove $\lambda_2(t) > \lambda_3(t)$ and $\lambda_3(t) > 0$ for all t near t_2 . Substitution of $\lambda_1(t_2) = 0$ into the adjoint equation yields $\dot{\lambda}_3(t_2) = 0$ and $\dot{\lambda}_2(t_2) = -z_1 r$ so that λ_2 is positive and decreasing near t_2 . Thus, $\lambda_2 > \lambda_3$ on (t^*, t_2) for some $t^* > t_1$.

Now assume, for a contradiction, that $\lambda_3(t^{**}) < 0$ for t^{**} in (t^*, t_2) . Then from the adjoint equation for λ_3 ,

$$\dot{\lambda}_3 = \mu'(\lambda_3 - \lambda_2) + \mu'\lambda_3.$$

Since $\lambda_3(t) < \lambda_2(t)$ for $t \in (t^*, t_2)$, it follows that $\dot{\lambda}_3(t) < 0$ for $t \in (t^*, t_2)$, and the terminal transversality condition cannot be satisfied. Thus, $\lambda_2(t) > \lambda_3(t) > 0$ for all $t \in (t^*, t_2)$.

Now Proposition (2.1) - (2.3) can be established.

Proposition 2.1: Both v_2 and z_1 take their maximum value at the terminal time.

Proof. The terminal transversality condition requires that $\lambda_1(t_2) = 0$. First, consider z_1 . At $t = t_2$, $\lambda_1 = \lambda_2 = 0$ implies that $\sigma_{z_2}(t_2) = br$. It follows from the switching function for z_1 that $z_1 = 1$ at $t = t_2$. For v_2 , note that at t near t_2 , $\sigma_{v_2} > 0$ by Lemma 2.3, hence $v_2 = 1$.

Proposition 2.2: If the control variables switch to $v_2 = 1$ and $z_1 = 1$ at some time in (t_1, t_2) , then a switch in v_2 occurs before a switch in z_1 .

Proof. Define t_v as the time at which v_2 switches to $v_2 = 1$ and t_z as the time at which z_1 switches to $z_1 = 1$. From the switching function σ_{v_2} , $\lambda_2 < \lambda_3$ if $t < t_v$ for t near t_v , and $\lambda_2 > \lambda_3$ if $t > t_v$ for t near t_v , with equality at $t = t_v$. Similarly, from σ_{z_1} , $\lambda_2 / (1 + \lambda_1) < r$ if $t < t_z$ and t is near t_z ; $\lambda_2 / (1 + \lambda_1) = r$ for $t = t_z$; and $\lambda_2 / (1 + \lambda_1) > r$ if $t > t_z$ for t near t_z . Ignoring z_1 for the moment, the adjoint equations relative to v_2 are

$$\dot{\lambda}_2 = -(1 + \lambda_1)z_1r + (\mu' + z_1)\lambda_2$$

for all t in (t_1, t_2) , and

$$\dot{\lambda}_3 = \begin{cases} \mu' \lambda_3 & \text{if } t \leq t_v \\ -\delta' \lambda_2 + (\delta' + \mu')\lambda_3 & \text{if } t_v < t, \end{cases}$$

and t is close to t_v . Now since $\lambda_2(t_v) = \lambda_3(t_v)$ and $\lambda_2(t) > \lambda_3(t)$ for $t > t_v$, it follows that $\dot{\lambda}_2(t) > \dot{\lambda}_3(t)$. Then at $t = t_v$, substitution of the adjoint equations into this inequality and simplifying yields

$$-[1 + \lambda_1(t_v)]z_1r + \lambda_2(t_v)z_1 \geq 0$$

Now consider two cases. Firstly, if $z_1 = 0$, then the above inequality is satisfied and $t_v < t_z$. Secondly, if $z_1 \neq 0$ then $\lambda_2 / (1 + \lambda_1) > r$, which implies that $t_v < t_z$. Notice that this proof does not assume any particular values for v_2 and z_1 prior to t_v and t_z , respectively.

Proposition 2.3: The control variables v_2 and z_1 only take their extreme values, i.e., singular control does not occur.

Proof. Consider z_1 and assume, for a contradiction, that singular control occurs over some interval. On this interval the switching function σ_{z_1} is identically zero so that $\lambda_2 = (1 + \lambda_1) r$, with adjoint equations

$$\begin{aligned}\dot{\lambda}_1 &= (1 + \lambda_1)(\mu' + \phi'_0 - z_1 b) \\ \dot{\lambda}_2 &= -(1 + \lambda_1)z_1 r + (\mu' + z_1)\lambda_2.\end{aligned}$$

Let t^* be the time at which control becomes singular in z_1 .

Integrating the adjoint equations forward yields

$$\begin{aligned}\lambda_1(t) &= [1 + \lambda_1(t^*)] \exp[(\mu' + \phi'_0)(t - t^*) - \int_{t_1}^{t_2} z_1(\tau) b(\tau) d\tau] - 1 \\ \lambda_2(t) &= \lambda_2(t^*) \exp[\mu'(t - t^*)].\end{aligned}$$

Since $\lambda_2 = (1 + \lambda_1) r$, solving for λ_1 yields $\lambda_1 = (\lambda_2 / r) - 1$.

Substituting the expression for λ_2 into this formula,

$$\lambda_1 = \frac{\lambda_2(t)}{r} \exp[\mu'(t - t^*)] - 1 .$$

Equating the two expressions for λ_1 and simplifying,

$$r(t) = r(t^*) \exp[-\phi'_0(t - t^*) + \int_{t_1}^{t_2} z_1(\tau) b(\tau) d\tau] .$$

Now over the interval of singular control, the state equation for r is

$$\dot{r} = [-\mu' - (\phi'_0 - z_1 b)]r .$$

Integrating this forward from t^* ,

$$r(t) = r(t^*) \exp[-(\mu' + \phi'_0)(t - t^*) + \int_{t_1}^{t_2} z_1(\tau) b(\tau) d\tau] .$$

Equating the two expressions for $r(t)$ gives $\mu' = 0$, a contradiction.

This implies that singular control in z_1 does not occur.

Now consider singular control in v_2 . Assume that singular control occurs over some interval, during which σ_{v_2} is identically zero and $\lambda_2 = \lambda_3$. Suppose that control becomes nonsingular at a time $t = t^{**}$. For $t > t^{**}$ there are two possible combinations of values for the control variables: $v_2 = 0$, $z_1 = 0$; and $v_2 = 1$,

$z_1 = 0$. The cases where control in v_2 switches from singular and $z_1 = 1$ cannot occur. Firstly, by Proposition 2.2, if control in v_2 switches from singular to $v_2 = 1$ at some $t = t^*$, then $z_1(t^*)$ cannot equal 1. Secondly, suppose control in v_2 switches from singular to $v_2 = 0$ at $t = t^{**}$, with $z_1(t^{**}) = 1$. Proposition 2.1, however, requires that control in v_2 switch to $v_2 = 1$ at $t < t_2$. Application of Proposition (2.2) yields a contradiction.

For the case where $v_2 = 0$, $z_1 = 0$, the adjoint equations for λ_2 and λ_3 over some $t > t^{**}$ are

$$\dot{\lambda}_2 = \mu' \lambda_2$$

$$\dot{\lambda}_3 = \mu' \lambda_3 .$$

Since $\lambda_2(t^{**}) = \lambda_3(t^{**})$, it follows that

$$\lambda_2(t) = \lambda_2(t^{**}) \exp[\mu'(t - t^{**})] = \lambda_3(t) .$$

Thus, if singular control occurs prior to $t = t^*$, then control remains singular until $t = t_2$. This contradicts Lemma (2.3). Thus, $v_2 = 0$ and $z_1 = 0$ cannot occur if control is singular up to $t = t^{**}$. The proof of the second case, $v_2 = 1$ and $z_1 = 0$, is analogous.

VITA

Carole Louise Hom was thrust upon an unsuspecting world on 16 January 1958. She grew up in sun-drenched suburbia, attended elementary school in East Palo Alto and Fremont, California, and trundled happily off to college barely in time to avoid the development boom in the Silicon Valley. Being a stubborn child, she insisted on lumping mathematics with biology and graduated from the University of the Pacific with a do-it-yourself degree in Mathematical Ecology in May 1975.

That autumn, Ms. Hom entered graduate school at The University of Tennessee, Knoxville where her hybrid academic orientation was nurtured gleefully by several faculty. She became interested in Slimy Things (much to the dismay of her very genteel mother) en route to earning the Master of Science degree in August 1982, and the Doctor of Philosophy degree in August 1986, both with majors in Ecology.

The author is a member of professional societies devoted to the study of ecology, mathematics, slime and lizard-squeezing, choral music, and scientific illustration, as well as assorted rabble-rousing political and environmental organizations.

Ms. Hom, with her cats and photographs of Slimy Things firmly in tow, returned to the bosom of the Dong/Hom family in late summer 1986 and began a period as a Visiting (i.e., Larval) Assistant Professor of Mathematics at the University of California, Davis. As of last report, she is not yet accustomed to her new identity.