PHYSIOLOGICAL AND ECOLOGICAL MEDIATION OF NATAL DISPERSAL IN BELDING'S GROUND SQUIRRELS

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ABSTRACT

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Natal dispersal, the permanent departure of an individual from its birth site, occurs in nearly all mammals, and tends to be sexually dimorphic in its occurrence. That is, one sex usually disperses at higher rates or over greater distances than the other. The physiological and ecological variables influencing this behavior are poorly understood. Here I attempted to elucidate the roles played by these variables in the mediation of natal dispersal behavior in Belding's ground squirrels (*Spermophilus beldingi*).

Males are the predominant dispersers in *S. beldingi*, as they are in most mammals. I examined the role of three classes of physiological factors in the mediation of the natal dispersal behavior of free-living male *S. beldingi*: energy availability, endogenous timing mechanisms, and gonadal steroid hormones. I also assessed ecological influences on natal dispersal behavior in both juvenile male and female *S. beldingi*. I manipulated body fat and energy availability of juvenile *S. beldingi* by provisioning them with food, and used unprovisioned juveniles as controls. I compared dispersal between juveniles born early vs. late during the breeding season. I manipulated early hormone exposure of female *S. beldingi* by treating them with testosterone (T) at birth, and used oil-treated females as controls. I combined hormone and body fat manipulations to evaluate the interaction between the two. I also collected blood from juveniles to measure circulating

concentrations of T. I regularly evaluated dispersal status, body mass, and body fat of subjects, while concurrently monitoring several ecological variables.

Juvenile male *S. beldingi* appear to delay dispersal until they have attained a threshold body mass. Body fat also importantly influences the timing of dispersal. Dispersing males tend to be fatter than same-aged males still residing in the natal area.

The energetic demands of depositing fat for hibernation appear to constrain dispersal by juvenile male *S. beldingi*. Juvenile males born late in the year, and which have little time to prepare for hibernation, exhibit accelerated acquisition of body fat, and postpone dispersal until their yearling summer. Thus, an endogenous timing mechanism appears to interact with body fat or fat-related variables in mediating natal dispersal behavior.

Concentrations of circulating T are undetectable in juvenile *S. beldingi*, suggesting that T does not activate natal dispersal during ontogeny. However, treatment of newborn females with T increases the likelihood that they will emigrate from the natal area. Moreover, food provisioning advances the timing of natal dispersal among both T-treated females and males, and these advances in timing appear to be mediated by increases in body fat.

All male *S. beldingi* eventually emigrate from their birth sites, regardless of ecological conditions. By contrast, the probability of dispersal by young female *S. beldingi* is influenced by demand for environmental resources. Young females may disperse to enhance their access to space in which to establish a territory.

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Chapter 1

INTRODUCTION

Most group-living vertebrates transfer between social units at least once during their lifetimes. The tendency to leave the natal group, also called dispersal or emigration behavior, occurs in most mammals and tends to be sexually dimorphic (Greenwood, 1980). Specifically, male mammals generally disperse with greater frequency or over greater distances than do female conspecifics. Although ecological variables promoting dispersal behavior and adaptive consequences of dispersal have been well studied (e.g. Pusey, 1987; Stenseth and Lidicker, 1992; Smale et al., 1997), the physiological mediation of this important and widely-occurring behavior remains poorly understood in mammals (Dingle, 1996). The goal of this work was to elucidate physiological processes that underlie emigration from the natal site in male mammals, using Belding's ground squirrels (*Spermophilus beldingi*) as a model species. I examined three classes of physiological variables as potential regulators of dispersal behavior: (1) availability of energy as reflected in body fat (Nunes and Holekamp, 1996), (2) internal timing mechanisms (Nunes et al., in press), and (3) gonadal steroid hormones (Smale et al., 1997). In the course of this work I was also able to observe ecological influences, such as competition for food and space, on emigration by those few female *S. beldingi* that leave the natal area (Nunes et al., 1997). This allowed me to examine causes and consequences of dispersal by female mammals.

ENERGY AVAILABILITY AND DISPERSAL

Energy availability often influences the behavior of animals, which must partition available energy according to priorities that ensure survival and optimize long-term reproductive success (Wade and Schneider, 1992). The possibility that the availability of energy might regulate the timing of dispersal is suggested by the fact that emigration from the natal area is potentially both risky and energetically costly (Smale et al., 1997). Emigration is associated with increased locomotor and exploratory behavior (Messier, 1985a; Holekamp, 1986; Rood, 1987). Moreover, dispersers exploring unfamiliar areas often appear to be more vulnerable to predation or other sources of mortality than are long-term residents (Metzgar, 1967; Snyder et al., 1976; Messier, 1985b), and dispersing individuals often encounter aggressive resistance from resident animals when attempting to immigrate into new areas (Holekamp, 1986; Pusey and Packer, 1987a; Smale et al., 1997). As a result, individuals may need to reallocate time spent foraging to vigilant and defensive behaviors during the dispersal movement. Changes in activity associated with dispersal may therefore increase energy requirements and decrease energy intake. Natural selection should therefore theoretically favor individuals that delay departure from the natal area until their energy reserves are adequate to support the potential demands of the dispersal movement (Nunes and Holekamp, 1996).

I examined the influences of energy availability, as reflected in body mass and body fat, on the dispersal behavior of juvenile male *S. beldingi*. If the timing of dispersal is in fact influenced by the amount of energy available to an individual, then altering energy availability should have an impact on when an individual leaves its birth site. I provided juvenile male *S. beldingi* with extra food to increase their reserves of energy stored as fat. I predicted that these provisioned *S. beldingi* should be better able to cope with the energetic demands of dispersal, and should emigrate from their natal areas at younger ages than unprovisioned controls.

TIMING MECHANISMS AND DISPERSAL

Many life history events that occur in seasonal or annual cycles are regulated by endogenous timing mechanisms (Zucker et al., 1991). When dispersal behavior is closely associated with seasonal events, it might also be influenced by endogenous timing mechanisms (Smale et al., 1997). In species exhibiting seasonal reproduction, dispersal frequently occurs during the breeding period (e.g., Berger, 1986, 1987; Pusey and Packer, 1987a; Sprague, 1992; Matsumura, 1993), and reproduction in seasonal breeders is often regulated by circannual or photoperiodic timing mechanisms (e.g., Zucker, 1988; Zucker et al., 1991; Bartness et al., 1993). Thus, it seems reasonable to expect that the timing of dispersal, puberty and reproduction in some seasonal breeders might be regulated physiologically by a common timing mechansim. For example, an internal oscillator that regulates the timing of changes in reproductive status might also mediate temporal variation in dispersal-related behaviors such as locomotion and exploration. “Dispersal restlessness” occurs seasonally in birds (Ritchison et al., 1992), and might also occur in mammals (Smale et al., 1997).

Timing mechanisms that regulate cyclic life history events other than reproduction might also mediate the timing of dispersal behavior. For example, many mammals in temperate habitats hibernate during predictable periods of limited food availability in winter, and many hibernators depend entirely on stored fat for survival during dormancy (Mrosovsky and Fisher, 1970; Davis, 1976; Mrosovsky, 1976; French, 1982). Hibernation and its associated patterns of fat deposition and usage exhibit annual rhythms generated by internal oscillators (Zucker et al., 1991). Because hibernation and dispersal both present the organism with energetic challenges, they might come into conflict. That is, an individual that uses energy to support dispersal behavior may compromise its ability to build fat reserves adequate to survive the winter (Nunes and Holekamp, 1996). It is plausible that after a critical date during the annual cycle, when little time remains until winter, an endogenous timing mechanism initiates fattening for hibernation. If body fat reserves are low at this critical date, this timing mechanism may inhibit dispersal behavior so that available energy can be used to build fat stores for overwinter survival. Thus, energetic and timing mechanisms might interact to mediate dispersal behavior in hibernators, and in other mammals in which energetic demands vary seasonally.

I tested the hypothesis that endogenous timing mechanisms influence natal dispersal in juvenile male *S. beldingi*. I compared dispersal behavior between three years, during one of which the active season was substantially delayed. If endogenous timing mechanisms regulate dispersal behavior, then a very late onset of the active season should be associated with a decrease in dispersal by juveniles. In particular, when the active season begins late, internal physiological processes should have been favored by natural selection which anticipate the approach of winter, and these should direct available energy toward building fat reserves for hibernation rather than toward fueling dispersal behavior. Thus, a greater proportion of juvenile *S. beldingi* should delay dispersal until the yearling summer in years when the juvenile active season begins very late, compared to years in which the active season is not delayed.

GONADAL STEROID HORMONES AND DISPERSAL

Hormones have been implicated in the control of long-distance movements in invertebrates, fish, amphibians, and birds (Dingle, 1996), and might also mediate mammalian dispersal (Smale et al., 1997). The specific possibility that mammalian dispersal might be influenced by gonadal steroid hormones is suggested by the sexual dimorphism in dispersal, and by its common occurrence around the time of puberty. Gonadal steroids can promote sex differences in behavior in two general ways (Phoenix et al., 1959). First, hormones can alter the physical and biochemical configuration of the developing central nervous system during fetal or neonatal life to organize tissue which mediates sexually dimorphic behavior patterns later in life. Second, hormones can act directly to induce more immediate behavioral changes in the mature animal. Phoenix et al. (1959) chose the terms "organizational" and "activational," respectively, to describe these two modes of hormone action. Organizational effects on behavior are often permanent and irreversible, whereas activational effects are generally temporary and require the concurrent presence of activating hormone. Either organziational, activational, or both effects might promote sexually dimorphic mammalian dispersal.

Temporal coincidence of dispersal with puberty is consistent with the hypothesis that rising levels of gonadal steroid hormones might activate dispersal behavior. Puberty is characterized by dramatic increases in circulating levels of gonadal steroid hormones that stimulate a variety of behavioral changes (Bronson and Rissman, 1986; Wilson, 1992). A close temporal association between dispersal and puberty has been observed in many species of mammals (e.g., Errington, 1963; Harcourt et al., 1976; Harcourt, 1978; Pusey, 1980; Berger, 1986, 1987; Pusey and Packer, 1987a; Rood, 1987, 1990; Matsumura, 1993). However, the hypothesis that gonadal steroid hormones activate dispersal has not yet been empirically tested in any of these species.

When emigration occurs well before puberty, as it does in many sciurid rodents and dasyurid marsupials (Holekamp, 1984a; Cockburn et al., 1985), gonadal steroid hormones are not likely to activate dispersal behavior. However, gonadal steroids might exert organizational effects during the prenatal or perinatal period to promote dispersal later in life. Androgens organize many non-sexual, dimorphic behaviors that all males of a species predictably exhibit during specific stages of development (Beatty, 1992). For example, perinatal androgen exposure promotes masculine patterns of play behavior in many mammals (e.g., Thor and Holloway, 1984, 1986). Theoretically, androgens might also organize a predisposition to emigrate from the natal area in the many species of mammals in which all males eventually disperse, such as *S. beldingi*, spotted hyenas (*Crocuta crocuta*), lions (*Panthera leo*), ponies (*Equus caballus*), marsupial mice (e.g., *Antechinus stuartii*, *A. swainsonii*), and various species of cercopithecine primates (Smale et al., 1997). However, in some species of mammals, such as Columbian ground squirrels (*S. columbianus*), the dispersal behavior of males is more flexible, and some males disperse while others remain in their natal areas. In these species, androgens probably do not organize a deterministic predisposition to leave the natal area, but might organize a masculine response to social or ecological variables which promote dispersal behavior.

Androgen exposure during early development might also masculinize the ways in which dispersal behavior is influenced by energetic variables (Smale et al., 1997). In the numerous species of mammals in which dispersers are predominantly male, the few females that disperse should confront many of the same energetic problems faced by males. Nevertheless, patterns of natal dispersal and key life history events often differ between males and females, and the specific manner in which fat-related variables regulate dispersal behavior might also differ. For example, in many mammals, females begin reproducing at earlier ages than males (e.g., Harcourt et al., 1976; Sherman and Morton, 1984; Berger, 1987; Bronson, 1989; Packer et al., 1995), and disperse at later ages (Smale et al., 1997). Consequently, females tend to experience the energetic rigors of reproduction earlier than males, and the energy requirements of emigration from the natal area might impose greater potential costs on the future reproductive success of young females than on that of young males. The energetic regulation of dispersal behavior may be fundamentally similar for young male and female mammals, but sex differences in some of its finer details might be mediated by gonadal hormones. Thus, endocrine mechanisms and energetic variables might work together to mediate dispersal behavior, just as timing mechanisms and energetic variables might interact.

I examined the possibility that testosterone (T) influences natal dispersal behavior in *S. beldingi*, and that the effects of T on dispersal are influenced by energy availability. I predicted that if if T activates natal dispersal in juvenile males, then circulating concentrations of T should increase near the time of dispersal. However, if T organizes natal dispersal behavior, then treatment of female *S. beldingi* with T at birth should masculinize physiological systems that promote dispersal behavior, and T-treated females should exhibit male-typical dispersal behavior later during their ontogeny. Finally, if energy availability interacts with organizational effects of T on dispersal, then altering energy availability by food provisioning should advance the timing of dispersal (Nunes and Holekamp, 1996) in both males and females treated with T at birth.

EVOLUTIONARY CAUSES OF DISPERSAL

Three hypotheses are commonly offered in the literature as evolutionary explanations for natal dispersal: individuals may disperse (1) to increase their access to environmental resources such as food, territories or nest sites, (2) to improve their access to mates, or (3) to avoid deleterious effects on fitness of close inbreeding (Lidicker, 1962, 1975; Packer, 1979; Dobson, 1982; Moore and Ali, 1984; Dobson and Jones, 1985; Pusey, 1987; Pusey and Packer, 1987a; Stenseth and Lidicker, 1992). As suggested by the striking sexual dimorphisms in dispersal behavior, the evolutionary causes of dispersal often differ between males and females (Dobson, 1979; Holekamp, 1984a; Lambin, 1994; Waser, 1996; Smale et al., 1997). In species in which ultimate causes of dispersal have been identified, males often disperse to avoid inbreeding or to enhance the probability of encountering receptive mates, whereas females usually disperse to gain better access to environmental resources such as food or space (Smale et al., 1997). As is the case in many mammals, female *S. beldingi* appear to leave the natal area to improve their access to environmental resources critical for raising young, whereas male *S. beldingi* appear to leave to increase their opportunities to mate with receptive or unrelated females (Nunes et al., 1997; Smale et al., 1997).

HYPOTHESES

In this work I examined five hypotheses suggesting explanations for particular aspects of dispersal behavior in *S. beldingi*:

(1) variables, reflecting availability of energy, such as body fat and body mass influence the timing of dispersal by juvenile male *S. beldingi*,

(2) (a) internal timing mechanisms regulate dispersal behavior in juvenile males and (b) in doing so, these interact with energetic variables such as body fat,

(3) gonadal steroid hormones exert (a) organizational and/or (b) activational effects on natal dispersal behavior in *S. beldingi*,

(4) endocrine mechanisms interact with energetic variables to mediate the dispersal behavior typical of juvenile male *S. beldingi*, and

(5) the probability of dispersal by juvenile female but not juvenile male *S. beldingi* is influenced by demand for environmental resources such as (a) food, (b) nest sites, or (c) space on which to establish a territory.

I tested these hypotheses using various experimental regimens of food provisioning and hormone manipulations. I gave juvenile *S. beldingi* extra food to manipulate body mass and body fat, and observed the influences of these variables on dispersal behavior. Because food provisioning is often associated with increases in local population density (Dobson, 1995; Boutin, 1990; Nunes et al., 1997), I was able to oberve the effects of local resource competition on dispersal behavior. To evaluate effects of endogenous timing mechanisms, I compared dispersal behavior of provisioned and unprovisioned juveniles between years with normal and unusually late active seasons. To examine endocrine influences on dispersal, I altered androgen levels of some provisioned and unprovisioned females at birth, and later observed whether they exhibited masculine dispersal behavior (Holekamp et al., 1984), and I also measured circulating androgen levels of juveniles.

SIGNIFICANCE OF THE RESEARCH

This research represents an attempt to elucidate the relationship between physiological substrates, ontogenetic development, and adaptive significance of an ecologically significant mammalian behavior. Dispersal profoundly influences the demography and genetic composition of vertebrate populations (Keith and Tamarin, 1981; Charlesworth and Charlesworth, 1987; Chepko-Sade and Halpin, 1987; Waser and Elliott, 1991; Nelson, 1993), so an understanding of its proximal and evolutionary causes should prove directly useful to ecologists and conservation biologists alike, as habitats of vertebrates become increasingly fragmented and isolated (e.g., Halpin, 1987; Bennett, 1990; Diffendorfer et al., 1995). Moreover, this work helps to fill a virtual information void in the physiology of mammalian dispersal behavior. It also sheds light on the extent to which gonadal steroid hormones can mediate nonsexual behavior patterns in mammals, and further elucidates the influence that these hormones exert on sex differences in mammalian behavior. Finally, and most importantly, this work is a novel endeavor to evaluate in free-living mammals the interaction between endocrine control of behavior and the regulation of energy balance. To date most studies of this interaction have been accomplished in the laboratory using species such as hamsters (*Mesocricetus auratus*;

Wade and Schnieder, 1992; Wade et al., 1996) and mice (*Mus musculus*; Campfield et al., 1995; Halaas et al., 1995; Pellymounter et al., 1995) whose ecology is very poorly understood. As these laboratory studies become progressively more molecular, it is important not to lose sight of the importance of ecological and evolutionary phenomena influencing interactions between physiology and behavior.

Chapter 2

GENERAL METHODS

SPERMOPHILUS BELDINGI

S. beldingi is a diurnal, semi-fossorial sciurid rodent inhabiting alpine and sub-alpine meadows of the western United States (Jenkins and Eshelman, 1984). Although populations of *S. beldingi* do not contain distinct social subdivisions as do those of some of the more social sciurids (King, 1955; Barash, 1973; Michener, 1983; Hoogland, 1995*),* *S. beldingi* is a group-living animal that engages in daily social interactions with conspecifics (Sherman, 1977). Populations of *S. beldingi* typically contain several clusters of related females (Michener, 1983). Within such clusters, each breeding female utilizes a separate burrow system, but interacts more frequently and more amicably with close female kin than with more distantly related females (Sherman, 1977, 1981a).

The life history of *S. beldingi* is characterized by four-month active seasons interspersed with eight-month hibernation periods (Sherman and Morton, 1984). The annual cycle of activity is rigidly constrained by extreme seasonal changes in climate (Morton and Sherman, 1978), and ontogenetic development after birth is accelerated compared to that of non-hibernating rodents of similar body size. Between weaning and entry into hibernation seven to twelve weeks later, the mean body mass of juveniles increases four to six fold. This rapid development presumably permits juveniles to attain body mass and fat reserves necessary for overwinter survival (Morton and Tung, 1971; Morton et al., 1974; Maxwell and Morton, 1975).

S. beldingi's breeding season ordinarily begins one to two weeks after emergence from hibernation in the spring, and lasts three to four weeks (Sherman and Morton, 1984). Adult males are reproductively competent when they emerge from hibernation, whereas yearling males generally do not exhibit descended testes and do not mate (Morton and Gallup, 1975). Variance in mating success is high, and only a small proportion of males successfully copulate during the breeding season (Sherman, 1976). Body mass of male *S. beldingi* is positively correlated with success in winning fights with other males during the breeding season, and also with mating success (Sherman, 1976). Adult and yearling female *S. beldingi* emerge from hibernation slightly later than do males, and most females (>60%) begin mating as yearlings. Females exhibit estrous behavior on only one day per year, and appear to exhibit mate choice behavior, accepting the mating attempts of some males while rejecting those of others (Sherman, 1976). Litters are multiply sired, typically by two to three males (Hanken and Sherman, 1981). Gestation lasts 24-25 days, and the lactation interval is 25-28 days (Holekamp, et al., 1984; Nunes, unpublished data). Young remain underground in their natal burrows until weaning. Females maintain maternal territories during gestation and lactation, and often act cooperatively with female kin in defending these territories (Sherman, 1977, 1981a). Female *S. beldingi* are able to discriminate between littermate sisters, non-littermate sisters, and other female squirrels, and this capacity to recognize kin appears to facilitate and mediate nepotistic interactions among females (Holmes and Sherman, 1982; Holmes, 1986a, 1986b).

Natal dispersal in *S. beldingi* occurs predominantly among males (Holekamp, 1984a). Most males (40-90%) emigrate from their natal areas as juveniles, and 100% of surviving males disperse by the end of the yearling year (Holekamp, 1984a; Nunes and Holekamp, 1996). By contrast, most females remain in their natal areas throughout their lives, and only about 8% of females ordinarily emigrate (Holekamp, 1984a; Nunes et al., 1997).

The probability of natal dispersal by male *S. beldingi* is not proximally influenced by social or ecological factors (Holekamp, 1986). All males eventually disperse regardless of population density, resource availability, ectoparasite load, conspecific aggression and the frequency of other social interactions with conspecifics. A suite of behaviors associated with dispersal, including increased locomotion, exploration and boldness, appear to be triggered at some point during ontogeny. These behaviors evidently lead to or otherwise facilitate emigration from the natal area. The trigger for these behaviors appears to be attainment of a particular body mass or body composition (Holekamp, 1986; Nunes and Holekamp, 1996).

STUDY SITE AND SUBJECT POPULATION

I studied a population of *S. beldingi* in a 20 hectare meadow at Tioga Pass (38° N, 119° W, elevation 3040 m) in Mono County, California, from 15 May to 26 Sept 1993, 15 May to 3 Sept 1994, 17 May to 5 Oct 1995, and 13 May to 2 Sept 1996. This site has been described in detail by Morton (1975) and Sherman (1976). I defined a juvenile as an individual born during the current year, a yearling as one born during the previous year, and an adult as one born at least two years earlier. I defined a reproductive female as one who was pregnant or lactating. From 1993-1996 I permanently marked 1098 squirrels (including 365 male and 380 female juveniles from 163 complete litters) with monel metal ear tags (National Band and Tag Co., Newport, KY).

The growing season at Tioga Pass is very short, and places considerable constraints on the population of *S. beldingi* that lives there (Morton and Tung, 1971; Morton et al., 1974; Maxwell and Morton, 1975; Morton, 1975; Sherman, 1976; Morton and Sherman, 1978; Sherman and Morton, 1979). When squirrels at Tioga Pass first emerge from hibernation in May, much of their habitat is still covered with snow, and they must rely on fat reserves for energy. During June and July, green vegetation is abundant and comprises the main part of the squirrels' diet. In August, the squirrels' diet shifts to seeds produced by grasses and other vegetation at that time of year. By September, much of the vegetation at Tioga Pass has dried out or senesced, and food availability is severely limited. The extreme brevity of the growing season at Tioga Pass has important consequences for *S. beldingi*. The active season of the squirrels is very short (about 3-4 months), and squirrels enter hibernation throughout August and September. Events such as reproduction, pre-hibernation fattening, dispersal, and physical and behavioral development must be exactly timed to fit into this very short period of activity.

TRAPPING, HANDLING, AND RECOVERY OF SQUIRRELS

I regularly live-trapped squirrels in the study area using Tomahawk live-traps (Tomahawk Live-Trap Co., Tomahawk, WI) baited with peanut butter. At each capture, I weighed squirrels to the nearest gram with spring balance scales (Avinet, Dryden, NY), and recorded reproductive status (Morton and Gallup, 1975; Holekamp, 1983) and capture location (distance and direction from nearest landmarks). I marked individual squirrels with unique symbols using hair dye to facilitate visual identification (Sherman, 1976; Holekamp, 1983).

I observed maternal burrows of lactating females daily to determine the dates on which their young first emerged above ground. I trapped juveniles within four days of their first appearance of the juveniles, during which time they remained close to their natal burrows and could be unambiguously assigned to litters (Sherman, 1976). I assigned birth dates to juveniles by subtracting 27 days, the mean duration of lactation, from the date of first emergence from the natal burrow (Holekamp et al., 1984). I observed squirrels from atop rocks and ladders, with the aid of 7 x 50 binoculars.

I trapped and ear-tagged 48 juvenile males and 47 juvenile females in 1993; 95 juvenile males and 107 juvenile females in 1994, 126 juvenile males and 130 juvenile females in 1995, and 107 juvenile males and 97 juvenile females in 1996. I considered juveniles to be recovered each summer if their dispersal status (see below) was known at 10 weeks of age in 1993, 1994, and 1995, or 9 weeks of age in 1996. I recovered 50.0% of juvenile males and 51.1% of juvenile females in 1993, 54.7% of juvenile males and 70.1% of juvenile females in 1994, 62.7% of juvenile males and 73.1% of juvenile females in 1995, and 58.9% of juvenile males and 63.9% of juvenile females in 1996.

EVALUATION OF DISPERSAL STATUS

I defined the natal area of each individual as a circle with a radius of 80 meters centered at the natal burrow, based on maximum size of maternal territories when young were weaned (Holekamp, 1984a, 1986). To determine ages at which juveniles dispersed, I observed each marked litter in the study area at least twice weekly. In the morning I noted whether marked squirrels emerged from burrows in their natal areas, and in the evening I noted whether they returned to their natal areas for the night. I considered juveniles to have disappeared during the week-long age interval in which they were no longer observed in their natal areas. I classified juveniles that had disappeared as dispersers only if I later recovered them outside their natal areas. To relocate juveniles that had disappeared, I systematically trapped and visually searched the study site and its surrounding areas, and examined all road kills on the nearby highway. During morning and evening observations, and during daily visual searches of the study area, I recorded the distances at which juveniles were observed from their natal burrows.

OBSERVATION OF BEHAVIOR

To evaluate the intensity of resource competition, I observed the behavior of reproductive females at the study site for a total of 143 hours during the summer of 1995 using Altmann's (1974) focal animal survey technique and Holekamp's (1983) ethogram for *S. beldingi*. Specifically, I defined vigilant behavior as posting or alarm calling, and aggressive behavior as threatening, chasing, attacking, or fighting a conspecific. I attempted to observe each marked reproductive female in the study site for 20 minutes at least twice weekly, during the morning and late afternoon when squirrels were most active (Docekal, 1978). I calculated rates of vigilant and aggressive behavior for individuals as the number of minutes during which each behavior was observed per hour of observation. I determined these rates for females during two week intervals comprising the first and second halves of both gestation and lactation, and included in calculations only those females for which I had a total of at least 20 minutes of behavioral observations during the relevant time interval.

ESTIMATION OF POPULATION DENSITY

I calculated the densities of females that weaned litters in the study area in 1993 and 1994. I noted the initial appearance above ground of all litters weaned in these areas during regular observations. I used natural landmarks such as stands of trees and bushes, ponds, creeks, lakeshore, rock outcrops and other areas not inhabited by *S. beldingi* to delineate the boundaries of local sites within the study area. I estimated geometric areas of these sites using aerial photographs obtained from the United States Geological Survey (USGS), and direct measurements obtained using a 30 meter measuring tape. I calculated densities as the total number of females that weaned litters per hectare.

I also monitored overall density of *S. beldingi* in the study area in 1993 and 1994. I conducted scan samples in the study area hourly during regular observations. During each scan, I counted all *S. beldingi* present in a transect of approximately 800 m^2 entirely within the area I was watching. I calculated geometric areas of transects using USGS aerial photographs and measurements obtained with a 30 meter measuring tape. I calculated average density per hectare prior to and after the first appearance of juveniles above ground each year.

PROVISIONING

To manipulate body mass and body fat of juveniles, I provided them with extra food. I gave additional food to juveniles from 15 litters in 1993, 18 litters in 1994, 21 litters in 1995, and 20 litters in 1996. I provided these juveniles daily with sunflower seeds (700-1000 grams/litter), peanuts (350-500 grams/litter), or peanut butter (75-150 grams/litter) *ad lib*. Sunflower seeds were the primary food supplement in 1993 and 1994, and peanuts were the primary supplement in 1995 and 1996. I gave juveniles peanut butter occasionally when I had no sunflower seeds or peanuts. I provided supplemental food in plywood feeding boxes (25 by 20 by 10 cm) open at one end, and situated 2 to 3 meters from the entrance of the natal burrow. I covered the feeding boxes to prevent access by birds.

Reproductive female *S. beldingi* who receive supplemental food channel excess energy to their offspring, which are heavier at weaning than offspring of unprovisioned mothers (Trombulak, 1991). Therefore, I provided each adult female in provisioned areas daily with extra food (700-1000 grams of sunflower seeds, 350-500 grams of peanuts, or 75-150 grams of peanut butter) on her maternal territory from mid-gestation through lactation. In addition, following their first appearance above ground, members of all litters weaned by provisioned females were further provisioned in the natal area until they were 10 weeks of age. Provisioned areas were separated by at least 100 meters from areas inhabited by unprovisioned mothers and their young to ensure that unprovisioned individuals did not have free access to supplemental food. Provisioned and unprovisioned areas of the study site were alternated between years to control for local environmental effects.

EVALUATION OF BODY FAT

I measured body fat of juvenile *S. beldingi* during the summers of 1994-1996 with a device that analyzes body composition of small animals (model SA-2, EM-SCAN, Springfield, IL). This device uses electrical conductivity of the body to non-destructively estimate lean (fat-free) mass of live animals (Walsberg, 1988). I calculated mass of body fat for live subjects as the difference between total body mass and lean mass, and expressed fat as a percentage of total body mass.

I formulated a species-specific equation for *S. beldingi* to predict lean mass from readings of electrical conductivity of the body (Scott et al., 1991). This equation was generated from data acquired from 11 squirrels captured near my study site in May and June of 1994, and from four squirrels captured in August of 1995. I obtained readings of electrical conductivity from these squirrels, sacrificed them via anesthetic overdose, and stored them at -20° C for approximately 5 months. I dried whole carcasses to constant mass in a convection oven at 60° C, and then homogenized each carcass. I determined mass of body fat of carcasses by ether lipid extraction in a Soxtec apparatus (System HT 1043 Extraction Unit, Tecator AB, Hogana, Sweden). Lipid content of carcasses was similar to that reported by Morton (1975) for *S. beldingi* captured during May and June, 1970-1972 (approximately 5-10 grams). I obtained lean mass by subtracting fat mass from mass of total body. My prediction equation was calculated from a linear regression of lean mass on readings of electrical conductivity (Scott et al., 1991) for *S. beldingi* (Figure 1). My determinations of the percentage of body mass composed of fat for juveniles were similar to those Morton et al. (1974) obtained by solvent lipid extraction

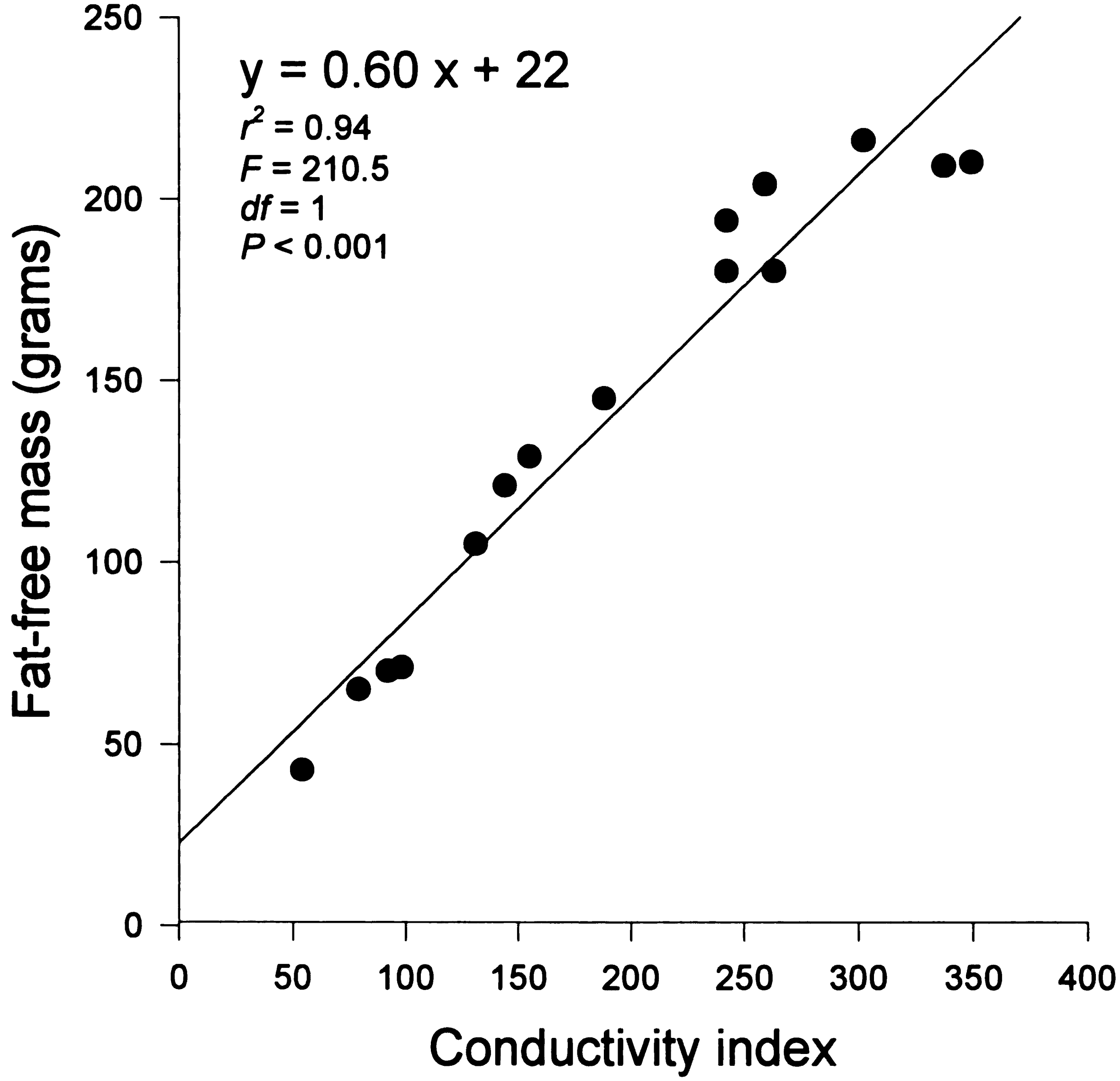

Figure 1 - Regression of fat-free mass on conductivity index in *S beldingi*. Values of the conductivity index were generated by a device which estimates lean body mass by evaluating electrical conductivity of the body.

(approximately 2% lipid before pre-hibernation fattening and 20-25% lipid at peak body mass).

For analysis of body composition of *S. beldingi*, I live-trapped subject animals and carried them 200-300 meters to a location on the periphery of the study site. I anesthetized squirrels with methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL) in a two liter anesthesia chamber before measuring body composition. After recovering from anesthesia (30-90 minutes), squirrels were released at their capture sites. The mean number times over the summer that body composition was measured in each juvenile was 3.9 ± 1.8 (SD) in 1994, 3.8 ± 1.7 (SD) in 1995, and 2.9 (SD) ± 1.5 in 1996.

HORMONE TREATMENTS

During the summers of 1993, 1995 and 1996 I treated newborn females with testosterone (T) to evaluate the effects of perinatal androgen exposure on dispersal behavior (Holekamp et al., 1984). I trapped pregnant females (1993: $n = 16$; 1995: $n = 69$; 1996: $n = 51$) on their maternal territories and transported them to a lab tent approximately 16 kilometers from the study site, where they were housed until they gave birth (1993: 6.1 ± 4.1 (SD) days; 1995: 5.2 ± 3.2 (SD) days; 1996: 7.9 ± 4.4 (SD) days). Pregnant females were kept individually in cages (60 by 40 by 15 cm), and were provided with nest boxes (25 by 20 by 10 cm, open at one end) and cotton nest material. I also gave pregnant females water, peanut butter, mouse chow, and fresh fruit *ad lib* daily. I checked nest boxes for the presence of young each day early in the morning, at midday, and in the evening. The date that young were first observed in a nest box was considered to be their date of birth. At birth, all females of a litter were injected subcutaneously either with T (500 µg in 0.05 ml sesame oil) or with sesame oil alone (0.05 ml). Males of a litter were not treated.

I returned each mother to her maternal territory with her litter within 24 hours of treatment, on the day after birth. I transported each mother along with her young and cotton nest material back to the study area in a small cooler, which was placed on the maternal territory at 0600 hours and allowed to settle for one hour. The cooler was then opened, after which the mother foraged and explored the surrounding area, usually for 30-60 min, before carrying her young and nest material to her maternal burrow. I stayed within three meters of the cooler until the mother came back for the last of her young. I warmed the young and defended them from predators and conspecifics when necessary.

BLOOD COLLECTION AND HORMONE ASSAYS

To evaluate circulating T levels of juveniles in relation to dispersal, I collected 89 blood samples (0.5-1.0 ml) from 71 juvenile males and 62 samples from 52 juvenile females in 1996. I anesthetized squirrels with methoxyflurane near their capture locations, and collected blood via cardiac puncture. I released squirrels at their capture sites after they recovered from anesthesia (30-90 minutes). I stored blood in liquid nitrogen (-196° C) for one to two months until I returned to Michigan, at which time I stored it at -20° C for an additional month. I then measured plasma T with I^{125} radioimmunoassay, using Coat-A-Count Total Testosterone assay kits (Diagnostic Products, Los Angeles, CA). Minimum assay sensitivity was 0.2 ng/ml.

STATISTICAL ANALYSES OF DATA

I monitored various dependent measures, including dispersal status, body mass, percentage of body mass composed of fat, age at dispersal, hourly rates of behavior, minimum distance from the natal burrow, litter size, and the number of females per litter. I analyzed frequency data (e.g., dispersal status) with chi-square tests or two-tailed Fisher's exact tests, depending on sample sizes (Sokal and Rohlf, 1981). I evaluated normally distributed body mass data with Analysis of Variance (ANOVA). Values of percent body fat were not distributed normally, so I transformed them to values that followed the normal distribution using the angular transformation, and analyzed these data with ANOVA. I analyzed discrete data (e.g., age at dispersal, litter size, number of females per litter) and data that were not normally distributed (e.g., rates of behavior) with nonparametric tests. I defined differences between groups to be significant when $P \leq 0.05$. I adjusted significance levels of multiple pairwise comparisons with Holm's procedure (Neter et al., 1996). Mean values of body mass, body fat, rates of behavior, and distance from the natal burrow are presented ± one standard error.

Most parametric and non-parametric statistical tests assume independence of observations. However, studies of free-living animals over time routinely yield multiple observations from the same individuals in the same and different time intervals (Hoogland, 1995). In my statistical analyses, I assumed dependence of data obtained from the same individual in the same time interval, and so averaged all data for each individual within each time interval. I assumed independence of data from the same individual in different time intervals (see Hoogland, 1995). Although data from the same individual are not truly independent across time intervals, in field studies of free-living animals they generally act as though they were (Dobson, personal communication).

In many of my statistical analyses, I used ANOVA to evaluate data over a series of age or time intervals. Although my data act as though they were independent across these intervals, they are not truly independent and so violate one of the basic assumptions of ANOVA. Thus, the results of my analyses with ANOVA should be interpreted with some caution.

Chapter 3

ONTOGENY OF MASS AND FAT ACQUISITION AND DISPERSAL

INTRODUCTION

Body mass, body size, and body condition may all exert important effects on life history traits of mammals (e.g., Iverson and Turner, 1974; Armitage et al., 1976; Barnes, 1984; Sauer and Slade, 1987; Trombulak, 1989, 1991; Kenagy et al., 1990a, 1990b; Dark et al., 1992; Bachman, 1993). It has been proposed that these variables influence temporal patterns of natal dispersal in various sciurid rodents. For example, late dispersal by smaller or lighter than average individuals, or by individuals in poor condition has been observed in Uinta ground squirrels *(S. armatus*; Balph and Stokes, 1963), round-tailed ground squirrels (*S. tereticaudus*; Dunford, 1977), Belding's ground squirrels (*S. beldingi*; Holekamp, 1984a), yellow-bellied marmots (*Marmota flaviventris*; Downhower and Armitage, 1981), and Gunnison's prairie dogs *(Cynomys gunnisoni*; Rayor, 1985). Holekamp (1986) noted that of eleven variables examined in juvenile *S. beldingi*, body mass was the most reliable predictor of an individual's dispersal status at any time. On the basis of this relationship between body mass and dispersal status, she proposed an hypothesis suggesting that dispersal by *male S. beldingi* is triggered by attainment during ontogeny of a particular body mass or body composition, or some combination of these two variables.

Here I evaluated the relationship between the ontogenetic development of body mass, body fat, and dispersal behavior in juvenile male *S. beldingi*. I manipulated body mass and body fat of juveniles by provisioning them with food, and used unprovisioned juveniles as controls. I monitored dispersal status of provisioned and unprovisioned juveniles throughout the active season. My results suggest that natal dispersal in *S. beldingi* is constrained by the energetic demands of depositing fat for hibernation. My results further indicate that juvenile male *S. beldingi* postpone dispersal until they have attained a threshold body mass, and that the percentage of body mass composed of fat may importantly influence the timing of dispersal.

METHODS

I trapped and ear-tagged 135 juvenile males from 65 complete litters during the summers of 1993 and 1994. I gave extra food to 35 males from 15 litters in 1993, and 40 males from 18 litters in 1994. I used 15 males from 10 litters in 1993 and 45 males from 22 litters in 1994 as unprovisioned controls. I measured body mass of these males weekly from 4-11 weeks of age in 1993 and 1994, and also monitored dispersal status weekly from 6-10 weeks of age and recorded minimum distances from the natal burrows at which I observed juveniles from 6-10 weeks of age. In 1994, I also measured body fat weekly from 6-11 weeks of age.

RESULTS

Body mass did not differ significantly between 1993 and 1994 for either provisioned males (*t*-test for independent samples, $P = 0.649$) or unprovisioned males (*t*-

test for independent samples, $P = 0.126$). Therefore, body mass data for the two years were combined for subsequent statistical analyses. Provisioned males were significantly heavier than unprovisioned males throughout the active season (Figure 2; ANOVA, $F = 98.7$, $df = 1$, $P < 0.001$).

Mean minimum distances from the natal burrow at which I observed juveniles increased steadily from six to ten weeks of age for both provisioned males (Figure 3; Kruskal-Wallis test, test statistic = 52.455, $P < 0.001$) and unprovisioned males (Figure 3; Kruskal-Wallis test, test statistic = 71.358, $P < 0.001$). Mean minimum distances from the natal burrow were significantly greater overall for provisioned than unprovisioned males at 6-10 weeks of age (Figure 3; Kruskal-Wallis test, $U = 757.5$, $P = 0.012$). In particular, provisioned juveniles were observed significantly farther from the natal burrow at 7 weeks of age (Figure 3; Kruskal-Wallis test, $U = 757.5$, $P = 0.002$).

Ages at which dispersal occurred also differed significantly between provisioned and unprovisioned males (Figure 4; $\chi^2 = 13.437$, $df = 4$, $P = 0.009$). Provisioned males began dispersing at a younger age than did control males (Figure 4; 6 weeks vs. 7 weeks), and had a younger modal age of dispersal (Figure 4; 7 weeks vs. 8 weeks). A larger cumulative percentage of provisioned than unprovisioned males had dispersed during each age interval from six to ten weeks of age, and significant differences were observed from six to eight weeks of age (Figure 5; 6 weeks: Fisher's exact test, $P = 0.038$; 7 weeks: Fisher's exact test, $P < 0.001$; 8 weeks: $\chi^2 = 5.291$, $df = 1$, $P = 0.021$). Mean body mass at dispersal did not differ significantly between provisioned and unprovisioned males (192.0 ± 10.1 grams, $n = 10$, vs. 183.0 ± 7.4 grams, $n = 15$, respectively; t-test for independent samples, $P = 0.470$). Body mass at dispersal was constant at about 185

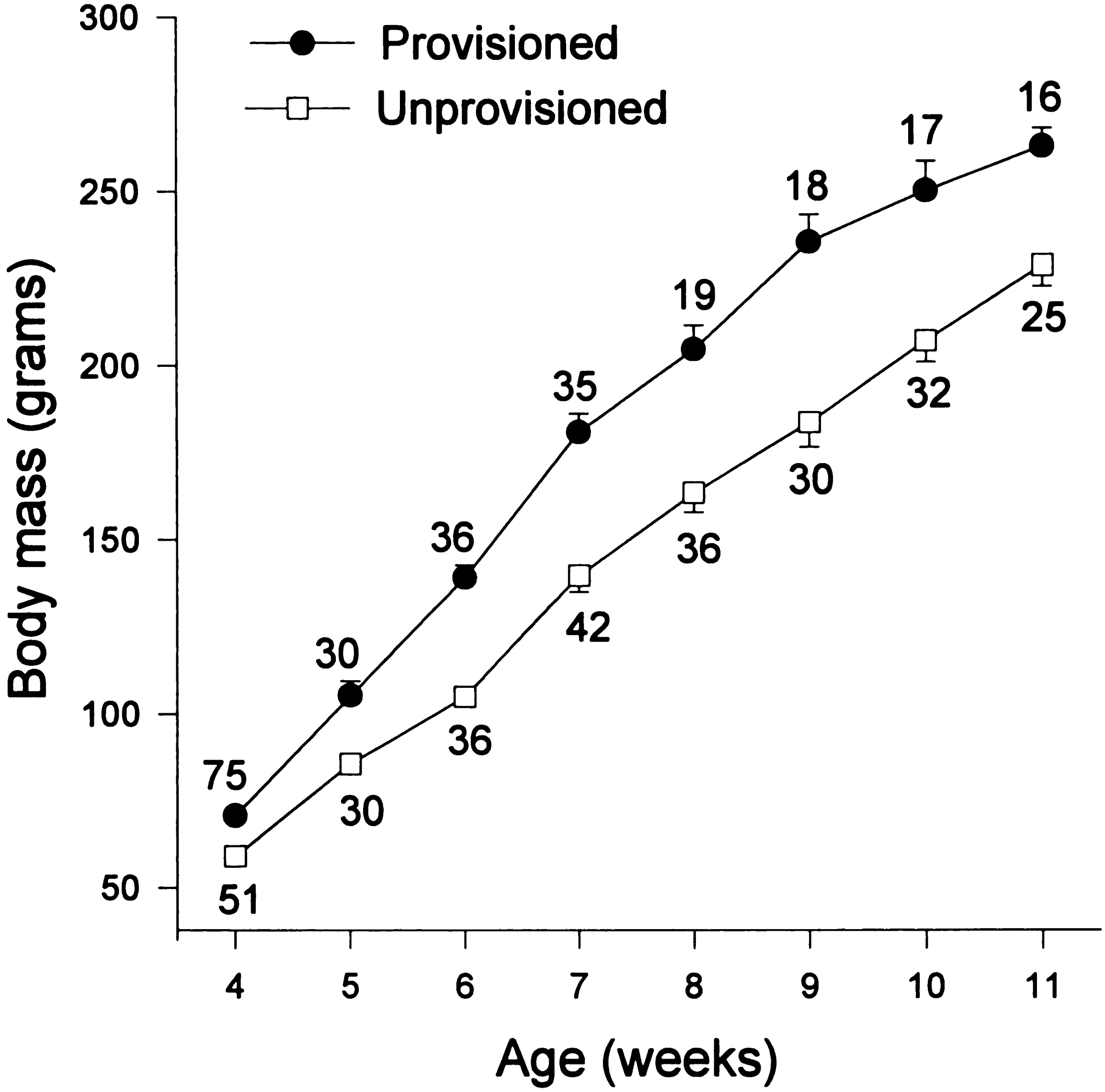

Figure 2 - Body mass of juvenile males, 1993-1994. Sample sizes are given on the graph.

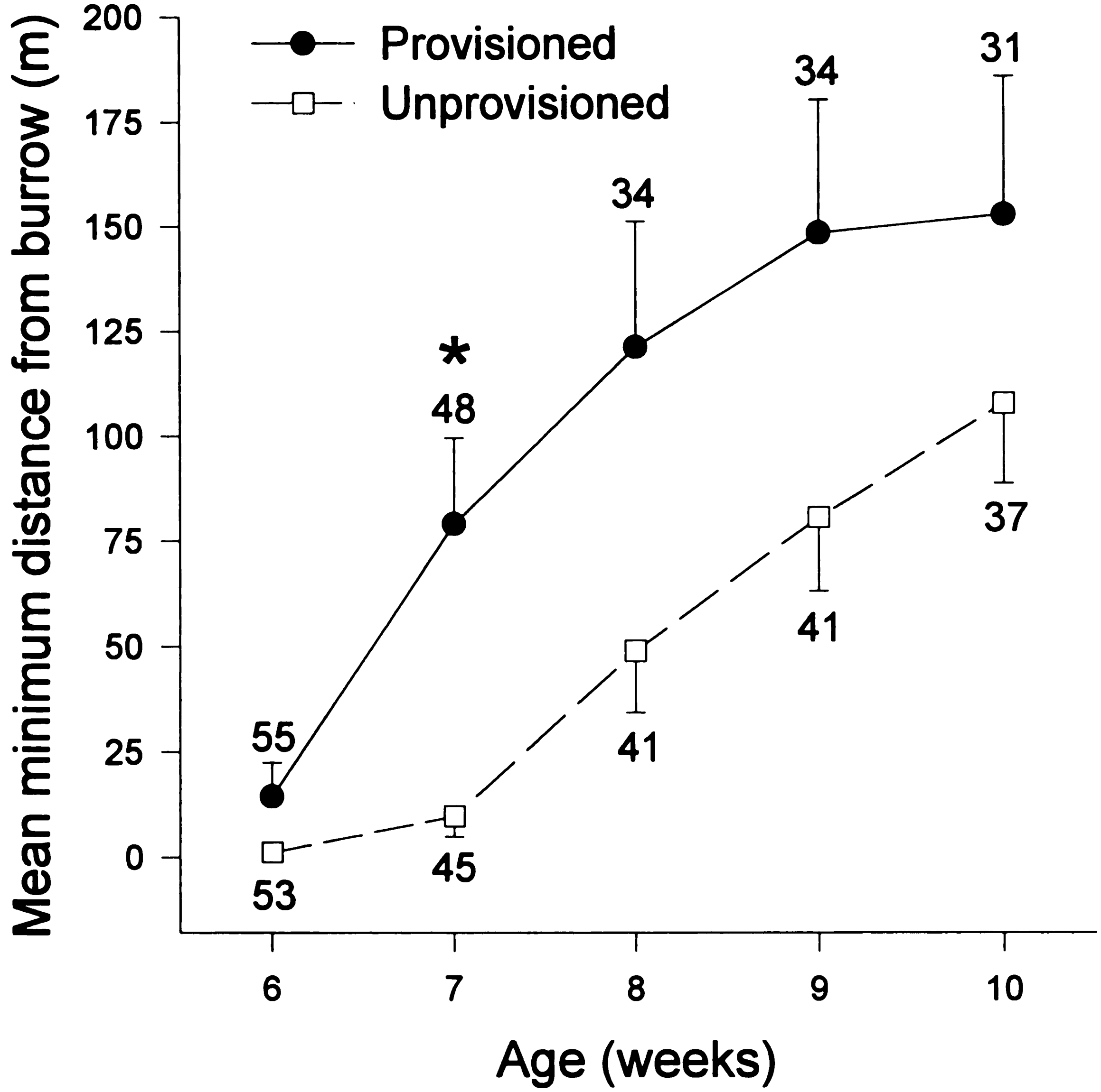

Figure 3 - Distances of juvenile males from their natal burrows, 1993-1994. The asterisk indicates a significant difference between provisioned and unprovisioned males. Sample sizes are given on the graph.

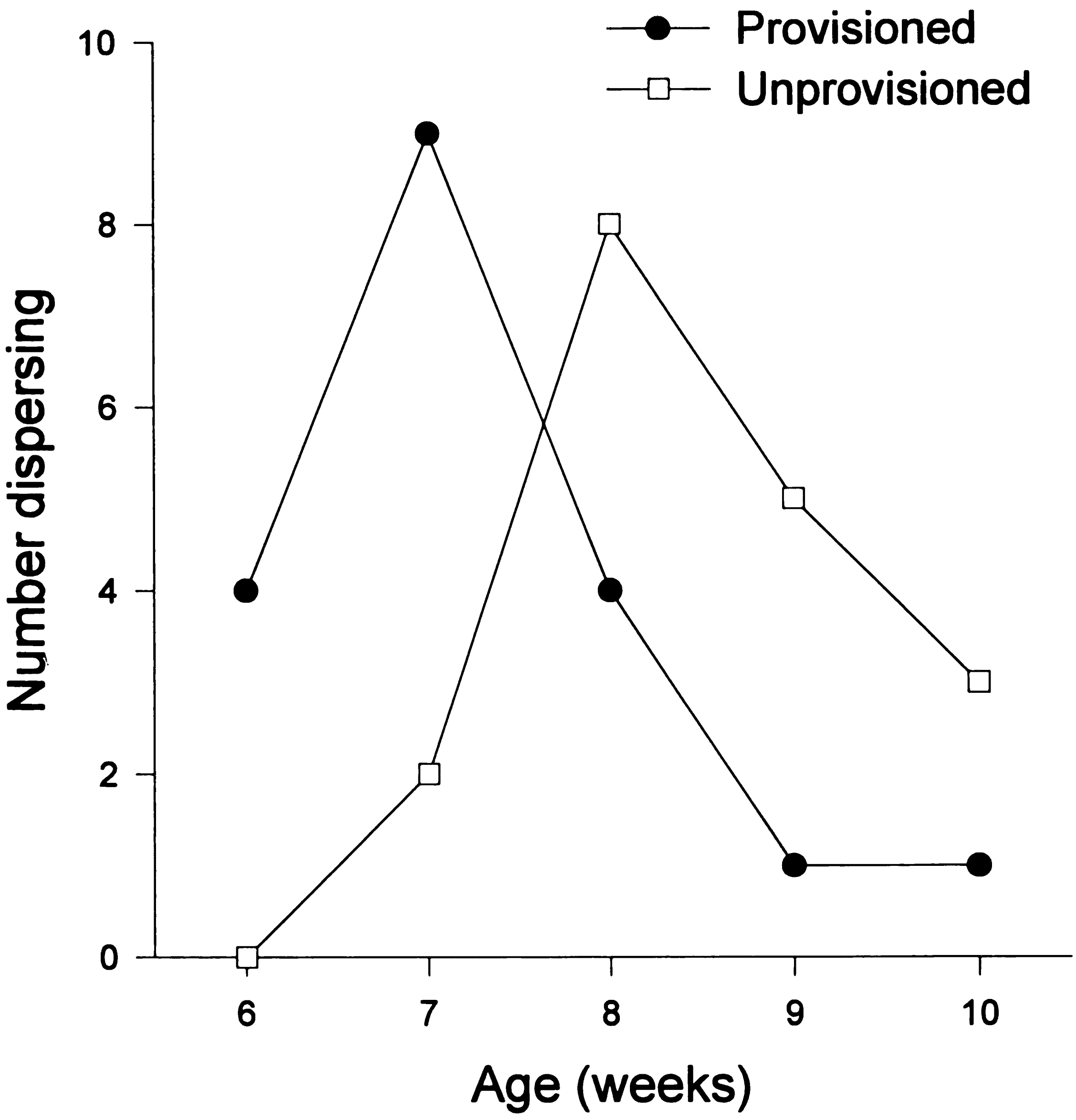

Figure 4 - Dispersal of juvenile males at various ages, 1993-1994.

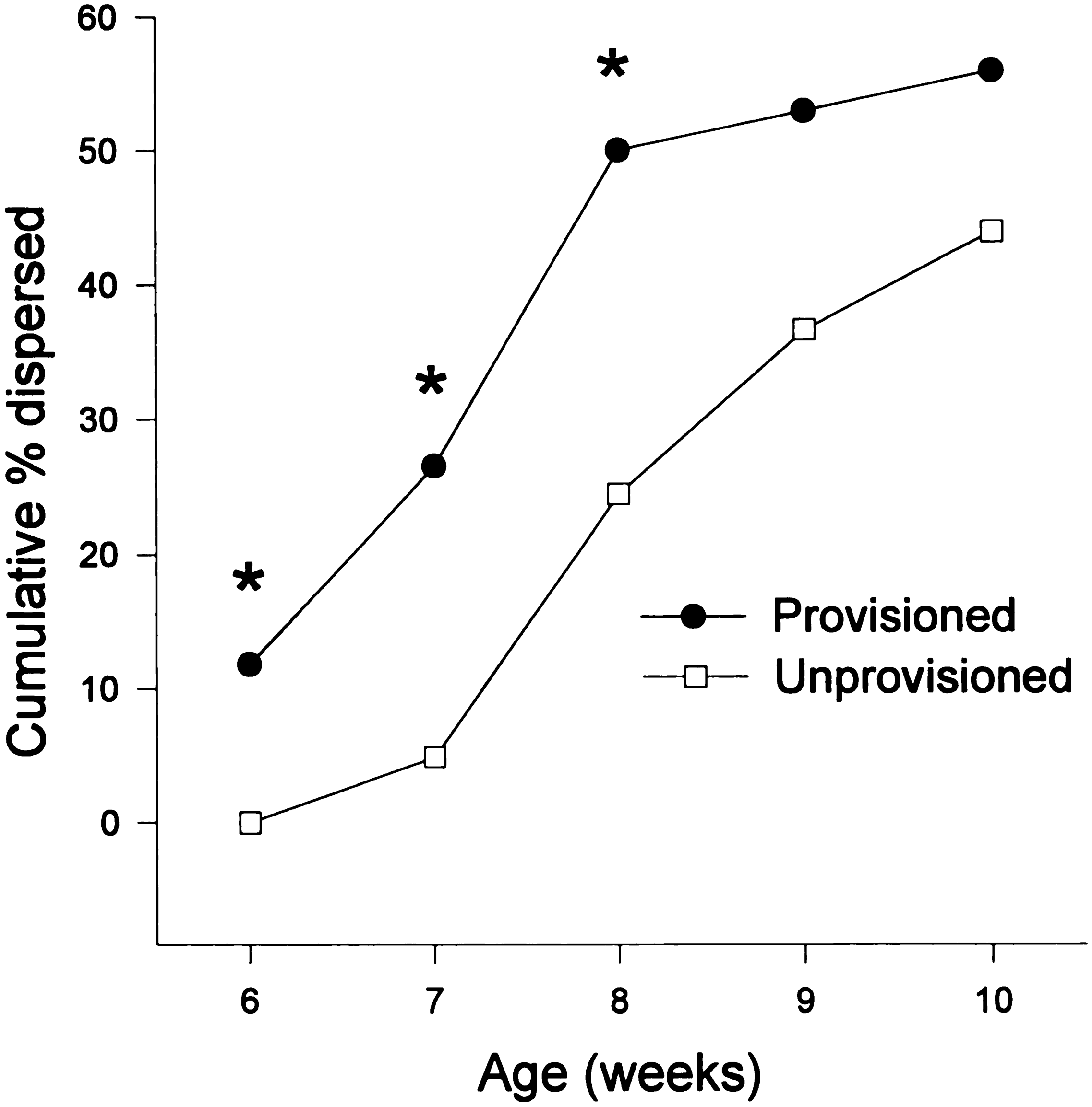

Figure 5 - Cumulative dispersal of juvenile males, 1993-1994. Asterisks indicate a significant difference between provisioned and unprovisioned males. Samples included 34 provisioned and 41 unprovisioned males.

grams, regardless of differences among individuals in age at dispersal (Figure 6; ANOVA, $P = 0.600$). This indicates that there may be a mass threshold for dispersal. Provisioned and unprovisioned juveniles did not differ in body mass relative to time of dispersal (Figure 7; ANOVA, $P = 0.850$). This further indicates that body mass importantly influences the timing of dispersal.

Seven males in the 1993 cohort and 17 males in the 1994 cohort were known to be alive at 55 weeks of age. All of these males, in both provisioned and unprovisioned groups, emigrated from their natal areas before reaching 55 weeks of age. Thus, food provisioning did not alter the probability that males would eventually leave their birth sites.

Fat, as a percentage of body mass, was greater in provisioned than unprovisioned juvenile males overall from 6-11 weeks of age (Figure 8; ANOVA, $F = 19.554$, $df = 1$, $P < 0.001$). In particular, provisioned males were fatter than their unprovisioned counterparts at 6 and 7 weeks of age (Figure 8; *t*-tests for independent samples; 6 weeks: $t = -4.214$, $df = 31$, $P < 0.001$; 7 weeks: $t = -4.053$, $df = 33$, $P < 0.001$). There was a marginally significant difference in body mass between provisioned and unprovisioned males at 8 weeks of age (Figure 8; *t*-test for independent samples; $t = -1.905$, $df = 29$, $P = 0.067$). At ages 9-11 weeks, however, body fat of provisioned males was similar to that of unprovisioned males (Figure 8). Thus, food availability or food quality appears to be a strong determinant of how much body fat a male has early, but not late, during his juvenile summer. Moreover, there appears to be a close association between body fat and dispersal. A greater proportion of provisioned than unprovisioned males dispersed early during ontogeny, by 6-8 weeks of age, when provisioned males tended to be fatter than unprovisioned males. However, after 8 weeks of age, there was no significant difference

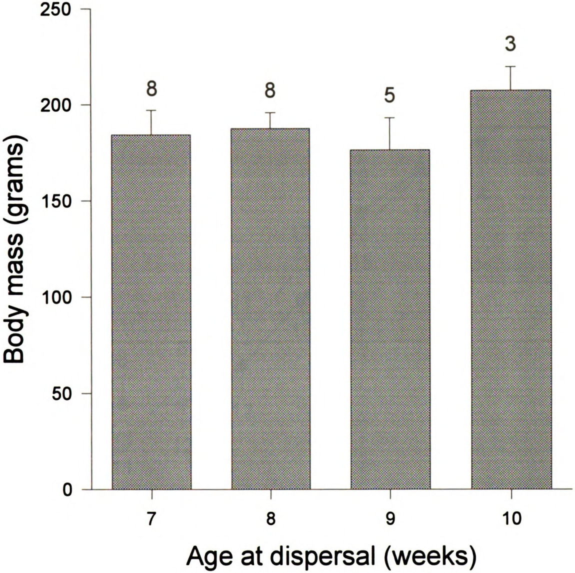

Figure 6 - Body mass of juvenile males at dispersal, 1993-1994. Sample sizes are given on the graph.

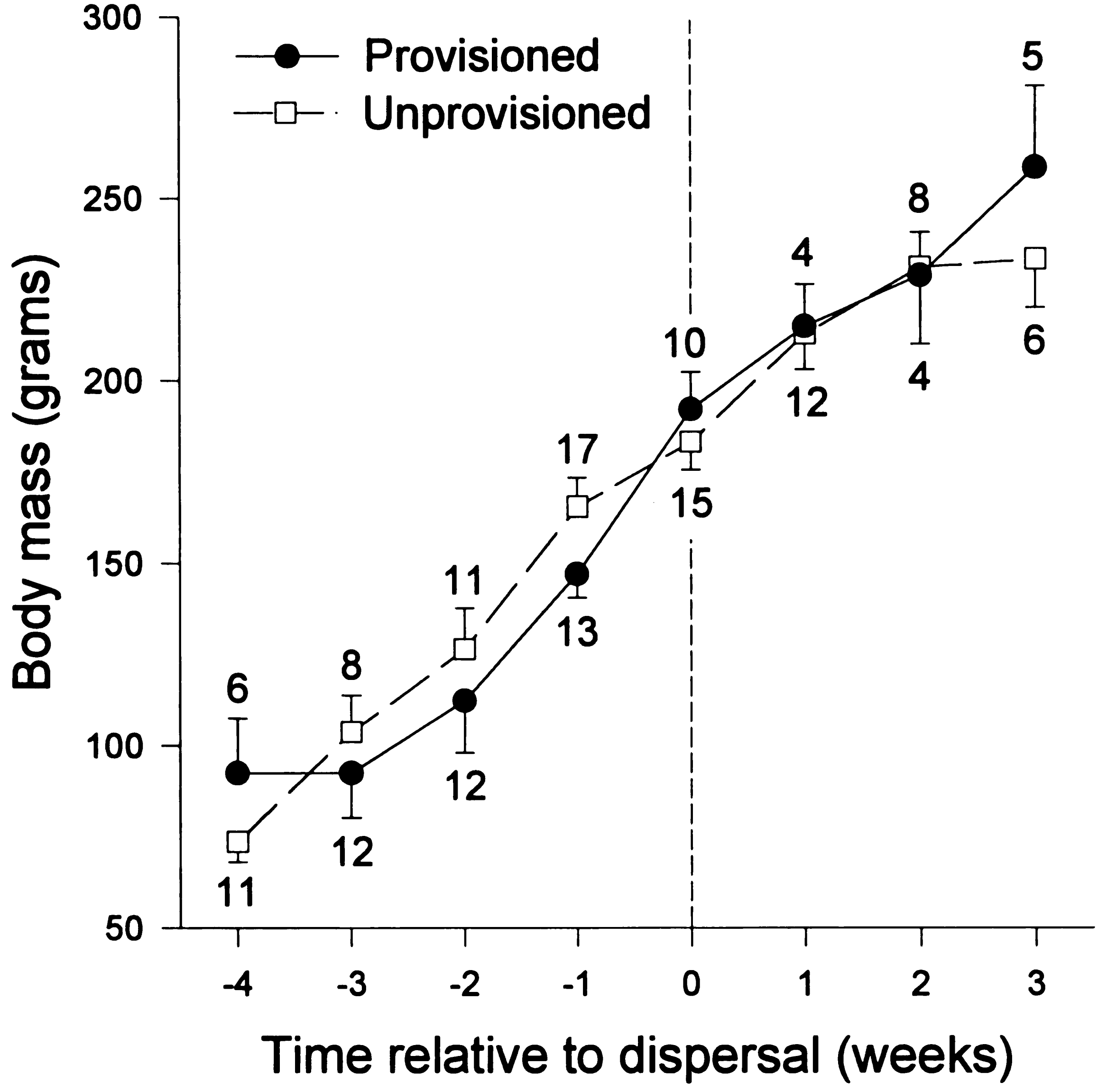

Figure 7 - Body mass of juvenile males relative to dispersal, 1993-1994. The vertical dashed line indicates the week of dispersal. Samples sizes are given on the graph.

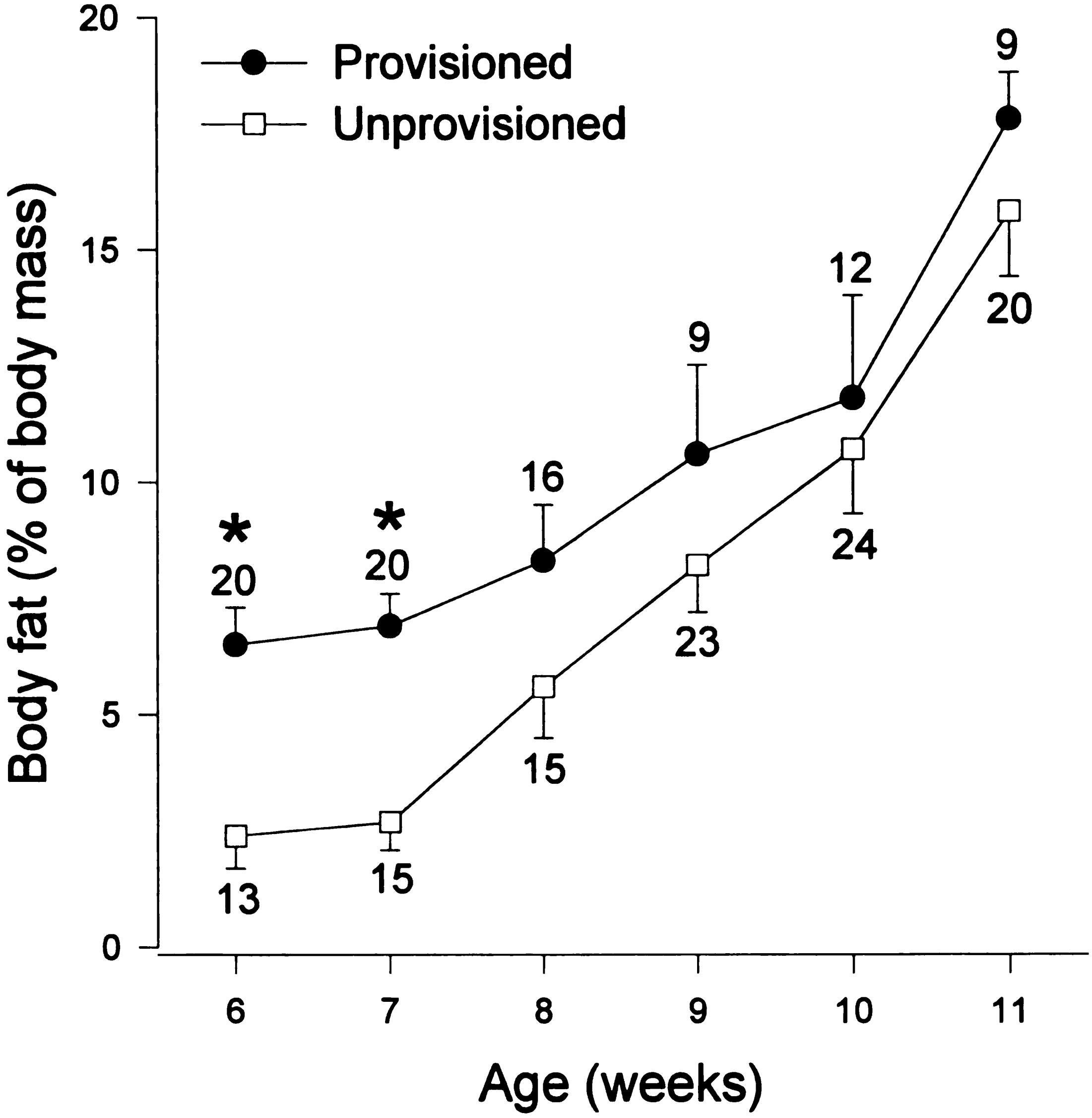

Figure 8 - Body fat of juvenile males, 1994. Asterisks indicate a significant difference between provisioned and unprovisioned males. Sample sizes are given on the graph.

between provisioned and unprovisioned males either in the proportion dispersed or in body fat.

During 1994, I recovered 25 dispersed juvenile males, Twenty-one of these (84%) emigrated between 27 July and 25 August. Fat, as a percentage of body mass, increased steadily in these juveniles, and differed significantly among 10-day intervals within this period (Figure 9; repeated measures ANOVA; provisioned: $F = 3.757$, $df = 2$, $P = 0.043$; unprovisioned: $F = 13.119$, $df = 2$, $P < 0.001$). Thus most dispersal behavior occurred during a period of rapid fat increase.

I compared body fat of males dispersing during the 7 to 10 week age intervals with that of males still residing in their natal areas. Fat, as a percentage of body mass, was greater overall for dispersing males than for males that had not yet dispersed (Figure 10; ANOVA, $F = 7.851$, $df = 1$, $P = 0.006$), and it was significantly greater in dispersing males than in undispersed males at 8 weeks of age (Figure 10; *t*-test for independent samples, $t = 3.490$, $df = 23$, $P = 0.002$).

DISCUSSION

Patterns of natal dispersal by juvenile male *S. beldingi* in 1993-1994 were similar to those observed by Holekamp (1984a). In both studies, natal dispersal began at 6-7 weeks of age, mean minimum distances at which juvenile males were observed from their natal burrows increased steadily throughout the active season, and 100% of surviving males dispersed before 55 weeks of age. This suggests that food provisioning left intact the normal dispersal behavior of males. This confirms prior studies, in which provisioning

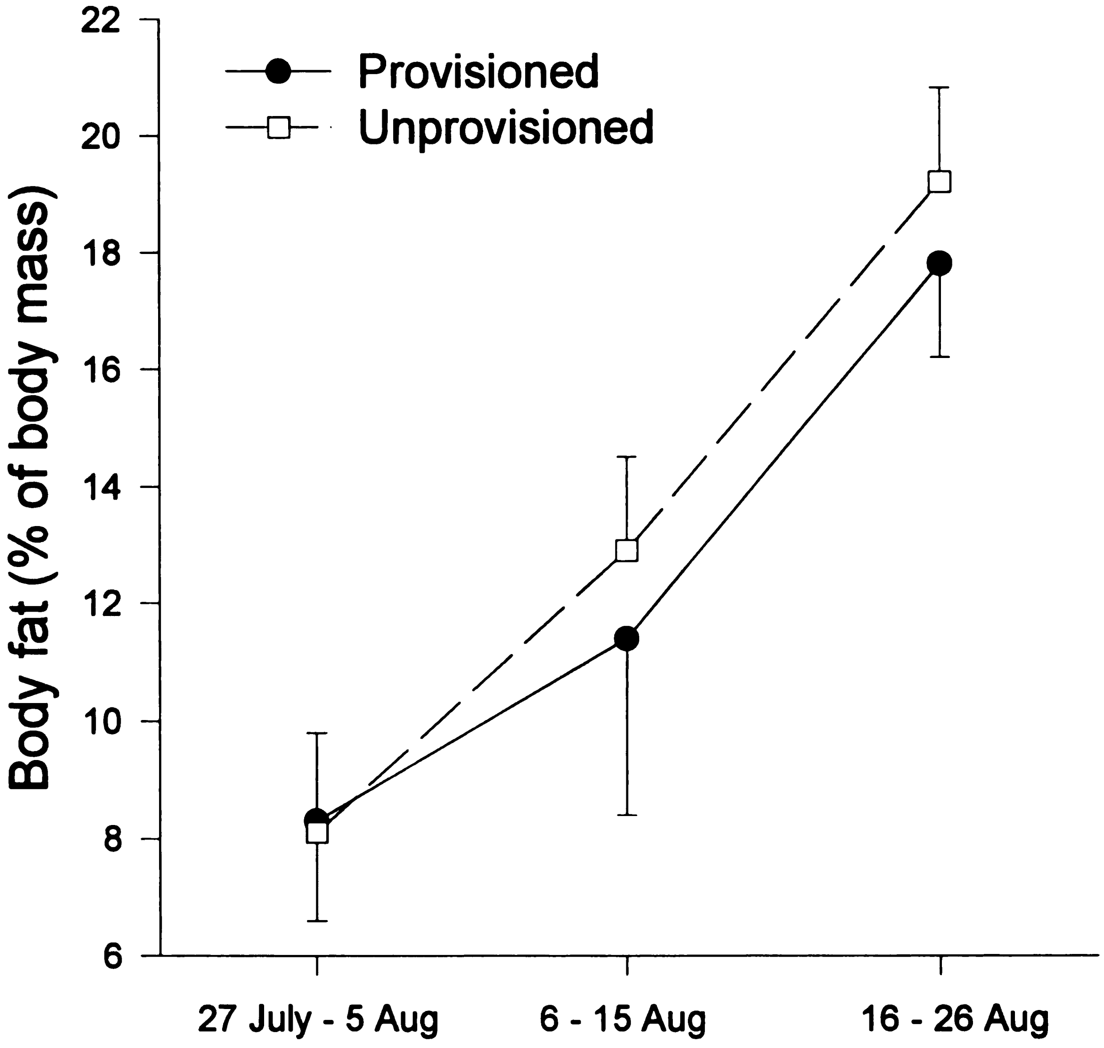

Figure 9 - Body fat of juvenile males during the primary dispersal period, 1994. Samples included repeated measures for 7 provisioned and 14 unprovisioned males.

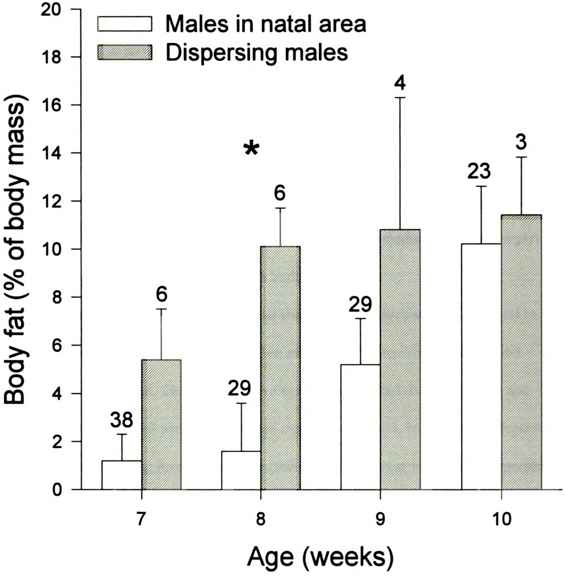

Figure 10 - Body fat of dispersing and natal males, 1994. Mean body fat is presented during each age interval for males dispersing during that interval, and for males still residing in their natal areas. The asterisk indicates a significant difference between dispersing and natal males. Sample sizes are given on the graph. Samples include both provisioned and unprovisioned males.

did not affect the probability of dispersal by male *S. beldingi* (Trombulak, 1991) or by male California ground squirrels (*S. beecheyi*; Dobson, 1979).

Holekamp (1984a, 1986) suggested that there was a relationship between body mass and dispersal in juvenile male *S. beldingi*, but noted that body mass alone could not explain the timing of natal dispersal, because some heavier than average *S. beldingi* delayed dispersal. She proposed that body composition also influences the timing of natal dispersal, and that juvenile male *S. beldingi* disperse only after attainment of some threshold of body mass or body fat. In this study, food provisioning caused an advance in the timing of dispersal behavior. I suggest that this effect was mediated, at least in part, by body fat or some variable correlated with body fat.

My results support the hypothesis that there is a mass threshold for dispersal in *S. beldingi*. Provisioned males were heavier than same-aged unprovisioned males, and dispersed at younger ages. However, relative to time of dispersal, body mass did not differ between provisioned and unprovisioned males. In addition, body mass at dispersal was fairly constant among juvenile males, regardless of the ages at which they dispersed. Thus juvenile males may need to attain a minimum degree of physical growth and development, as reflected in body mass, prior to dispersing.

Body fat appears to exert a strong influence on the timing of dispersal. A greater proportion of provisioned than unprovisioned males dispersed in the 6-8 week age intervals, when provisioned males were fatter than their unprovisioned counterparts. However, from 9 to 10 weeks of age, neither body fat nor the cumulative percentage of males dispersed differed significantly between provisioned and unprovisioned males.

Moreover, body fat was greater in dispersing males than in same-aged males still residing in their natal areas.

Juvenile *S. beldingi* enter hibernation when body mass is about 200-260 grams, and when body fat is about 20-25% of total body mass (Morton et al., 1974). Attainment of a specific minimum body mass and percentage of body mass composed of fat by the end of the summer for juveniles may be critical to both the survival and future reproductive success of male *S. beldingi*. Overwinter survival of juveniles is positively correlated with pre-hibernation body mass in *S. beldingi* (Trombulak, 1991), and other hibernators (e.g., Sullivan and Sullivan, 1982; Sullivan et al., 1983; Murie and Boag, 1984; Dobson and Kjelgaard, 1985). Furthermore, a positive correlation between body mass and mating success has been observed in *S. beldingi* (Sherman, 1976). Energetic costs of dispersal behavior may slow the storage of body fat required to sustain the young animal throughout hibernation. Juvenile males may therefore delay dispersal until they achieve sufficient body mass and body fat to prevent these costs from compromising mass and fat acquisition necessary during the juvenile summer.

Early dispersal from the natal site and immigration into a new area may confer a variety of advantages on juvenile mammals, as it has been proposed to do in some species of birds. Juvenile crested tits (*Parus cristatus*) that disperse early are more likely to settle in favorable habitats, whereas late dispersers are more likely to immigrate into marginal habitats (Lens and Dhont, 1994). Juvenile male marsh tits (*Parus palustris*) are less apt to be targets of aggression and are more successful at establishing themselves in new areas when they disperse at early ages (Nilsson and Smith, 1988; Nilsson, 1989a, 1989b). Thus, plasticity of dispersal behavior in response to body mass and body fat may enable juveniles to coordinate physical and physiological readiness with optimal timing of dispersal.

Body mass, body fat, and availability of metabolic fuels have been shown to influence the timing of energetically expensive life history events such as reproduction (e.g. French, 1982; Barnes, 1984; Bushberg and Holmes, 1985; Wade and Schneider, 1992). Little is known about the actual energetic costs of dispersal behavior. However, the apparent sensitivity of dispersal behavior in *S. beldingi* to influences of body mass and body fat suggests that energetic requirements may constrain dispersal in *S. beldingi* and other mammals, as they do reproduction. Natal dispersal is associated with increased locomotor and exploratory activity (Holekamp, 1986; Ritchison et al., 1992). In addition, dispersers often encounter elevated levels of conspecific aggression when attempting to immigrate into new areas (Holekamp, 1986; Pusey and Packer, 1987a). Thus, dispersing males may need to reallocate time spent foraging to vigilant and defensive behavior. Moreover, dispersing individuals may need to be more vigilant as they explore unfamiliar areas, where they may be more vulnerable to predation (Metzgar, 1967; Snyder et al., 1976). Bachman (1993) observed a behavioral tradeoff in *S. beldingi* in which the amount of time spent alert was inversely proportional to the amount of time spent feeding. Thus, increases in activity associated with dispersal may not only increase energy requirements, but may also decrease energy intake.

Kenagy (1980) observed a specific temporal relationship between circannual cycles of hibernation and reproduction in male golden-mantled ground squirrels (*S. lateralis*). Testicular development in *S. lateralis* begins during the final three months of hibernation, and is complete when hibernation ends. Energy to support hibernation and testicular development is derived from stored fat. Males also rely on fat reserves during mating, which occurs immediately after emergence from hibernation. Testicular development is inhibited in underweight *S. lateralis*, which apparently do not have sufficient fat reserves to support both hibernation and reproduction (Barnes, 1984). Thus, the energy demands of hibernation and reproduction conflict in *S. lateralis*.

My results here suggest that a similar conflict occurs in *S. beldingi* between the energy demands of dispersal and those of hibernation. Most dispersal by juvenile males in 1993 and 1994 occurred when the percentage of body mass composed of fat was rapidly increasing, which suggests that dispersal competes with pre-hibernation fattening for available energy. Dispersal might be suppressed when, for a juvenile with a given percentage of body mass composed of fat, insufficient time remains in the active season to take in enough energy both to fuel dispersal behavior and to build fat reserves adequate for overwinter survival.

Chapter 4

INTERACTION OF TIME OF YEAR AND ENERGETIC VARIABLES

INTRODUCTION

Little is known about the mechanisms that regulate the timing of dispersal behavior. However, Smale et al. (1997) suggested that in species such as *S. beldingi*, which exhibit seasonal cycles of reproduction and behavior, the timing of dispersal might be influenced by circannual or other seasonal time-keeping mechanisms. Moreover, the amount of energy stored as fat should theoretically have an important influence on the timing of dispersal. Animals should delay dispersal until their fat reserves are adequate to meet the potential energy requirements of the dispersal movement (Nunes and Holekamp, 1996). Dispersing juvenile male *S. beldingi* do in fact tend to be fatter than same-aged males still residing in the natal area (Nunes and Holekamp, 1996). Here I explored the possibility that fat-related energetic variables interact with circannual or other seasonal time-keeping mechanisms to mediate dispersal in juvenile male *S. beldingi*.

Many behaviors, physiological traits, and life history events in ground-dwelling squirrels exhibit circannual rhythmicity (e.g., Davis, 1976; French, 1982; Barnes and York, 1991; Zucker et al., 1991). Similarly, body mass in ground squirrels undergoes predictable, endogenously driven annual rhythms, which persist in the absence of environmental cues (e.g. Pengelley and Fisher, 1963; Davis, 1976; Mrosovsky, 1976; Mrosovsky and Powley, 1977; Zucker and Boshes, 1982; Smale et al., 1986; Zucker et al., 1991). Changes in the size of fat stores are responsible for these changes in body mass (Dark et al., 1984, 1985; Forger et al., 1986). In juvenile *S. beldingi*, mass and fat appear to be regulated at seasonally appropriate levels that steadily increase prior to hibernation, and gradually decrease during hibernation (French, 1982; Bachman, 1993, 1994; Nunes and Holekamp, 1996).

Dispersing and depositing fat in preparation for hibernation both present energetic challenges to *S. beldingi*. Increases in activity associated with dispersal may compromise an individual's capacity to gain fat needed for survival during winter. Thus, at the phase of the annual cycle during which squirrels prepare for hibernation, dispersal and fattening might conflict with each other, and emigration might be delayed when it would compromise an individual's ability to acquire fat stores necessary for overwinter survival (Nunes and Holekamp, 1996). Circannual or other time-keeping mechanisms might be involved in the physiological processes determining whether an individual disperses or uses available energy to build fat reserves.

Heavy snowfall during the spring and summer of 1995 substantially delayed the active season of *S. beldingi* compared to its active seasons in 1994 and 1996. This fortuitous situation allowed me to examine an hypothesis suggesting that energy stores and time of year interact in juvenile male squirrels to mediate dispersal behavior. In particular, I tested the prediction of the hypothesis that juveniles having little time to fatten for hibernation should use their time and energy to build fat reserves necessary for overwinter survival rather than for dispersal. Thus, these animals should delay emigration from the natal area until the yearling summer. By contrast, individuals having time to store sufficient fat to support both hibernation and dispersal should emigrate as juveniles.

METHODS

In this phase of my research I examined body mass, body fat and dispersal status of juvenile male *S. beldingi* that were unprovisioned. During the summers of 1994-1996, I trapped and ear-tagged 182 juvenile males from 79 complete litters. I marked 45 males from 22 litters in 1994, 84 males from 33 litters in 1995, and 53 males from 24 litters in 1996. I measured body mass weekly from 4-11 weeks of age, and body fat weekly from 7-10 weeks of age. I also monitored dispersal status of juveniles each week from 6-10 weeks of age in 1994 and 1995, and from 6-9 weeks of age in 1996.

RESULTS

Compared to 1994 and 1996, initial emergence of litters from natal burrows was substantially delayed by heavy spring snowfall in 1995 (Figure 11). Litters emerged from 2-22 July in 1994, from 10-24 Aug in 1995, and from 4-26 July in 1996. The median date of litter emergence was 7 July in 1994 and 9 July in 1996, but was considerably later, 17 Aug, in 1995.

The number of young that females weaned in 1995 was not affected by delays resulting from heavy spring snowfall. Mean litter size did not differ significantly between years from 1994-1996 (ANOVA; $P = 0.567$). Mean litter size was 4.182 ± 0.340 (SE) in 1994, 4.667 ± 0.353 (SE) in 1995, and 4.667 ± 0.339 (SE) in 1996.

Body mass of juveniles overall from 4-11 weeks of age differed significantly between years from 1994 through 1996 (Figure 12; ANOVA, $F = 45.580$, $df = 2$, $P < 0.001$). I also observed a significant interaction of age with year in the comparison of body mass between years from 1994-1996 (Figure 12; ANOVA, $F = 3.136$, $df = 14$, $P <$

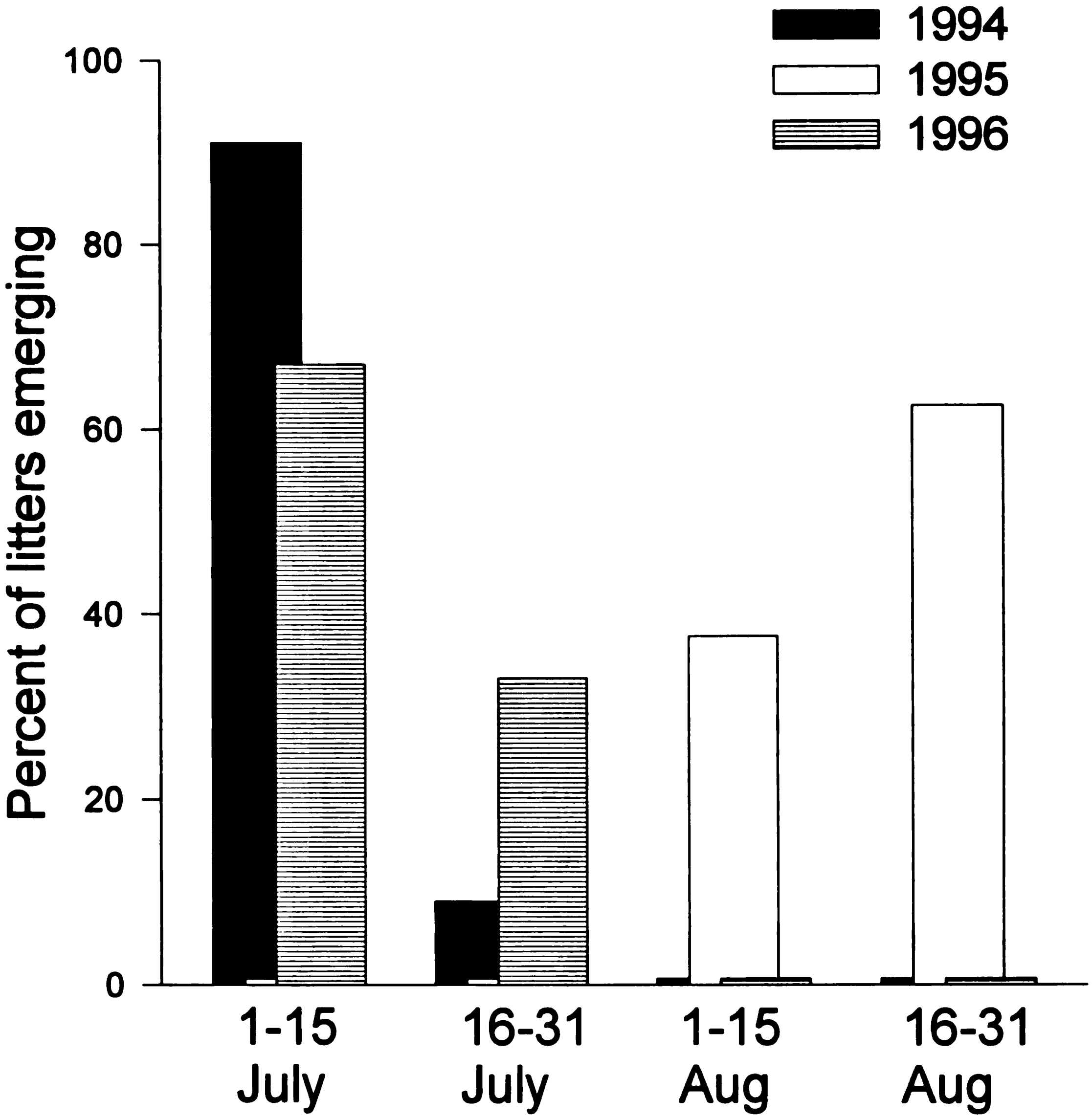

Figure 11 - First emergence of litters from the natal burrow, 1994-1996. Samples included 22 litters in 1994, 33 litters in 1995, and 24 litters in 1996.

0.001). In particular, patterns of mass acquisition in 1995 differed from those in 1994 and 1996. Beginning at about 7-8 weeks of age, male body mass increased more rapidly in 1995 (Figure 12). Juvenile males (aged 8-11 weeks) in 1995 were significantly heavier than were juvenile males in either 1994 or 1996 (Figure 12; *t*-tests for independent samples; 1995 vs. 1994: 8 weeks: $t = -3.381$, $df = 63$, $P = 0.001$; 9weeks: $t = -3.270$, $df = 68$, $P = 0.002$; 10 weeks: $t = -3.167$, $df = 59$, $P = 0.002$; 11 weeks: $t = -3.996$, $df = 26$, $P < 0.001$; 1995 vs. 1996: 8 weeks: $t = 4.967$, $df = 56$, $P < 0.001$; 9 weeks: $t = 3.743$, $df = 55$, $P < 0.001$; 10 weeks: $t = 3.872$, $df = 47$, $P < 0.001$; 11 weeks: $t = 3.006$, $df = 10$, $P = 0.013$). Juvenile females in 1995 also gained body mass more rapidly, beginning at about 7-8 weeks of age, than did juvenile females in either 1994 or 1996 (Nunes, unpublished data).

Body fat at ages 7-11 weeks differed significantly between years from 1994-1996 (Figure 13; ANOVA, $F = 14.862$, $df = 2$, $P < 0.001$). There was also a significant interaction of age with year in the comparison of body fat between years (Figure 13; ANOVA, $F = 3.417$, $df = 8$, $P = 0.001$). Starting at about 9 weeks of age, fat deposition occurred more rapidly in 1995 than in either 1994 or 1996. Body fat at ages 9-11 weeks was significantly greater in 1995 than it was in either 1994 or 1996 (Figure 13; *t*-tests for independent samples; 1995 vs. 1994: 9weeks: $t = -3.972$, $df = 58$, $P < 0.001$; 10 weeks: $t = -4.061$, $df = 57$, $P < 0.001$; 11 weeks: $t = -3.508$, $df = 23$, $P = 0.002$; 1995 vs. 1996: 9 weeks: $t = -4.549$, $df = 47$, $P < 0.001$; 10 weeks: $t = 5.186$, $df = 45$, $P < 0.001$; 11 weeks: $t = 3.031$, $df = 9$, $P = 0.014$). Juvenile females also fattened more rapidly in 1995, beginning at about 9 weeks of age, than did juvenile females in either 1994 or 1996.

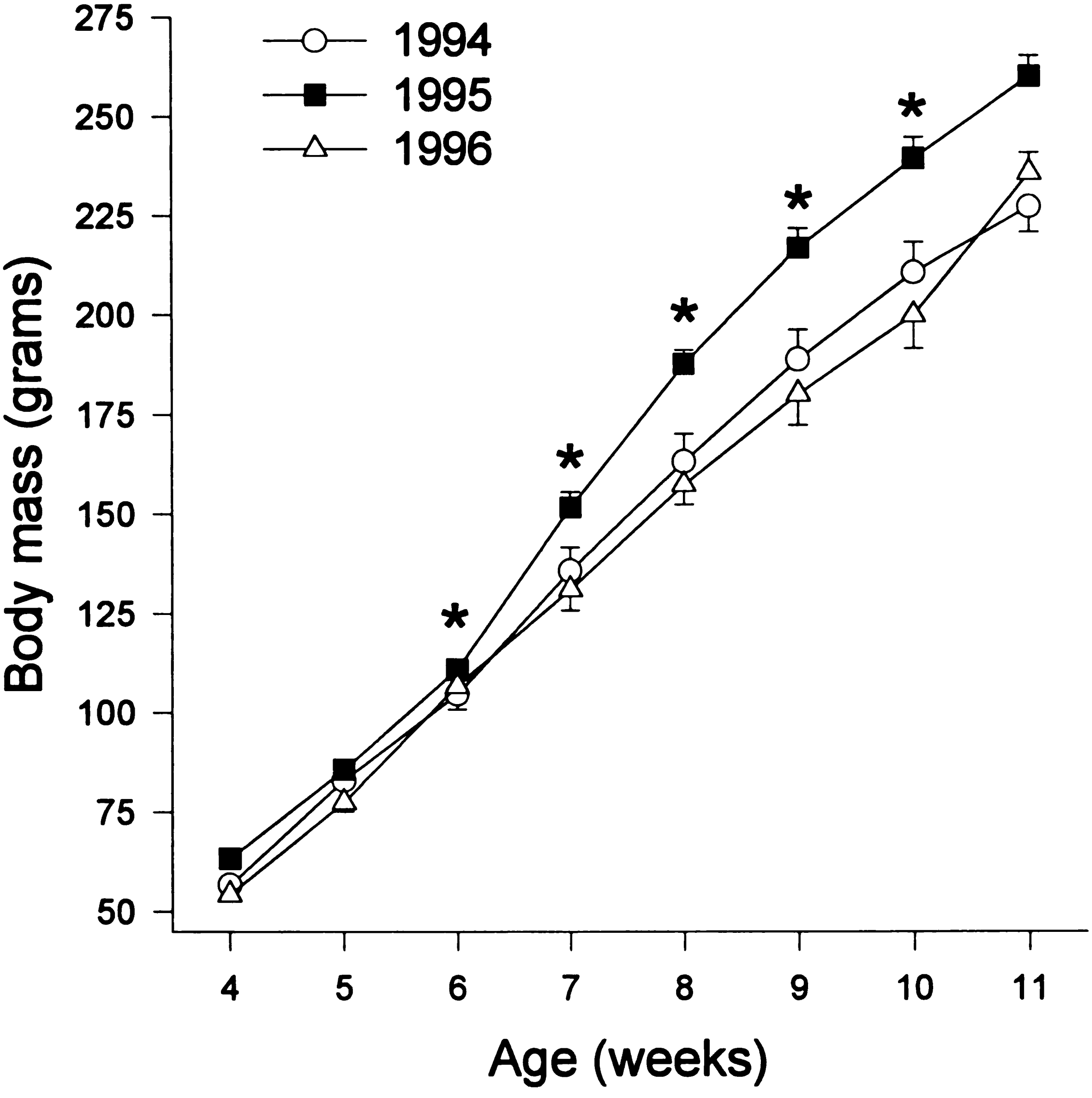

Figure 12 - Body mass of juvenile males, 1994-1996. Asterisks indicate that body mass in 1995 differed significantly from than in both 1994 and 1996. Sample sizes are as follows: 1994: 42, 25, 29, 30, 27, 27, 25, 22; 1995: 83, 48, 37, 45, 38, 43, 36, 8; 1996: 45, 32, 23, 20, 20, 14, 13, 4.

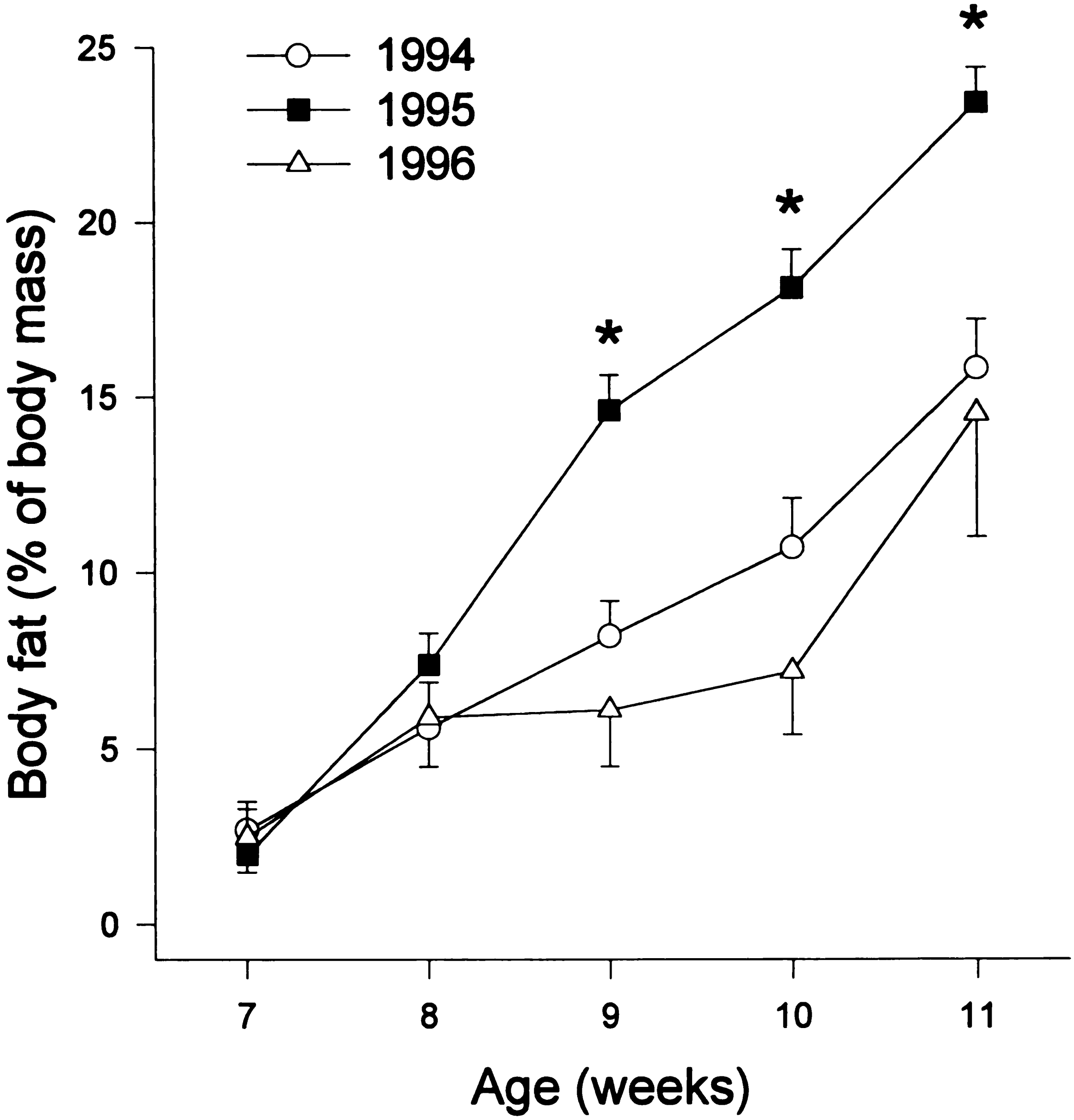

Figure 13 - Body fat of juvenile males, 1994-1996. Asterisks indicate that body fat in 1995 differed significantly from that in both 1994 and 1996. Sample sizes are as follows: 1994: 15, 15, 23, 24, 20; 1995: 22, 34, 37, 35, 7; 1996: 18, 16, 12, 12, 4.

I evaluated dispersal behavior of juveniles in the 1994-1996 cohorts whose dispersal status was known at 9 weeks of age. Compared to 1995, juvenile males were more likely to disperse at 7-9 weeks of age in 1994 (Figure 14; Mantel-Haenszel test, $\chi^2 = 16.058$, $P < 0.001$), and in 1996 (Figure 14; Mantel-Haenszel test, , $\chi^2 = 28.932$, $P < 0.001$). However, dispersal behavior at 7-9 weeks of age did not differ significantly between 1994 and 1996 (Figure 14; Mantel-Haenszel test, $\chi^2 = 1.908$, $P = 0.167$).

I also evaluated dispersal of males in the 1994 and 1995 cohorts whose dispersal status was known at 10 and 55 weeks of age. A greater proportion of juvenile males dispersed by 10 weeks of age in 1994 than in 1995 (Figure 15; $\chi^2 = 12.286$, $df = 1$, $P < 0.001$). All males of the 1994 and 1995 cohorts that were still residing in their natal areas at 10 weeks of age, and that were known to have survived their first winter, were still present in the natal area at the start of the yearling summer. Moreover, all surviving males of these cohorts dispersed by 55 weeks of age (Figure 15). Thus, most males born in 1995 postponed dispersal until their yearling year. Rates of survival through the first winter did not differ significantly between males in the 1994 and 1995 cohorts (10/30 vs. 13/51, respectively; $\chi^2 = 0.571$, $df = 1$, $P = 0.450$).

I examined the influence of calendar date on body fat of juvenile male *S. beldingi*, beginning when juvenile males first reached 6 weeks of age. I monitored body fat during 10-11 day intervals from 10 July to 31 August in 1994 and 1996, and from 21 August to 5 October in 1995 (Figure 16). I ordered these intervals chronologically from first to fifth, and compared body fat between years in corresponding intervals. Overall, body fat differed significantly between years from 1994-1996 (Figure 16; ANOVA, $F = 6.903$, $df = 2$, $P = 0.001$). I also observed a significant interaction of time interval with year in my

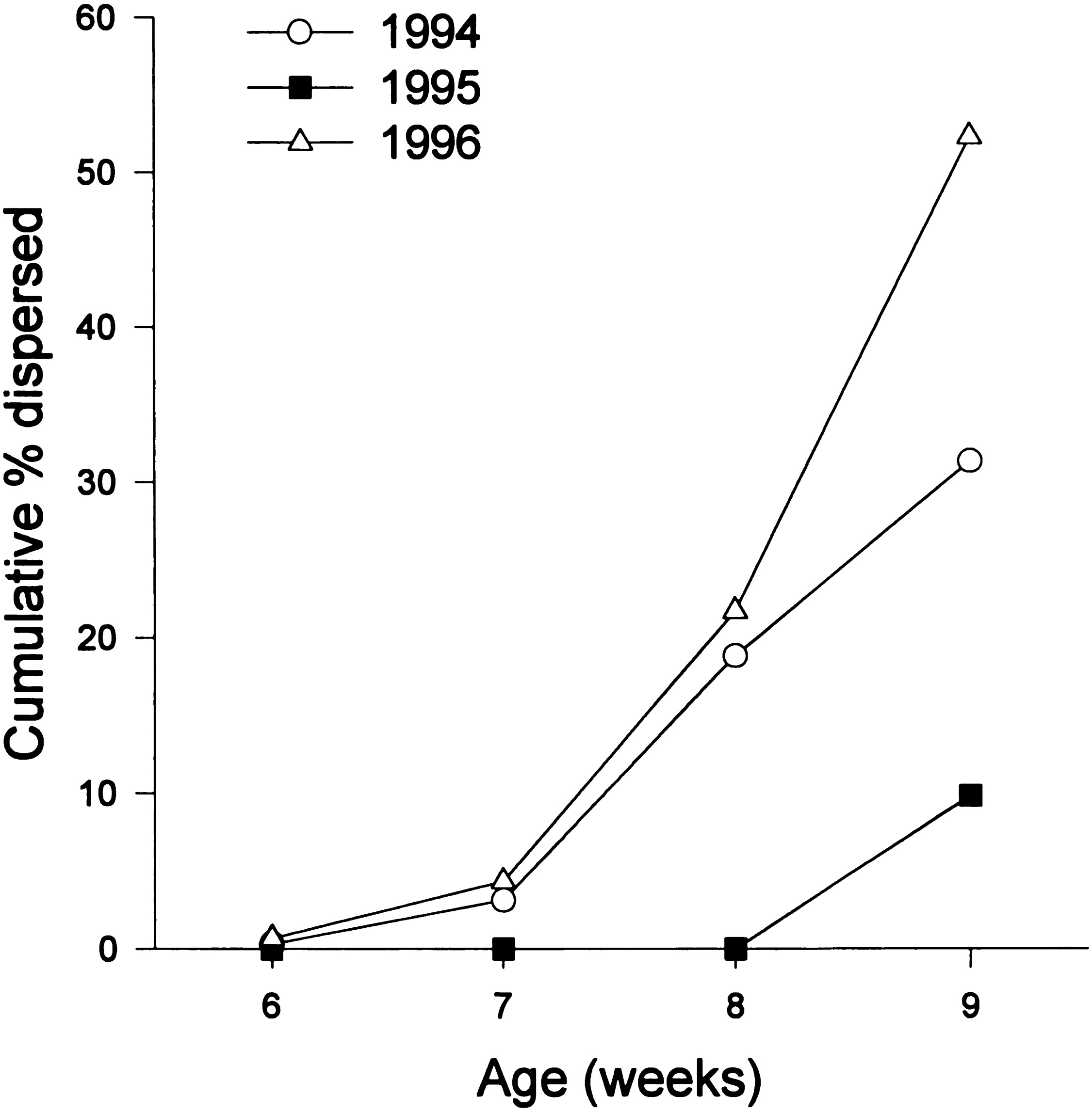

Figure 14 - Dispersal of juvenile males, 1994-1996. Samples included 32 males in 1994, 51 males in 1995, and 23 males in 1996.

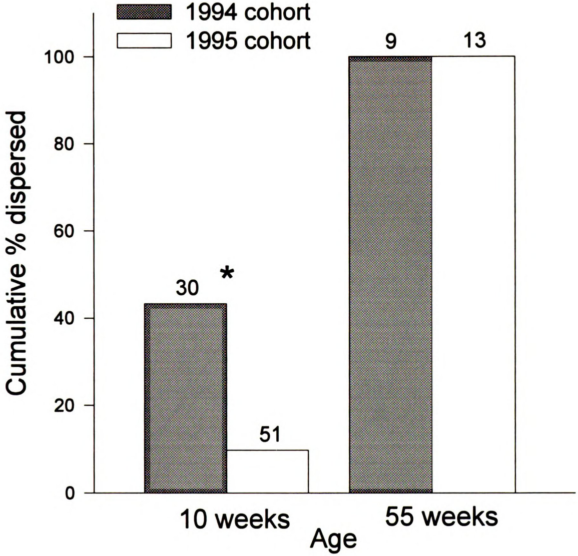

Figure 15 - Dispersal of males in the 1994 and 1995 cohorts. The cumulative percentage of individuals dispersed is presented for juvenile males at 10 weeks of age and for yearling males at 55 weeks of age. The asterisk indicates a significant difference between the 1994 and 1995 cohorts. Sample sizes are given on the graph.

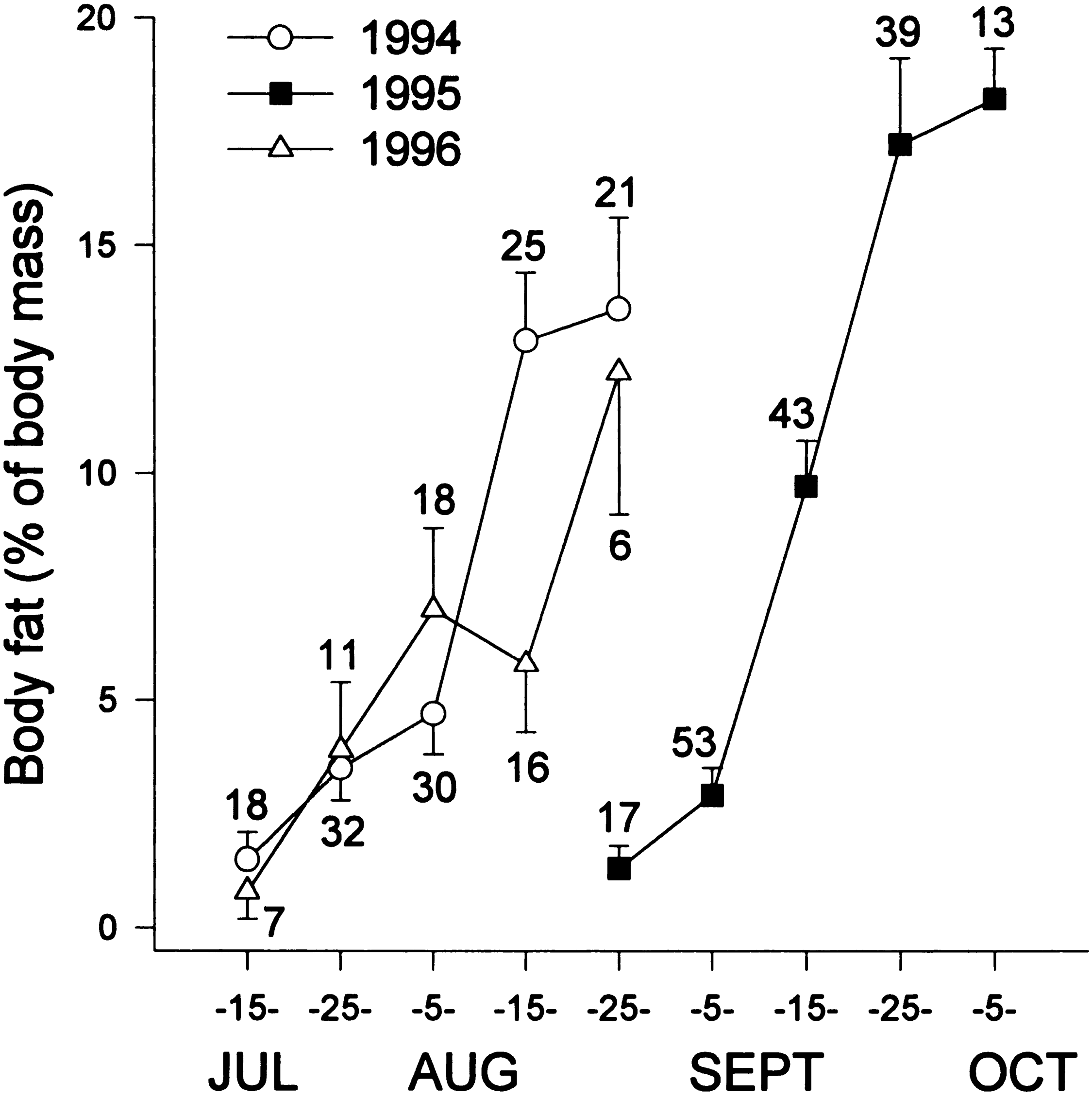

Figure 16 - Body fat of juvenile males during the active season, 1994-1996. Changes in body fat over time are presented. Sample sizes are given on the graph.

comparison of body fat (Figure 16; ANOVA, $F = 4.348$, $df = 8$, $P < 0.001$). I compared body fat in corresponding time intervals between years using *t*-tests for independent samples, and adjusted significance levels using Holm's procedure (Neter et al., 1996). Body fat of juvenile males in 1994 and 1996 differed only during the fourth time interval (Figure 16; $t = 2.993$, $df = 39$, $P = 0.005$). However, juvenile males were heavier in 1995 than in 1994 during the third through fifth time intervals (Figure 16; third interval: $t = -3.727$, $df = 71$, $P < 0.001$; fourth interval: $t = -2.151$, $df = 62$, $P = 0.035$, fifth interval: $t = -2.408$, $df = 25$, $P = 0.024$). This difference in body mass between 1994 and 1995 was statistically significant during the third interval, but was only marginally significant during the fourth and fifth intervals, after significance levels were adjusted. Juvenile males were heavier in 1995 than in 1996 during the fourth and fifth time intervals (Figure 16; fourth interval: $t = 5.783$, $df = 53$, $P < 0.001$; fifth interval, $t = 2.442$, $df = 17$, $P = 0.026$). This mass difference between 1995 and 1996 was statistically significant during the fourth interval and marginally significant during the fifth interval, after significance levels were adjusted. Thus, patterns of fat deposition differed between years. In particular, juvenile males acquired fat more rapidly during the last part of the active season in 1995 than did males in either 1994 or 1996. Males appear to exhibit accelerated pre-hibernation fattening when they are born late in the year.

I also evaluated the influence of calendar date on dispersal behavior in juvenile male *S. beldingi*. I monitored the cumulative percentage of juvenile males dispersed during 10-11 day intervals from 10 July to 31 August in 1994 and 1996, and from 21 August to 5 October in 1995 (Figure 17). I ordered these intervals chronologically from first to fifth, and compared dispersal behavior between years. I observed no difference in dispersal between 1994 and 1996 (Figure 17; Mantel-Haenszel test, $\chi^2 = 0.227$, $P = 0.634$). However, dispersal in 1995 occurred at significantly lower rates than it did in 1994 (Figure 17; Mantel-Haenszel test, $\chi^2 = 47.171$, $P < 0.001$) and 1996 (Figure 17; Mantel-Haenszel test, $\chi^2 = 51.438$, $P < 0.001$). It appears that dispersal seldom occurs after early September in the population of *S. beldingi* at Tioga Pass, regardless of how much fat juveniles have managed to store by then (Figure 17).

I used discriminant analysis to evaluate variables that might be reliable predictors of whether an individual dispersed by the end of the juvenile summer. In particular, I examined date of birth, body mass, and body fat during the last 10 days of the primary dispersal period each year (20-30 August 1994, 20-30 September 1995, and 15-25 August 1996). I observed no difference between 1994 and 1996 in date of birth (Kruskal-Wallis test, $P = 0.113$), body mass (ANOVA, $P = 0.250$), or body fat (ANOVA, $P = 0.927$). I also observed no interaction of year with age in my comparison of mass and fat between 1994 and 1996 (mass: ANOVA, $P = 0.900$; fat: ANOVA, $P = 0.484$). Thus, I combined data from these years for this analysis. In 1994 and 1996, date of birth, and mass and fat at the end of the dispersal period were dependable predictors of whether or not an individual dispersed as a juvenile (date of birth: $F = 6.486$, $df = 1$, $P = 0.015$; mass: $F = 8.374$, $df = 1$, $P = 0.006$, fat: $F = 7.436$, $df = 1$, $P = 0.010$). In 1995, however, when all litters were born very late during the year, date of birth, mass, and fat were not reliable predictors of dispersal status at the end of the juvenile summer (date of birth: $F = 2.445$, $df = 1$, $P = 0.125$; mass: $F = 0.219$, $df = 1$, $P = 0.642$; fat: $F = 0.852$, $df = 1$, $P = 0.361$).

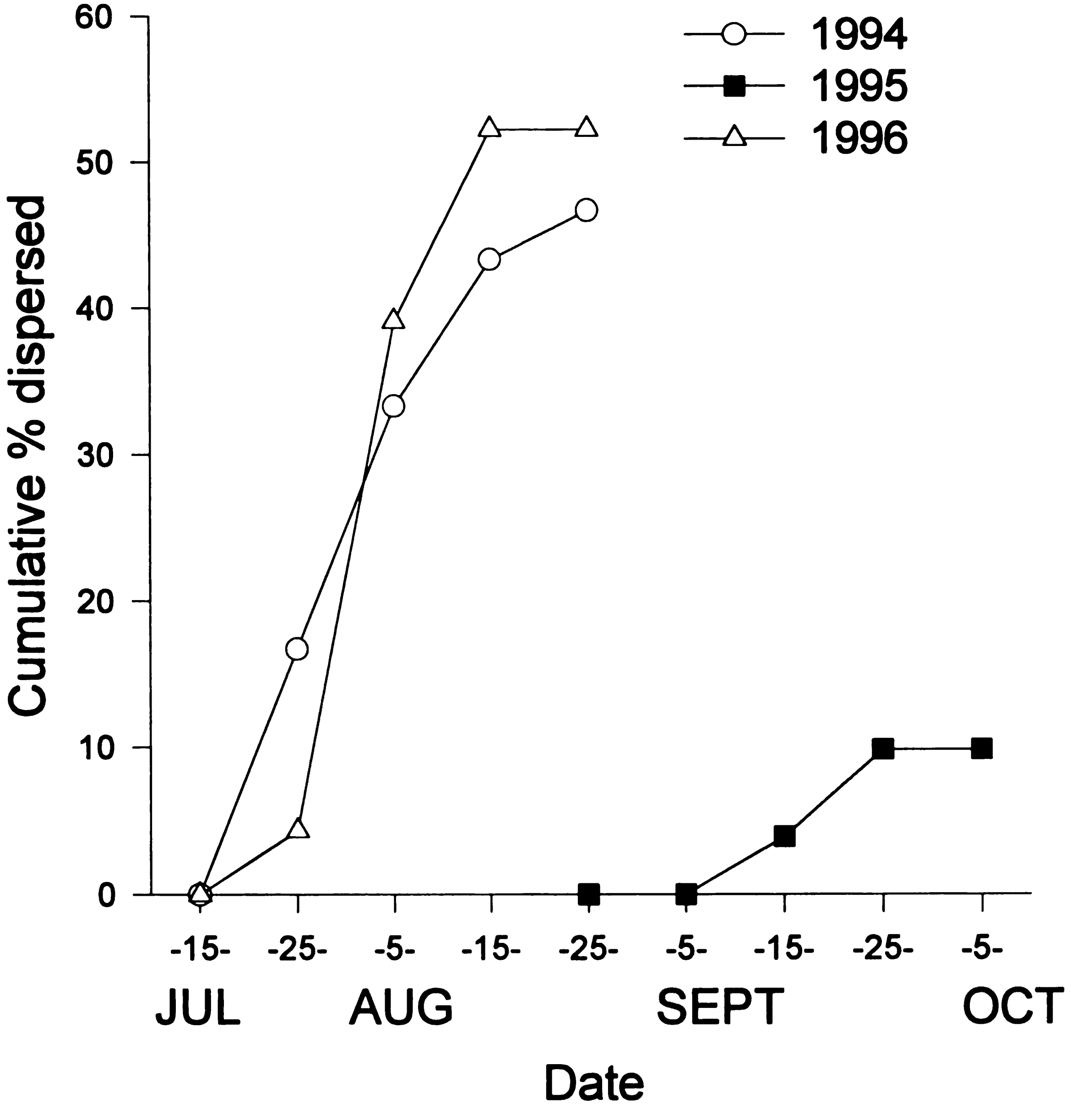

Figure 17 - Dispersal of juvenile males during the active season, 1994-1996. Changes over time in the percentage of individuals dispersed are presented for 32 males in 1994, 51 males in 1995, and 23 males in 1996.

DISCUSSION

The active season of juvenile *S. beldingi* began substantially later in 1995 than in 1994 and 1996. As a result little time was available to males in the 1995 cohort for pre-hibernation fattening, and more than 90% of these males postponed dispersal until the yearling summer. Moreover, juvenile males gained body mass and deposited fat more rapidly in 1995 than in 1994 or 1996. In my prior work examining the influences of body mass and body fat on the timing of dispersal by young male *S. beldingi*, most emigration by juvenile males occurred during the period of pre-hibernation fattening, when body mass and body fat were rapidly increasing (Chapter 3; Nunes and Holekamp, 1996). Moreover, dispersing males had greater percentages of their bodies composed of fat than did same-aged males remaining in their natal areas (Chapter 3; Nunes and Holekamp, 1996). Together with these earlier results, my data from 1994-1996 are consistent with the hypothesis that fat reserves interact with time of year to determine whether a male disperses during his juvenile summer or one year later. Specifically, when seasonal time constraints prevent an individual from acquiring enough energy to fuel both dispersal and pre-hibernation fat deposition, dispersal appears to be inhibited. Presumably, one consequence is that more energy can be stored as fat to sustain the animal through its first winter.

Delaying dispersal until the yearling summer may potentially be quite costly for male *S. beldingi*. Late during the active season when most juvenile dispersal occurs, adult *S. beldingi* are hibernating or fattening for the winter, and do not interact extensively with juveniles (Sherman, 1976; Sherman and Morton, 1979; Holekamp, 1984a; Nunes and Holekamp, 1996). By contrast, yearling males disperse during the early and middle parts of the active season, when adults are mating or when pregnant and lactating females are defending maternal territories, and levels of adult aggression are consequently high (Sherman, 1976, 1977; Holekamp, 1984a; Nunes and Holekamp, 1996; Nunes et al., 1997). Thus, as they attempt to settle in their new home areas, juvenile dispersers should encounter less conspecific aggression than do yearling dispersers. I suggest that this reduced resistance from conspecifics might also confer an energetic advantage on juvenile over yearling immigrants. If indeed juvenile dispersers expend less energy in agonistic interactions with adults, then they should be able to devote more time to foraging.

Patterns of body mass and body fat gain among juvenile male *S. beldingi* in 1995 differed significantly from those observed in 1994 and 1996. Juvenile males gained mass and fattened more rapidly in 1995, when they were born late in the season. Thus, ontogenetic patterns of mass and fat gain in juvenile males are not determined by age alone, but appear to also be influenced by time of year. Seasonal cycles in ground squirrels are generated by an endogenous circannual timing mechanism that is entrained to changes in daylength (Zucker et al., 1991). My data suggest that, in juvenile male *S. beldingi*, this timing mechanism regulates body mass and body fat at seasonally appropriate levels. Although the growing season of *S. beldingi's* habitat varies between years and it is possible that the differences I observed in mass and fat gain between years were due to interannual variation in food availability, I believe this unlikely. The active season of *S. beldingi* always coincides with the growing season, so food is abundant while *S. beldingi* are active, and squirrels hibernate during periods of limited food availability (Morton, 1975; Sherman, 1976). Sciurid rodents in general are not limited by food availability during the active season (e.g., Armitage and Downhower, 1974), and in fact may consume as little as 2% of the total energy available to them (e.g., Kilgore, 1972; Svendsen, 1973).

My results suggest that a circannual timing mechanism in juvenile male *S. beldingi* interacts with a physiological mechanism monitoring fat reserves to regulate the timing of dispersal behavior. It appears that perhaps the central nervous system assesses body fat in relation to the amount of time remaining until hibernation, and uses this information in determining whether dispersal occurs or is delayed. I propose a model (Figure 18) in which a circannual timing mechanism dictates minimum fat levels appropriate for particular calendar dates (Mrosovsky, 1976; Dark et al., 1984, 1985; Forger et al., 1986), and a physiological signal informs the central nervous system about whether these levels of fat have been achieved (Schneider, 1992; Wade and Schneider, 1992; Campfield et al., 1995; Wade et al., 1996). During the juvenile summer, dispersal behavior is triggered only when actual body fat rises above a seasonally appropriate threshold.

Various chemical signals have been identified that might inform the central nervous system about energy availability, or about the size of body fat stores. For example, metabolic fuels such as glucose regulate estrous cycles in golden hamsters (*Mesocricetus auratus*) and appear to be detected by the area prostrema of the hind brain (Morin, 1986; Schneider and Wade, 1989; Schneider, 1992; Wade and Schneider, 1992; Schneider and Zhu, 1994). Metabolic fuel levels might also be involved in the regulation of dispersal behavior in juvenile male *S. beldingi*. Circulating levels of metabolic fuels, such as glucose and fatty acids, might vary with adipose tissue mass, and convey to the central nervous system information about overall body fat mass. Another possibility is that a chemical signal released by adipose tissue in proportion to overall mass of body fat is

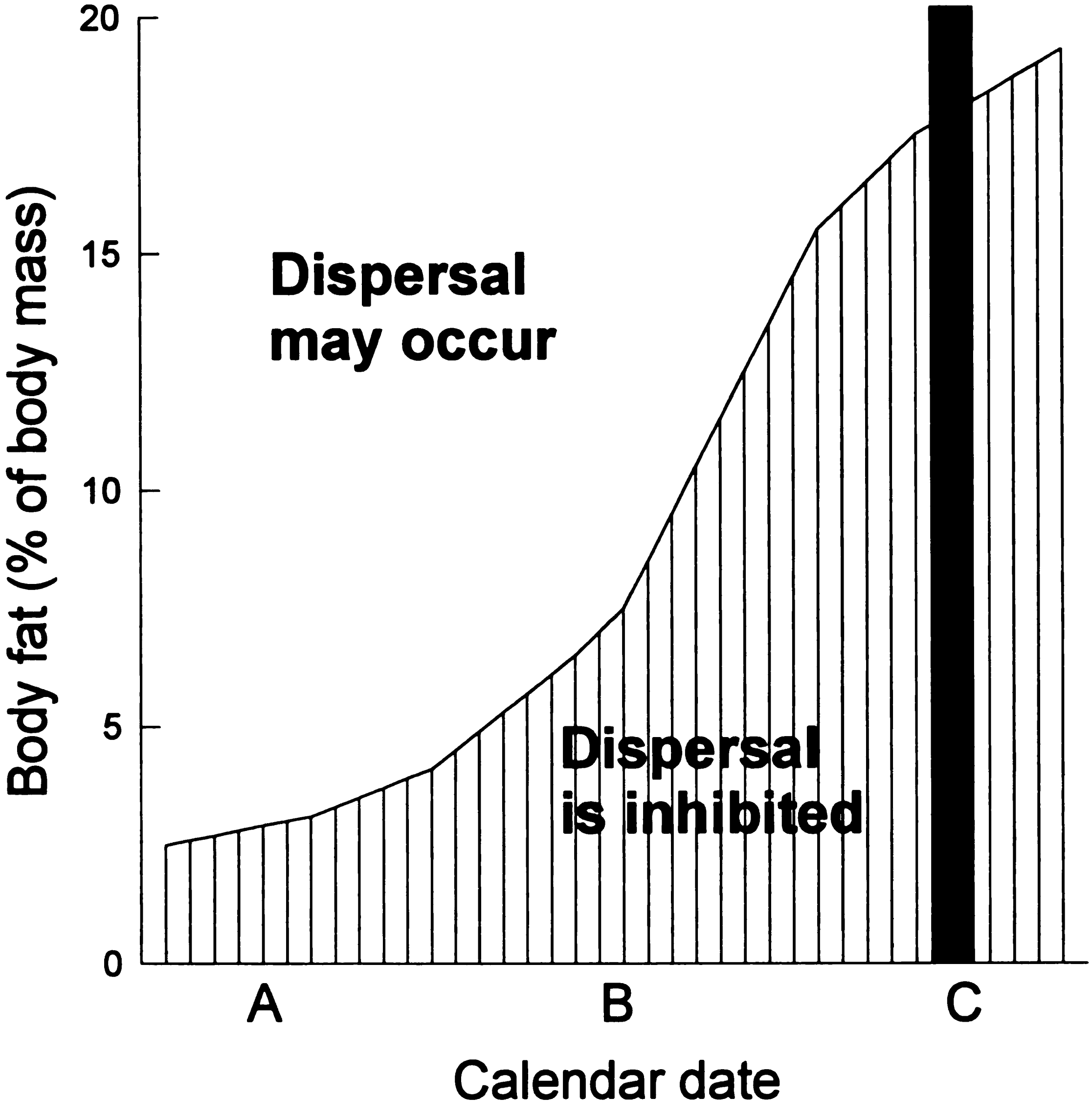

Figure 18 - A model of circannual and energetic regulation of dispersal. According to this model, dispersal is inhibited when body fat is below a seasonally appropriate threshold, represented on the graph by the solid curve. When the juvenile active season begins at date "A," the fat threshold is initially low, and available energy can be used to support dispersal behavior. When the active season begins at date "B," the fat threshold increases rapidly, available energy is stored as fat needed for survival during hibernation, and dispersal behavior is inhibited. After date "C," dispersal is inhibited regardless of the size of fat reserves.

detected by the central nervous system (Campfield et al., 1995). Interestingly, a peptide (leptin) that regulates energy balance via effects on feeding and activity levels has been isolated in laboratory mice and humans (Halaas et al., 1995; Maffei et al., 1995; Pellymounter et al., 1995; Cohen et al., 1996). It is tempting to speculate that leptin, or a molecule with similar functions, might play a role in regulating the timing of dispersal behavior in *S. beldingi*.

My results suggest that juvenile dispersal in *S. beldingi* is restricted to a particular phase of the annual cycle, and that it is inhibited very late in the active season regardless of the size of body fat reserves. Seasonal inhibition of dispersal in *S. beldingi* appears similar, for example, to seasonal suppression of reproduction in female golden-mantled ground squirrels (*S. lateralis*), in which luteinizing hormone (LH) is secreted in a circannual rhythm, limiting estrus to a specific phase of the circannual cycle (Zucker and Licht, 1983a). In male *S. lateralis*, sensitivity of LH secretion to negative feedback from gonadal steroids also follows a circannual pattern (Zucker and Licht, 1983b), and the capacity for reproductive maturation appears to be limited to a particular period within the circannual cycle (Barnes and York, 1990). It appears that dispersal behavior by juvenile male *S. beldingi* similarly occurs only within a specific phase of the circannual cycle, and is inhibited throughout the remainder of the year.

Chapter 5

ENDOCRINE INFLUENCES ON DISPERSAL

INTRODUCTION

The male bias typical of mammalian dispersal suggests that it might be mediated by gonadal androgens (Smale et al., 1997). Early work in behavioral endocrinology revealed that testosterone (T) can affect sexually dimorphic behavior in mammals by "organizing" physiological substrates of behavior during early development, or by "activating" physiological processes that mediate behavior later in life (Phoenix et al., 1959). Because concentrations of circulating T are higher in males than in females during specific stages of ontogeny, T plays an important role in the organization and activation of many male-typical behaviors (Toran-Allerand, 1978; Arnold, 1980; McEwen, 1981; Arnold and Breedlove, 1985; Moore, 1991; Beatty, 1992; Breedlove, 1992).

Prior work by Holekamp et al. (1984) indicated that T probably does not exert activational effects on natal dispersal behavior in juvenile male *S. beldingi*. Male *S. beldingi* that were castrated at weaning, and thus deprived of their primary source of T, still dispersed from the natal area. This prior work by Holekamp et al. (1984) also provided preliminary evidence that T might exert organizational effects on the natal dispersal behavior of *S. beldingi*. The proportion of females treated with T at birth that emigrated from the natal area by 60 days of age did not differ significantly from the proportion of males that dispersed by 60 days of age. However, Holekamp et al. (1984)

released mothers of T-treated females in areas other than where they had been captured. Holekamp et al (1984) used females treated with oil at birth as controls, but all of these that were weaned disappeared from their natal areas, and none were later found. Thus, Holekamp et al. (1984) were unable to definitively rule out the possibility that T-treated females dispersed in response to release of their mothers at a new site, or some variable other than perinatal T exposure.

Here, I replicated the findings of Holekamp et al. (1984), and collected new data to test hypotheses suggesting that natal dispersal in *S. beldingi* is mediated by activational or organizational effects of T. I predicted that, if T exerts activational effects on dispersal behavior, then concentrations of circulating T should increase around the time of dispersal. I also exploited variation in reproductive maturation among yearling males to test an additional prediction of the activational hypothesis. Specifically, if T activates natal dispersal behavior, then yearlings with large, descended testes, and thus presumably with elevated concentrations of circulating T, should be likelier to emigrate from the natal area than should yearling males showing no testicular development. Finally, I examined the prediction of the organizational hypothesis that female *S. beldingi* treated with T at birth should disperse, whereas oil-treated control females should remain near their birth sites.

METHODS

During the summer of 1995, I ear-tagged 126 juvenile males and 130 juvenile females from 29 complete litters. During the summer of 1996, I ear-tagged 108 juvenile males and 95 juvenile females from 46 complete litters.

Tests of the Activational Hypothesis

To evaluate circulating T concentrations of juveniles in relation to dispersal, I collected 89 blood samples (0.5-1.0 ml) from 71 juvenile males and 62 samples from 52 juvenile females in 1996. I regularly collected blood samples beginning about 1 week after the first young of the year were weaned, and continuing until near the end of the primary dispersal period (13 July to 20 August). These samples were assayed for T concentrations at Michigan State University during October of 1996.

I determined whether body mass and body fat of free-living juvenile males prior to hibernation influence the likelihood that they will become reproductively mature as yearlings. I also evaluated whether reproductive status of yearling males influences the timing of their emigration from the natal area. During the summer of 1995, I manipulated body mass of juvenile *S. beldingi* by providing extra food to 21 litters, and used 33 litters as unprovisioned controls. I gave supplemental food to mothers of provisioned litters during lactation, and to their young for 6 weeks after they were weaned. I measured body mass of juveniles from these litters weekly from ages 4-11 weeks, and body fat weekly from ages 6-11 weeks. I then evaluated reproductive condition (Morton and Gallup, 1975) of these squirrels during their yearling summer in 1996. When I observed squirrels mating during regular observations, I recorded the identity of the squirrels, as well as the date, time, location, and duration of copulation.

Test of the Organizational Hypothesis

To test the organizational hypothesis, I manipulated early androgen exposure of *S. beldingi*. During the summer of 1995, I treated newborn females from 11 provisioned litters and 18 unprovisioned litters with testosterone (T), and used oil-treated females from 10 provisioned and 15 unprovisioned litters as controls. I monitored dispersal status of these females and their brothers weekly from 6-10 weeks of age. I also regularly monitored dispersal status of these squirrels during their yearling summer in 1996, until 55 weeks of age.

Hormone and provisioning manipulations were combined to elucidate the interaction between endocrine and energetic variables in the mediation of natal dispersal behavior. However, because of heavy snowfall during the winter and spring, juvenile dispersal occurred at very low rates in 1995 (Nunes et al., in press), and I was unable to evaluate this interaction. I repeated my experimental manipulation of hormones and food availability in 1996, and discuss the results in Chapter 4.

RESULTS

Provisioning Effects on Reproductive Development of Males

During May of 1996, I trapped 28 males from the 1995 cohort, 15 of which had been provisioned as juveniles, and 13 of which had been unprovisioned. None of the males from the unprovisioned group exhibited signs of reproductive competence (e.g., large, scrotal testes and pigmented scrotum; Morton and Gallup, 1975). By contrast, nine males from the provisioned group were in reproductive condition. Thus, males were more likely to become reproductively mature as yearlings if they were provisioned as juveniles (Fisher's exact test, $P = 0.001$). Reproductive yearling males in 1996 were significantly heavier than non-reproductive yearlings at the end of their juvenile summer during the previous year (Figure 19; *t*-test for independent samples, $t = 6.940$, $df = 22$, $P < 0.001$).

Similarly, body fat in 1996 was greater in reproductive than non-reproductive yearlings at the end of the juvenile summer during the previous year (Figure 20; *t*-test for independent samples, $t = 2.472$, $df = 21$, $P = 0.012$). Thus, body mass and body fat at the end of the juvenile summer appear to influence whether or not free-living male *S. beldingi* become reproductively competent as yearlings.

The Activational Hypothesis

Concentrations of circulating T did not differ between juvenile males and juvenile females of the 1996 cohort, and in all cases fell below assay detectability levels (0.2 ng/ml). Thus, I found no measurable increases in circulating T associated with natal dispersal behavior, as predicted by the activational hypothesis.

Of the nine reproductively mature yearlings I trapped in 1996, one dispersed as a juvenile during the summer of 1995, seven emigrated from the natal area in 1996, and one disappeared in 1996 and was never recovered. None of the reproductive yearling males that dispersed or disappeared in 1996 did so before the end of the mating period, which I estimated to be 12-29 May, based on observations of mating and dates of litter emergence. Of the 19 non-reproductive yearling males that I trapped in 1996, five dispersed as juveniles in 1995, 12 dispersed as yearlings in 1996, and two disappeared in 1996 and were never recovered. Eight of the 12 non-reproductive yearlings that dispersed in 1996 did so between 12-29 May, during the breeding period. Thus, yearling males with small, non-scrotal testes were significantly more likely to disperse during the breeding season than were yearling males with large, descended testes (Figure 21; Fisher's exact test, $P = 0.013$). In fact, all yearlings with large, scrotal testes delayed dispersal until after the

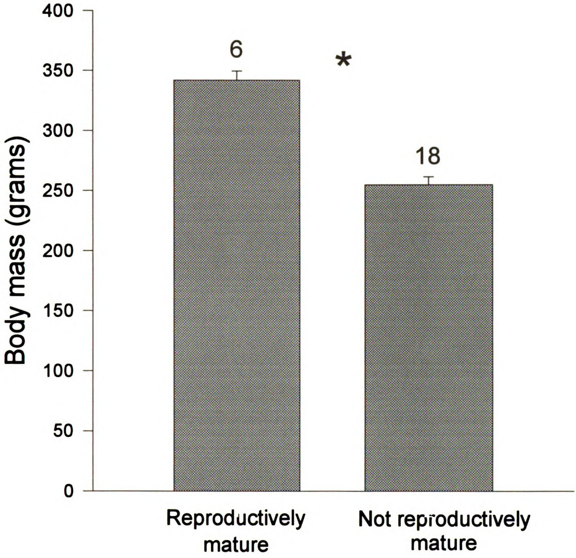

Figure 19 - Body mass of yearling males at the end of their juvenile summer. The asterisk indicates a significant difference between males that did and did not achieve reproductive maturity as yearlings. Sample sizes are given on the graph.

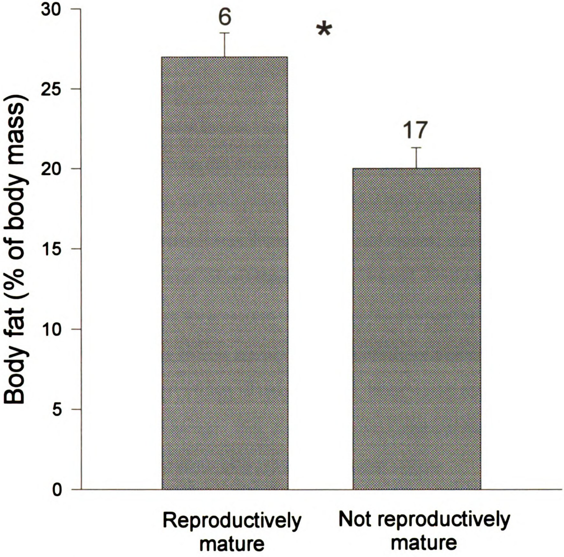

Figure 20 - Body fat of yearling males at the end of their juvenile summer. The asterisk indicates a significant difference between males that did and did not achieve reproductive maturity as yearlings. Sample sizes are given on the graph.

breeding period, when size of testes begins to diminish (Morton and Gallup, 1975), as concentrations of circulating T presumably do also (e.g., Holekamp and Talamantes, 1992).

The Organizational Hypothesis

Among groups (T-treated females, oil-treated females, and males) in the 1995 cohort, the proportion of juveniles that dispersed at ages 7-10 weeks did not differ significantly between squirrels that were provisioned and those that were unprovisioned (T-treated females: no T-treated females dispersed; oil-treated females: Mantel-Haenszel test, $\chi^2 = 2.151$, $P = 0.142$; males: Mantel-Haenszel test, $\chi^2 = 0.032$, $P = 0.859$). Similarly, among these groups, the proportion of individuals that dispersed by 55 weeks of age did not differ significantly between provisioned and unprovisioned squirrels (T-treated females: Fisher's exact test, $P = 0.249$; oil-treated females: Fisher's exact test, $P = 0.538$; males: all surviving males dispersed). Thus, dispersal data were combined for provisioned and unprovisioned individuals within these groups for statistical analysis.

Heavy winter and spring snowfall had a dramatic effect on the timing of natal dispersal behavior in 1995 (Nunes et al., in press), and less than 10% of surviving members of the 1995 cohort dispersed by the end of their juvenile summer (Figure 22). The proportion of T-treated juvenile females that dispersed from 7-10 weeks of age in 1995 did not differ significantly from that of oil-treated females (Figure 22; Mantel-Haenszel test, $\chi^2 = 1.634$, $P = 0.201$) or males (Figure 22; Mantel-Haenszel test, $\chi^2 = 0.933$, $P = 0.334$). However, by 55 weeks of age, 80% of surviving T-treated females and 100% of surviving males in the 1995 cohort had dispersed, whereas only 13% of oil-

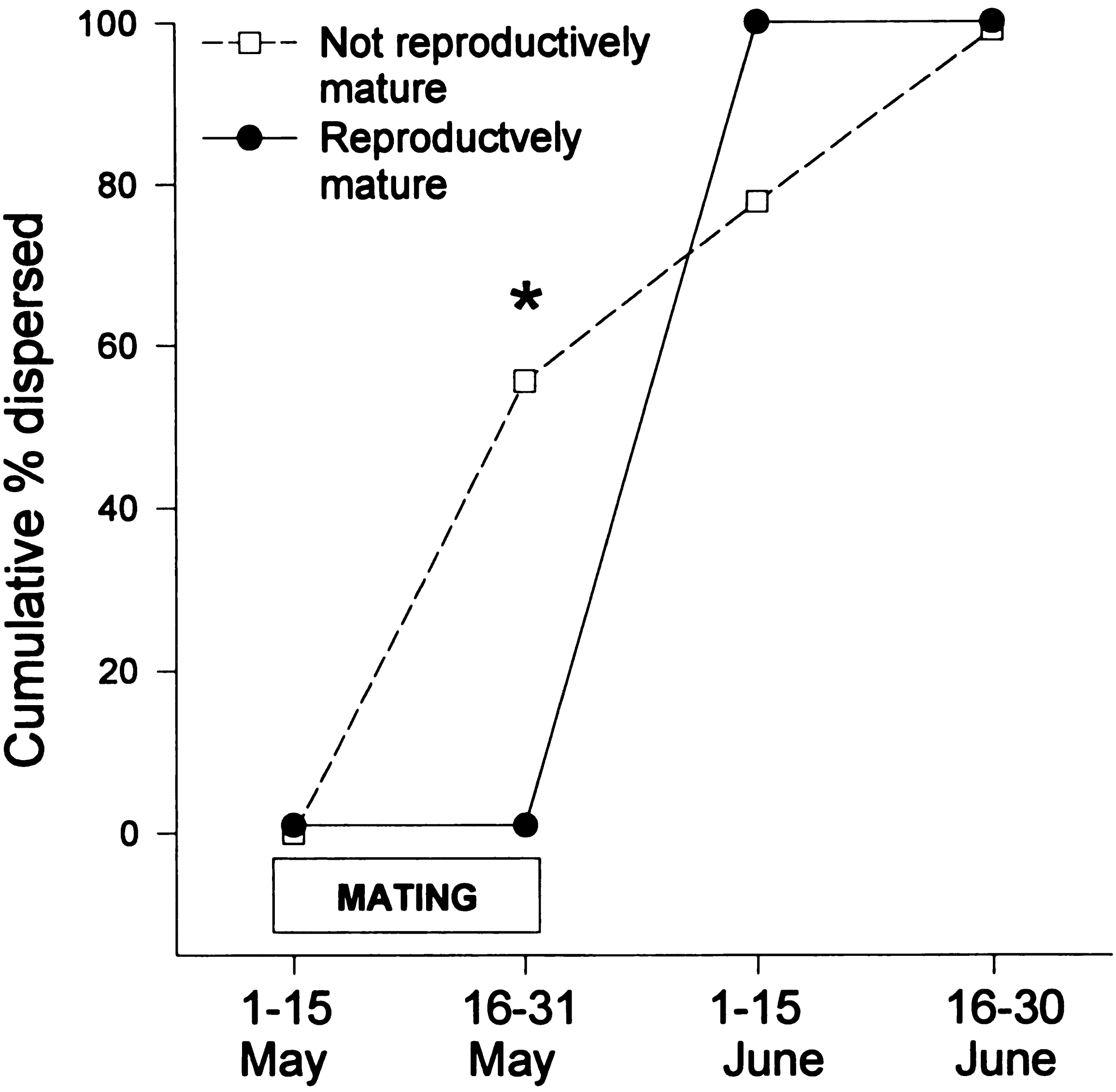

Figure 21 - Dispersal of yearling males, 1996. The asterisk indicates a significant difference between males that did and did not achieve reproductive maturity as yearlings. Samples include 7 males that achieved reproductive maturity and 12 that did not.

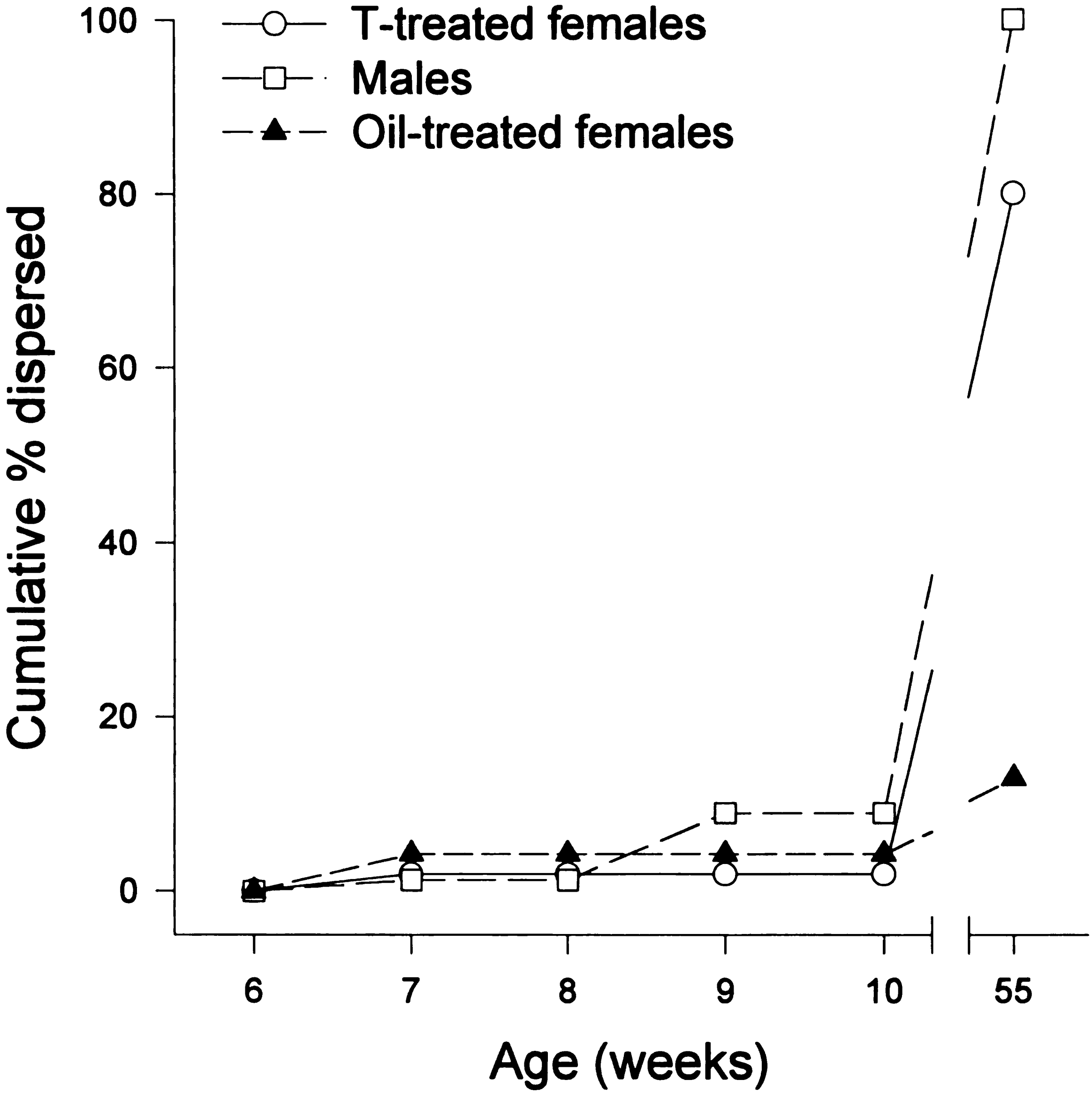

Figure 22 - Dispersal of the 1995 cohort. The cumulative percentages of juveniles that dispersed from 6 through 10 weeks of age, and of yearling that dispersed by 55 weeks of age, are presented. Sample of juveniles included 49 T-treated females, 79 males, and 46 oil-treated females. Samples of yearlings included 20 T-treated females, 24 males, and 23 oil-treated females.

treated females had emigrated (Figure 22). The proportion of T-treated females in the 1995 cohort that dispersed by 55 weeks of age differed significantly from that of both oil-treated females (Figure 22; $\chi^2 = 19.447$, $P < 0.001$) and males (Figure 22; Fisher's exact test, $P = 0.036$), but was more similar to that of males (Figure 22). Thus the probability of natal dispersal by T-treated females more closely resembled that of male than that of female peers.

DISCUSSION

Together with the results obtained by Holekamp et al. (1984), my data offer strong evidence that T does not activate natal dispersal behavior in *S. beldingi*. The activational hypothesis predicts that early castration should inhibit dispersal behavior, but this does not occur (Holekamp et al., 1984). Furthermore this hypothesis predicts that concentrations of circulating T should be relatively high around the time that dispersal occurs, but my work shows that this is not the case in this species. Holekamp et al. (1984) measured circulating T throughout the juvenile summer in *S. beldingi* that were born in the laboratory to mothers caught in the wild. Plasma T was undetectable throughout the summer in lab-reared juveniles. Here I found that circulating T was also undetectable in free-living juveniles throughout the juvenile summer, when most males usually disperse. The possibility remains that dispersal is activated by changes in T concentrations below the 0.2 ng/ml detectability level of the assay I used, but the fact that castrated males disperse (Holekamp et al., 1984) makes this possibility unlikely. Here I also found that yearling males were less likely to disperse when they had large, scrotal testes than when their testes were small and undescended. Size of testes in male ground squirrels is positively correlated with blood concentrations of T (Barnes, 1984; Holekamp and Talamantes, 1992), and male *S. beldingi* with large testes presumably have higher levels of circulating T than do males with small, abdominal testes. Thus, if T has concurrent effects on natal dispersal behavior in this species, they appear to be inhibitory rather than stimulatory.

Most male *S. beldingi* emigrate from the natal area during their juvenile summer, two years before achieving reproductive maturity (Morton et al., 1974). When dispersal occurs well before males reach puberty, the behavior probably is not likely to be activated by gonadal androgens (Smale et al., 1997). However, in many species there is a close temporal association between dispersal and puberty (e.g., Errington, 1963; Pusey and Packer, 1987a, 1987b; Rood, 1987, 1990; Matsumura, 1993; Smale et al., 1997). The onset of reproductive maturity is characterized by dramatic increases in circulating concentrations of gonadal steroid hormones, which stimulate a variety of behavioral changes (Bronson and Rissman, 1986; Wilson, 1992). Thus, elevations in concentrations of circulating androgens might be likely to promote dispersal behavior in species unlike *S. beldingi* in which males typically emigrate from the natal area shortly after attaining puberty (Smale et al., 1997).

My results indicate that around the time of birth in *S. beldingi*, T organizes masculine patterns of natal dispersal behavior. The proportion of female *S.*

beldingi treated neonatally with T that emigrated from their birth sites by 55 weeks of age was intermediate between that of females treated with oil at birth, and that of males, but was closer to that of males. Holekamp et al. (1984) observed that female *S. beldingi* treated with T at birth were as likely as males to emigrate from the natal area by 60 days of age, and also exhibited increases in exploratory and locomotor behavior and boldness that normally accompany dispersal by young male *S. beldingi*. Thus, my work with *S. beldingi* and the work by Holekamp et al. (1984) represent the first demonstration of organizational T effects on any behavior pattern in free-living animals. Laboratory studies with captive animals have shown that perinatal androgen exposure influences a variety of noncopulatory behaviors such as play, locomotion, aggression, and learning (e.g., Williams et al., 1990; Beatty, 1992). My results provide field verification that androgens can organize noncopulatory behavior. My results also strongly suggest that natal dispersal in *S. beldingi* is among those behaviors, such as play and spatial learning, which apparently require no subsequent activational supplementation of perinatal organizational hormone effects (Goy and McEwen, 1980; Holekamp et al., 1984; Williams et al., 1990).

In many species of mammals, all males eventually leave their natal areas (e.g., Dobson, 1979; Hanby and Bygott, 1987; Pusey and Packer, 1987a, 1987b; Matsumura, 1993; Rutberg and Kuiper, 1993; Smale, 1997). Gonadal androgens might often organize a male-typical propensity to emigrate from the natal area when all males of a species ultimately disperse (Smale et al., 1997). However, in many mammalian species, not all males leave the natal area (e.g., Harcourt et al., 1976; Harcourt, 1978; Wiggett and Boag, 1992, 1993). In species for which the costs of dispersal sometimes favor philopatric behavior by males, androgenic organization of a deterministic predisposition to leave the natal area might well be maladaptive (Holekamp et al., 1984; Smale et al., 1997). However, in these species, androgens might organize a masculine response to social or ecological variables which promote dispersal behavior.

The action of gonadal steroid hormones facilitates many features of reproductive behavior, including courtship, establishment and defense of breeding territories, sexual partner preference, and copulation (McEwen, 1981; Baum et al., 1990; Breedlove, 1992; Mansukhani et al., 1996; Rudd et al., 1996). The role of T in organizing male-typical dispersal behavior in *S. beldingi* offers physiological evidence of a link between dispersal and reproduction (see also O'riain et al., 1996). Prior studies suggest two ways in which natal dispersal might represent an important male reproductive strategy. First, emigration from the natal area can improve the quality of a male's offspring by reducing the probability of inbreeding (e.g., Pusey, 1980, 1987; Caley, 1987; Charlesworth and Charlesworth, 1987; Keane, 1990; Wolff, 1992). Second, natal dispersal can enhance a male's access to unfamiliar females that are more willing to mate with him than are females in his natal area (e.g., Peirera and Weiss, 1991; Alberts and Altmann, 1995). One or both of these might link dispersal behavior with male reproductive success in *S. beldingi*.

Chapter 6

INTERACTION OF ENDOCRINE AND ENERGETIC VARIABLES

INTRODUCTION

Organizational effects of the gonadal steroid testosterone (T) strongly influence the probability that individual *S. beldingi* will someday emigrate from the natal area (Holekamp et al., 1984; Chapter 5). Perinatal treatment of female *S. beldingi* with T induces male-like dispersal behavior during the juvenile or yearling summer. The determinants of when during its early life an individual will disperse are currently unknown. However, energy availability appears to play an important role in timing dispersal behavior in *S. beldingi* (Nunes and Holekamp, 1996; Nunes et al., in press). Here, I attempted to ascertain whether energetic variables influence the timing of natal dispersal behavior in this species, and whether endocrine and energetic factors interact to mediate the occurrence of this behavior pattern.

Hormones influence the development of many behaviors, and often interact with energetic variables in the regulation of reproduction and behavior (e.g., Zucker and Boshes, 1982; Kenagy, 1980; Barnes, 1984; Smale et al., 1986; Beatty, 1992; Wade and Schneider,1992; Wade et al., 1996). Because of the potential risks and energetic costs associated with dispersal, an individual's decision regarding whether or not to emigrate at any given time should be influenced by energy stores available to support this movement in addition to the normal maintenance and growth functions necessary for survival (Nunes and Holekamp, 1996; Smale et al., 1997; Nunes et al., in press). The amount of energy stored as fat does in fact appear to importantly influence the timing of natal dispersal by juvenile male *S. beldingi*. Dispersing males tend to be fatter than same-aged males still residing in the natal area (Nunes and Holekamp, 1996). Moreover, juvenile male *S. beldingi* born very late in the year delay dispersal until their yearling summer, presumably because they do not have sufficient time during the juvenile summer to take in enough energy both to fuel dispersal behavior and to build fat reserves necessary to survive overwinter hibernation (Nunes et al., in press).

Energy availability may also influence the timing of dispersal by those few juvenile female *S. beldingi* that leave their natal areas (Smale et al., 1997). Female *S. beldingi* that disperse during the juvenile summer tend to be heavier than same-aged females still residing in their natal areas (Holekamp, 1984; Nunes et al., 1997). However, the timing of dispersal differs between juvenile male and female *S. beldingi*. Furthermore, when juvenile females disperse, they generally do so at significantly older ages than do juvenile males (Nunes et al., 1997).

Here I examined an hypothesis suggesting that early hormone exposure interacts with current energy availability to mediate the natal dispersal behavior typical of male *S. beldingi*. I predicted that the timing of natal dispersal by juvenile female *S. beldingi* treated with T at birth should respond to experimental manipulations of body fat in the same manner as does the timing of dispersal by juvenile males. Juvenile male *S. beldingi* fattened by food provisioning disperse from the natal area at younger ages than do unprovisioned juvenile males (Nunes and Holekamp, 1996; Nunes et al., 1997a). If perinatal androgen exposure and energy availability interact to mediate natal dispersal, then T-treated females receiving supplemental food should disperse at younger ages than should unprovisioned T-treated females. Furthermore, T-treated females receiving extra food should emigrate at younger ages than should any provisioned oil-treated females that disperse.

METHODS

During the summer of 1996, I trapped and ear-tagged 203 juvenile *S. beldingi*, including 108 males and 95 females from 46 complete litters.

I concurrently manipulated body mass, body fat and perinatal androgen exposure of these free-living juveniles. I treated newborn females with testosterone (T) and used oil-treated and untreated females as controls. Within each treatment group, I manipulated body fat of females and their brothers by provisioning them with food, and used unprovisioned females and their brothers as controls. I treated newborn females from 14 litters with T, treated females from 19 litters with oil, and used females from 13 litters as untreated controls. I gave extra food to seven T-treated litters, nine oil-treated litters, and six untreated litters, and I used seven T-treated litters, ten oil-treated litters, and seven untreated litters as unprovisioned controls. I monitored body mass of juveniles weekly from 4-11 weeks of age, and measured body fat weekly from 6-11 weeks of age. I observed the dispersal status of juveniles weekly from 6-9 weeks of age.

RESULTS

Body mass did not differ significantly between T-treated, oil-treated, and untreated females (provisioned: ANOVA, $P = 0.193$; unprovisioned: ANOVA, $P = 0.174$) and so body mass data of these females were combined within provisioned and unprovisioned groups for statistical analysis. Overall, provisioned juveniles were significantly heavier than unprovisioned juveniles from 4-9 weeks of age among both females (Figure 23; ANOVA, $F = 129.068$, $df = 1$, $P < 0.001$) and males (Figure 24; ANOVA, $F = 56.087$, $df = 1$; $P < 0.001$). Provisioned females were significantly heavier than unprovisioned females in each week-long age interval from 4-9 weeks of age (Figure 23; t-tests for independent samples; 4 weeks: $t = -6.014$, $df = 74$, $P < 0.001$; 5 weeks: $t = 4.868$, $df = 58$, $P < 0.001$; 6 weeks: $t = -4.696$, $df = 51$, $P < 0.001$; 7 weeks: $t = -6.303$, $df = 41$, $P < 0.001$; 8 weeks: $t = -4.190$, $df = 45$, $P < 0.001$; 9 weeks: $t = -2.565$, $df = 38$, $P = 0.014$). Similarly, provisioned males were significantly heavier than unprovisioned males from 4-8 weeks of age (Figure 24; t-tests for independent samples; 4 weeks: $t = -4.464$, $df = 97$, $P < 0.001$; 5 weeks: $t = -3.272$, $df = 75$, $P = 0.002$; 6 weeks: $t = -3.046$ $df = 59$, $P = 0.003$; 7 weeks: $t = -3.693$, $df = 51$, $P = 0.001$; 8 weeks: $t = -2.890$, $df = 44$, $P = 0.006$).

Provisioning also had notable effects on body fat, particularly among younger juveniles. Body fat did not differ between T-treated, oil-treated, and untreated females (provisioned: ANOVA, $P = 0.227$; unprovisioned: ANOVA, $P = 0.149$), and so body fat data of these females were combined within provisioned and unprovisioned groups for statistical analysis. Overall, provisioned juveniles had significantly greater body fat from 6-9 weeks of age than did their unprovisioned counterparts among both females (Figure 25; ANOVA, $F = 12.575$, $df = 1$, $P < 0.001$) and males (Figure 26; ANOVA, $F = 4.784$, $df = 1$, $P = 0.030$). However, body fat differed significantly only at 6 and 7 weeks of age among both females (Figure 25; t-tests for independent samples, 6 weeks: $t = -2.489$, $df = 44$, $P = 0.017$; 7 weeks: $t = -5.363$, $df = 38$, $P < 0.001$) and males (Figure 26; t-tests for

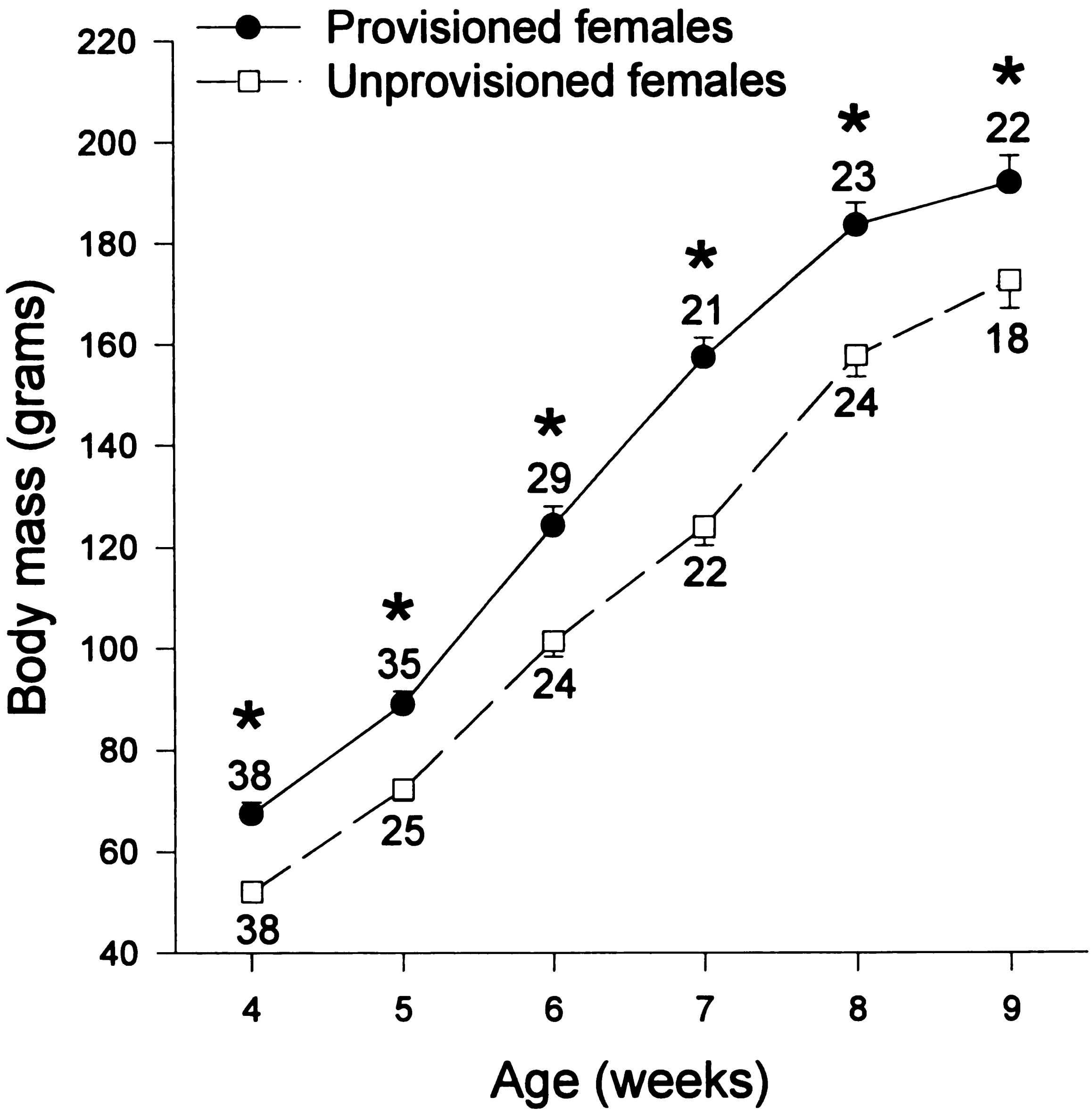

Figure 23 - Body mass of juvenile females, 1996. Asterisks indicate a significant difference between provisioned and unprovisioned females. Sample sizes are given on the graph.

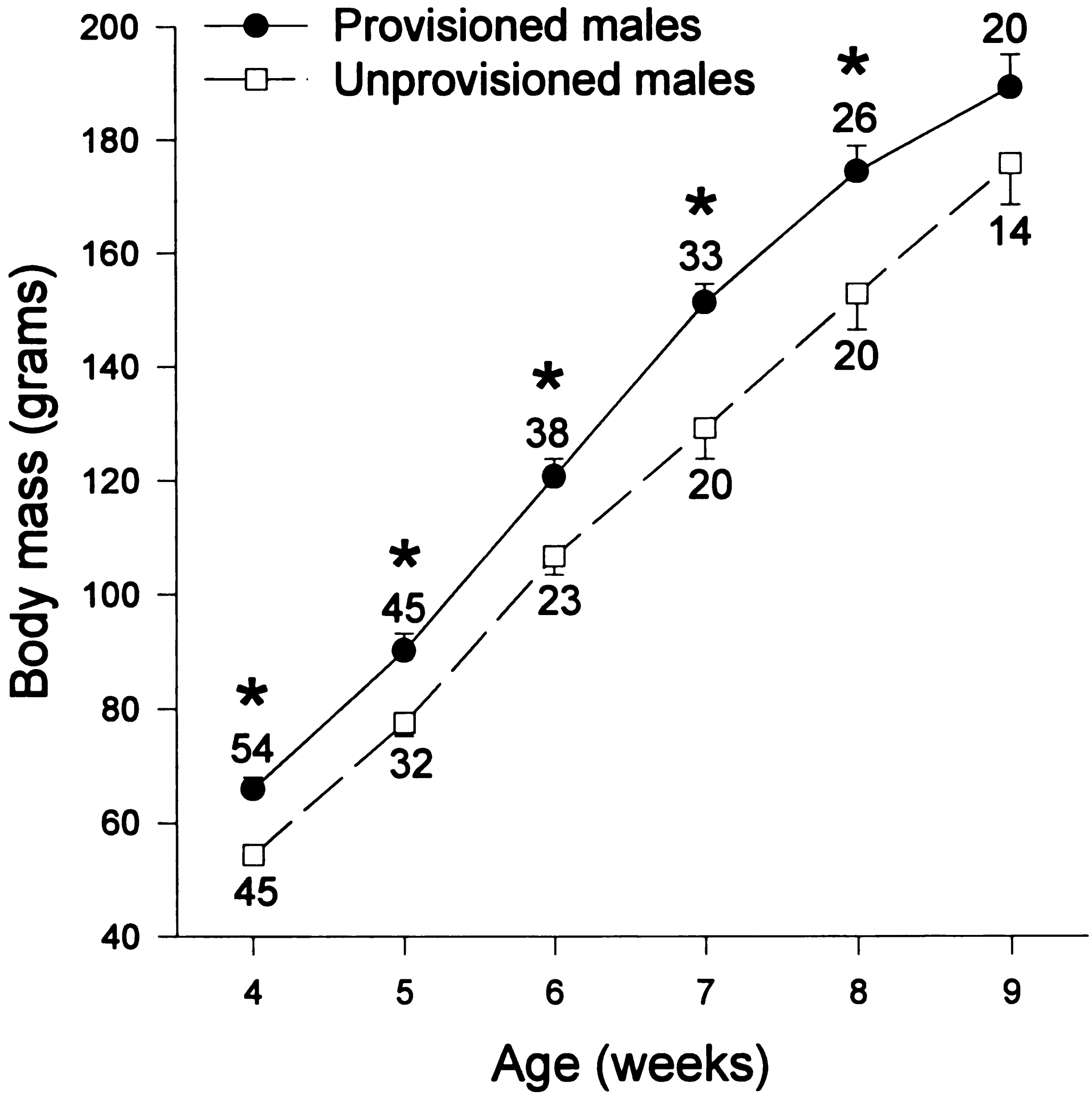

Figure 24 - Body mass of juvenile males, 1996. Asterisks indicate a significant difference between provisioned and unprovisioned males. Sample sizes are given on the graph.

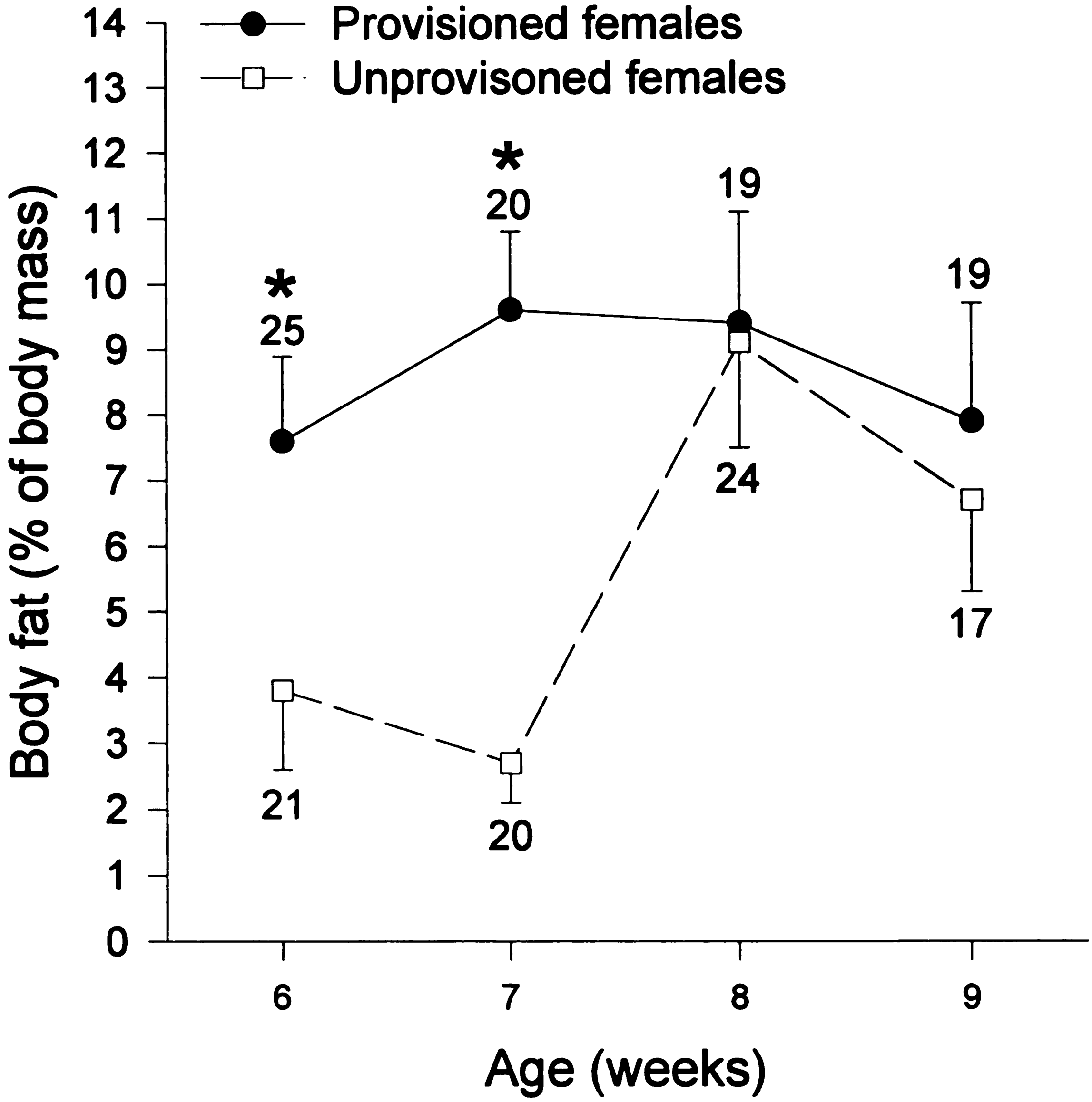

Figure 25 - Body fat of juvenile females, 1996. Asterisks indicate a significant difference between provisioned and unprovisioned females. Sample sizes are given on the graph.

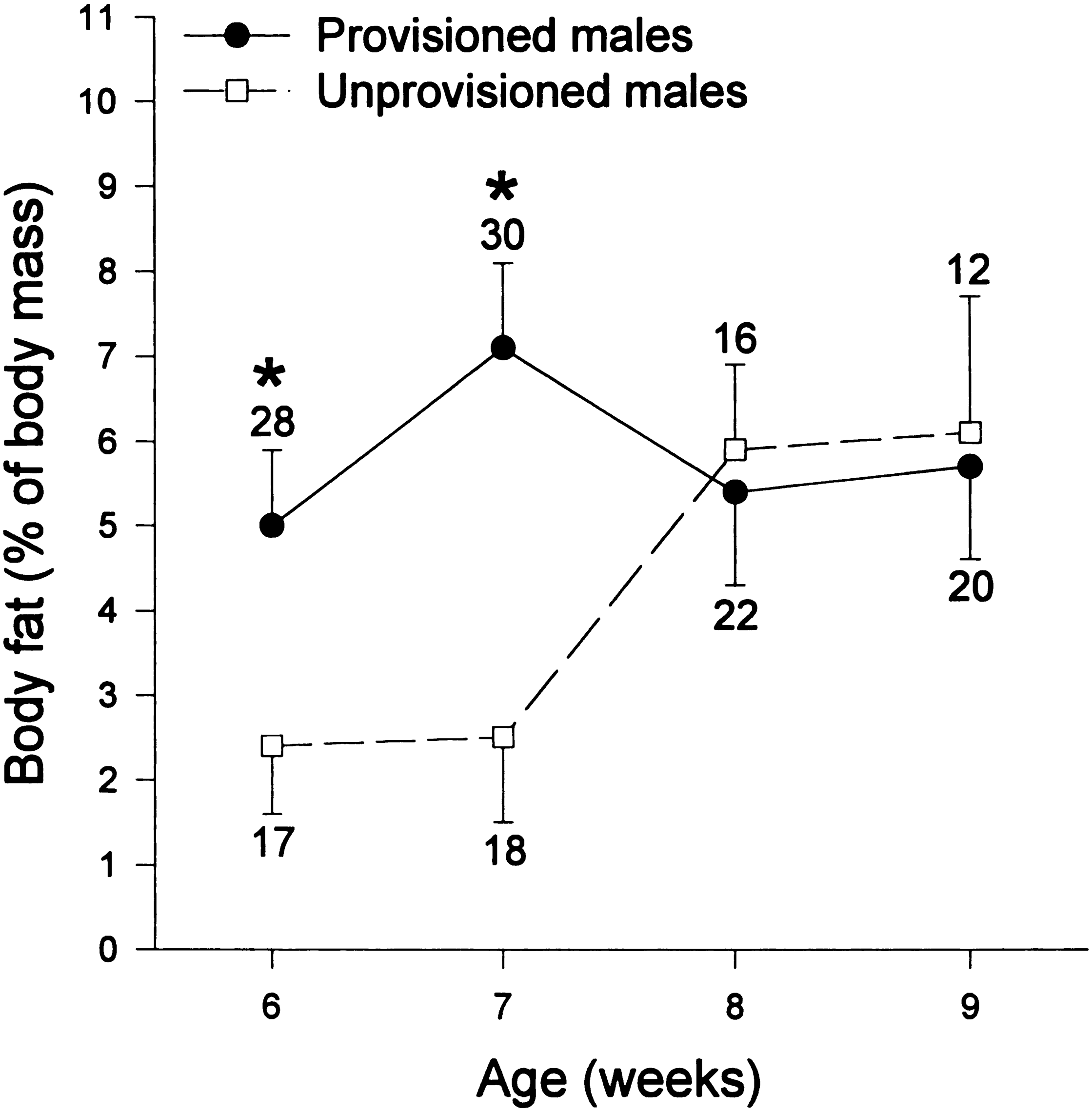

Figure 26 - Body fat of juvenile males, 1996. Asterisks indicate a significant difference between provisioned and unprovisioned males. Sample sizes are given on the graph.

independent samples, 6 weeks: $t = -2.348$, $df = 43$, $P = 0.024$; 7 weeks: $t = -3.407$, $df = 46$, $P = 0.001$).

Hormone treatments influenced the probability of dispersal by female *S. beldingi*. The cumulative percentage of oil-treated females that dispersed from 7-9 weeks of age did not differ significantly from that of untreated females (provisioned: $n = 10$ oil-treated, $n = 10$ untreated, Mantel-Haenszel test, $\chi^2 = 0.134$, $P = 0.715$; unprovisioned: $n = 6$ oil-treated, $n = 13$ untreated, no females dispersed), and so oil-treated and untreated females were combined within provisioned and unprovisioned groups for statistical analyses, and are referred to here as "control" females. The percentage of T-treated females that dispersed between 7-9 weeks of age differed significantly from that of control females in both provisioned (Figure 27; Mantel-Haenszel test, $\chi^2 = 13.513$, $P < 0.001$) and unprovisioned groups (Figure 27; Mantel-Haenszel test, $\chi^2 = 14.982$, $P < 0.001$). However, the percentage of T-treated females that dispersed between 7-9 weeks of age did not differ significantly from that of males in either provisioned (Figure 27; Mantel-Haenszel test, $\chi^2 = 0.253$, $P = 0.615$) or unprovisioned groups (Figure 27; Mantel-Haenszel test, $\chi^2 = 0.103$, $P = 0.748$). Thus, dispersal behavior of T-treated females more closely resembled that of males than that of control females.

Provisioning advanced the timing of dispersal among both males and T-treated females. The median age of dispersal was younger in provisioned than unprovisioned T-treated females (7 weeks vs. 9 weeks, Kruskal-Wallis test, $U = 2.0$, $P = 0.007$). The median age of dispersal was also younger in provisioned than unprovisioned males (7.5 weeks vs. 9 weeks, Kruskal-Wallis test, $U = 80.5$, $P = 0.023$).

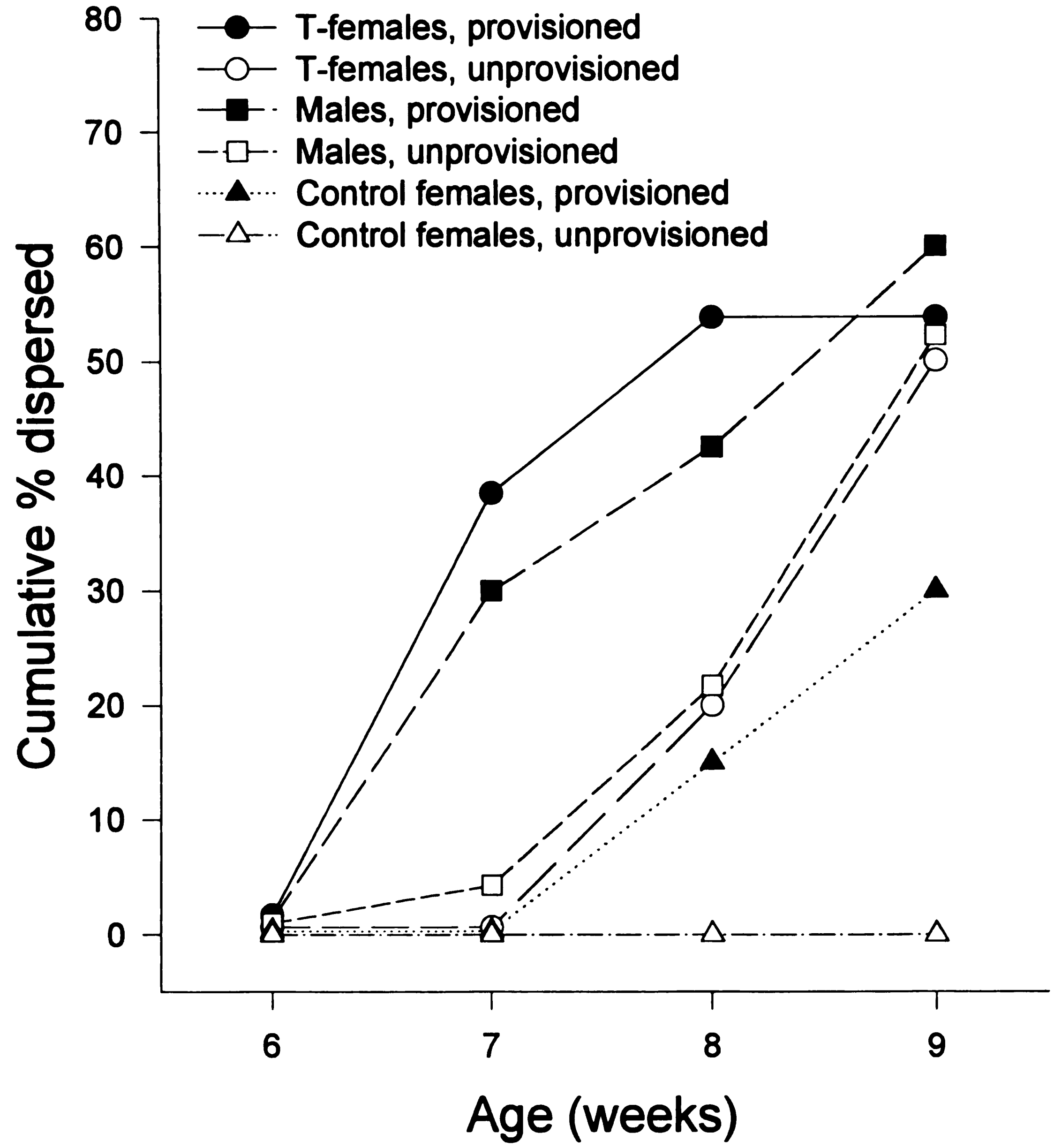

Figure 27 - Dispersal of juveniles, 1996. Samples included 13 provisioned T-treated females, 10 unprovisioned T-treated females, 40 provisioned males, 23 unprovisioned males, 20 provisioned control females, and 19 unprovisioned control females.

Provisioning also influenced the probability of dispersal by control females. Provisioned control females were more likely to disperse than were unprovisioned control females (Mantel-Haenszel test, $\chi^2 = 9.556$, $P = 0.002$), but only half as likely to disperse as were either group of T-treated females (Figure 27). The timing of dispersal by provisioned control females differed from that by provisioned T-treated females and provisioned males. Provisioned control females that dispersed did so at a significantly older median age than did provisioned T-treated females (9 weeks vs. 7 weeks, respectively; Kruskal-Wallis test, $U = 39.0$, $P = 0.006$) and at a somewhat older median age than did provisioned males (9 weeks vs. 7.5 weeks, Kruskal-Wallis test, $U = 105.0$, $P = 0.068$). Thus, perinatal T-treatment appears to have caused the timing of dispersal by young females to respond to food provisioning in a male-typical way.

I also observed an association between body fat and dispersal. By 7 weeks of age, when body fat was significantly greater in provisioned than unprovisioned juveniles, a greater proportion of provisioned than unprovisioned juveniles had dispersed among both T-treated females (Figure 28; Fisher's exact test, $P = 0.046$) and males (Figure 29; Fisher's exact test, $P = 0.022$). However, by 9 weeks of age, when body fat no longer differed between provisioned and unprovisioned juveniles, the proportion of juveniles that had dispersed was similar in provisioned and unprovisioned groups among both T-treated females (Figure 28; Fisher's exact test, $P = 1.000$) and males (Fig. 29; $\chi^2 = 0.365$, $P = 0.546$). This suggests that the advances in the timing of dispersal associated with food provisioning are mediated by body fat or fat-related variables.

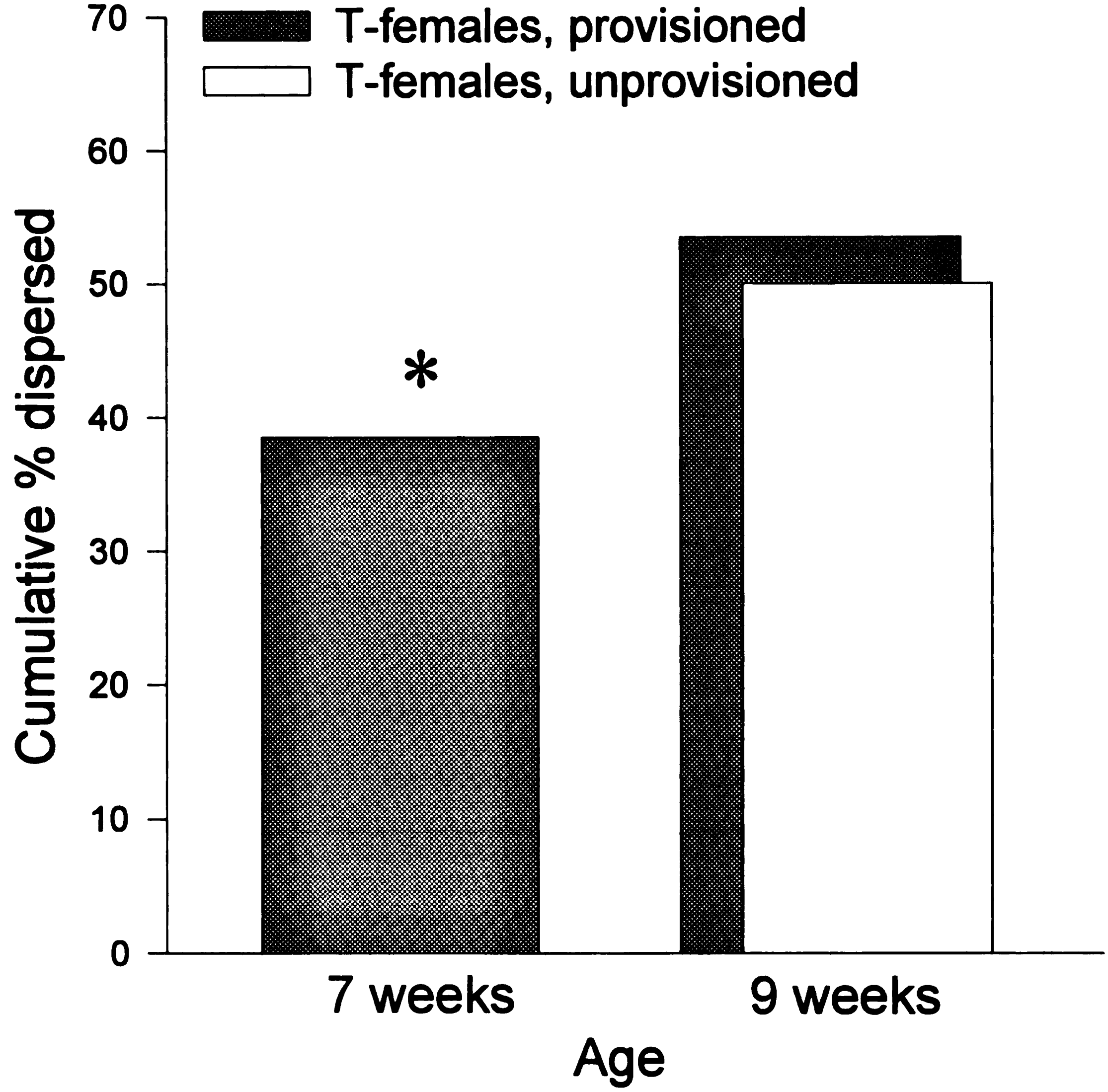

Figure 28 - Dispersal of T-treated females, 1996. The asterisk indicates a significant difference between provisioned and unprovisioned females. Samples included 13 provisioned and 10 unprovisioned T-treaed females.

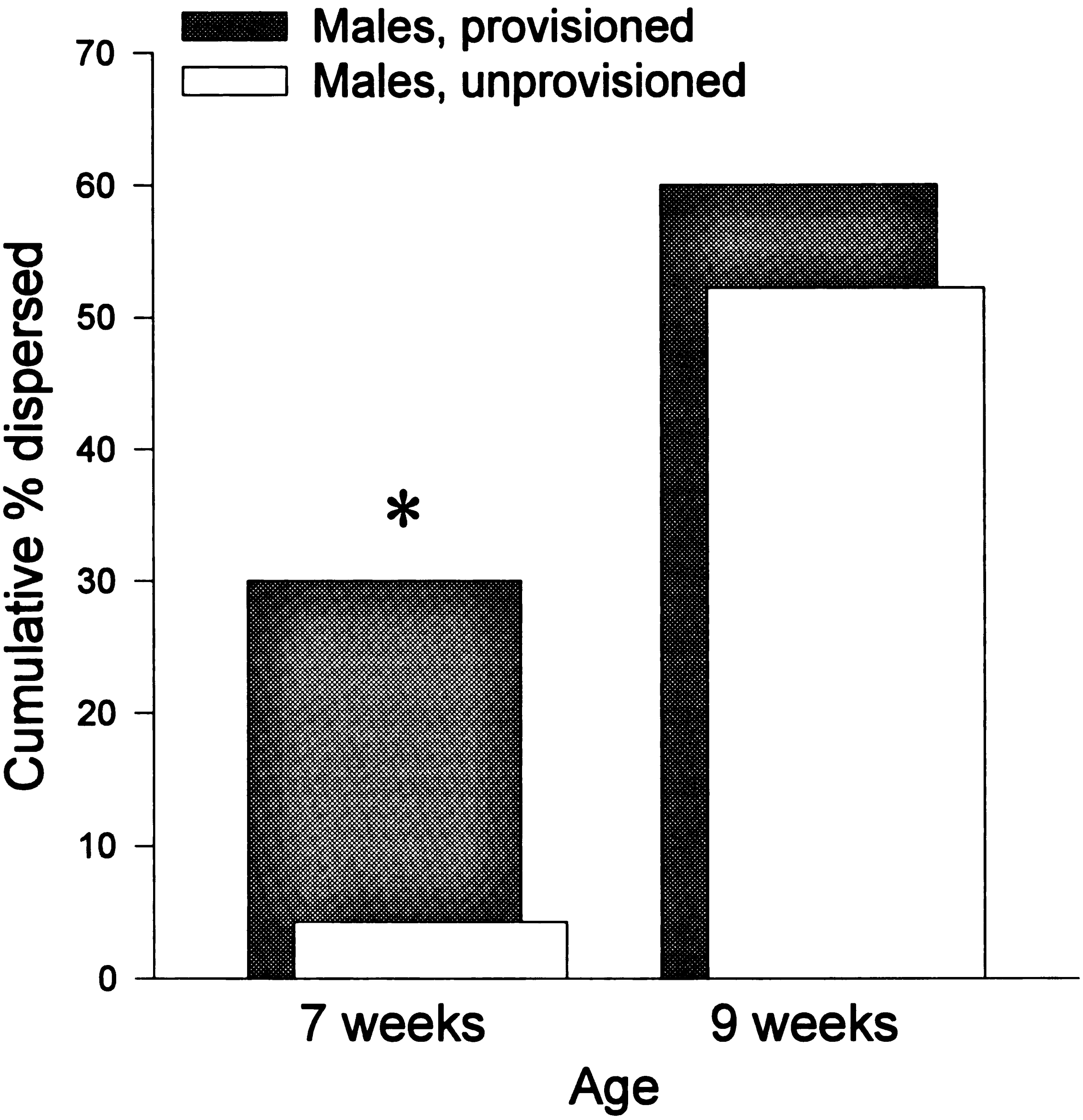

Figure 29 - Dispersal of males, 1996. The asterisk indicates a significant difference between provisioned and unprovisioned males. Samples included 40 provisioned and 23 unprovisioned males.

DISCUSSION

Perinatal T-treatment increased the probability that female *S. beldingi* would emigrate from the natal area, supporting the hypothesis that T organizes masculine dispersal patterns in this species (Holekamp et al., 1984). Furthermore, food provisioning advanced the timing of dispersal among both T-treated females and males. These results indicate that T organizes masculine timing of dispersal as well as a male-typical predisposition to emigrate from the natal area. Provisioned juveniles were heavier than unprovisioned juveniles throughout the summer. However, provisioned juveniles were fatter than their unprovisioned counterparts only until 7 weeks of age. Moreover, a greater proportion of provisioned than unprovisioned males and T-treated females had dispersed only until 7 weeks of age. Nunes and Holekamp (1996) observed that juvenile male *S. beldingi* disperse after attaining a threshold body mass. My results here indicate that once a mass threshold for dispersal has been reached, the timing of masculine dispersal behavior may be more importantly influenced by body fat than by body mass; body mass might affect dispersal only insofar as it reflects body fat (Smale et al., 1997). My results further suggest that physiological signals related to body fat convey information about environmental conditions such as food availability to endogenous mechanisms mediating dispersal behavior.

Provisioning increased the proportion of control female *S. beldingi* that dispersed. This replicates prior work by Nunes et al. (1997) in which food provisioning caused juvenile female *S. beldingi* to emigrate from the natal area. Nunes et al. (1997) observed that population density and density of mothers weaning litters was greater in provisioned than unprovisioned areas of their study site, and that mothers residing in provisioned areas exhibited higher rates of aggression and vigilance than did mothers in unprovisioned areas. They suggested that provisioned females emigrated from their natal areas in response to increased competition for non-food resources such as space in which to establish a territory. In this prior work, provisioned females that dispersed did so at significantly older ages than did provisioned males. Furthermore, only about 40% of provisioned females ultimately dispersed, whereas 100% of males eventually left their natal areas. Here we similarly observed that provisioned control females that dispersed left home at older ages and in smaller proportions than did either provisioned T-treated females or provisioned males. This further suggests that the effects of energy availability on the timing of dispersal are influenced by T exposure near the time of birth.

Endocrine factors interact with energetic variables in the mediation of a wide array of reproductive events among mammals. For example, hormonal and metabolic variables have been shown to interact in the control of reproductive maturation in both male and female mammals (Barnes, 1984; Bushberg and Holmes, 1985; Bronson and Rissman, 1986; Bronson, 1987, 1989; Wilson, 1992), and in the control of estrous cycling and fertility among females (Wade and Schneider, 1992; Wade et al., 1996). Here I show for the first time that endocrine and energetic variables may also interact in the mediation of dispersal behavior. Moreover, our results reveal in free-living mammals an interaction between endocrine control of behavior and the regulation of energy balance. To date, most studies of this interaction have been accomplished in the laboratory, using species such as hamsters (*Mesocricetus auratus*) and mice (*Mus musculus*) about whose ecology very little is known (e.g. Campfield et al., 1995; Halaas et al., 1995; Pellymounter et al., 1995; Wade and Schneider, 1992; Wade et al., 1996).

It was recently reported that a unique dispersive phenotype occurs occasionally in colonies of naked mole rats (*Heterochephalus glaber*; O'riain et al., 1996). Approximately 95% of these dispersive *H. glaber* are males. *H. glaber* that disperse are morphologically, physiologically, and behaviorally distinct from other colony members. Dispersers tend to be extraordinarily fat, have elevated blood concentrations of luteinizing hormone, and are attracted to rather than aggressive toward unfamiliar conspecifics. Dispersive phenotypes in *H. glaber*, *S. beldingi*, and perhaps many other mammals might share a suite of defining characteristics that differ between species only in degree. The situation in *H. glaber* may represent an extreme development of the same basic control system operating in *S. beldingi*, in which endocrine and energetic variables interact to mediate dispersal. Interestingly, data from both *H. glaber* and *S. beldingi* suggest the existence of key links between mediation of dispersal behavior and reproductive physiology (O'riain et al., 1996; Chapter 5).

I propose here a model (Figure 30) which describes possible interactions between endocrine and energetic variables in the mediation of natal dispersal behavior in male mammals. According to this model, perinatal androgen exposure organizes neural systems promoting male-typical natal dispersal behavior, as well as the responsiveness of these systems to energetic variables such as body fat. When an individual male has sufficient fat stored to meet the potential energetic demands of the dispersal movement, this information is conveyed by physiological systems that monitor fat stores or fuel availability to systems that mediate dispersal, which then initiate masculine patterns of dispersal behavior.

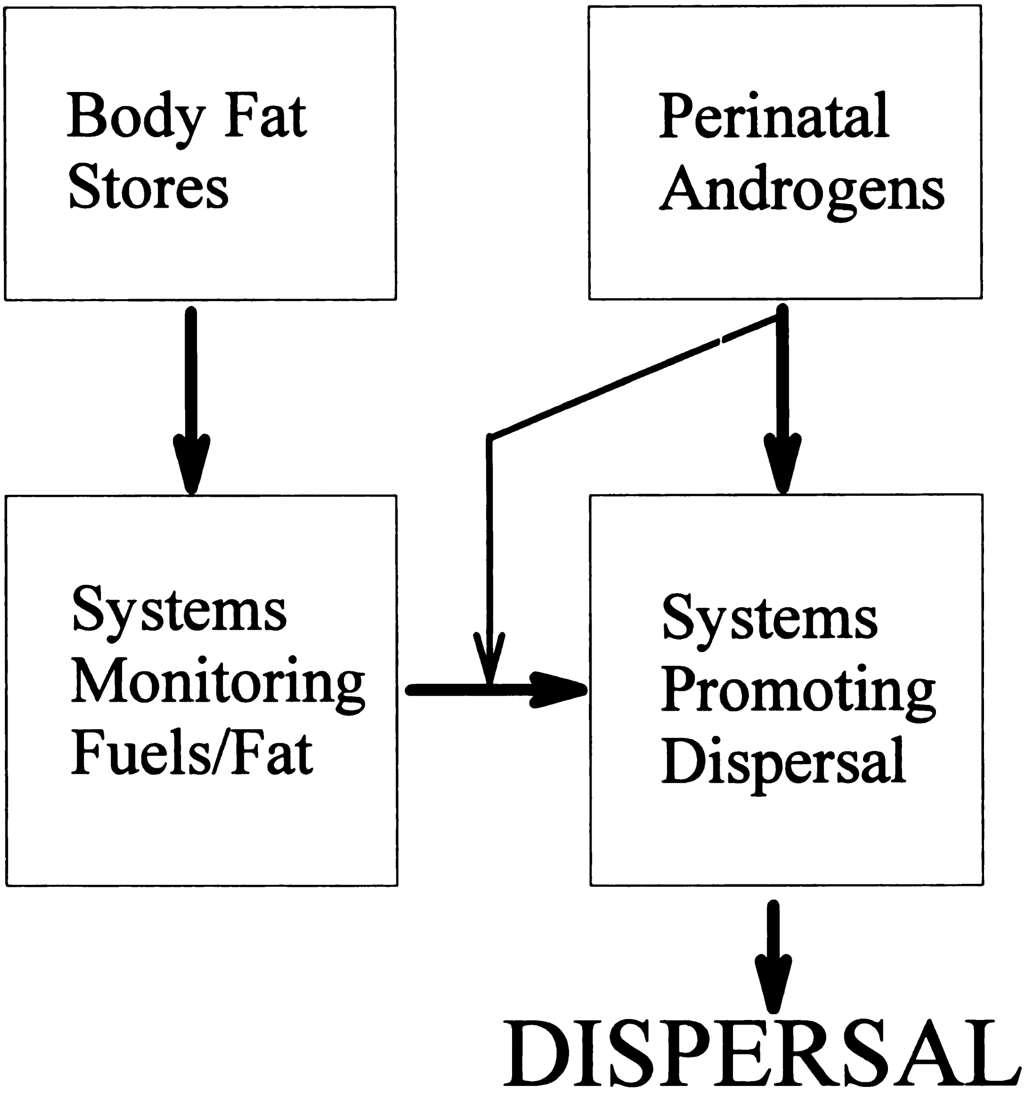

Figure 30 - A model of endocrine and energetic regulation of dispersal. According to this model, early androgen exposure masculines physiological systems that promote dispersal behavior, as well as the way in which these systems respond to energetic variables such as body fat. When an individual has sufficient fat stored to meet the potential energetic demands of dispersing, this information is conveyed by systems that monitor fuel availability to systems that promote dispersal, which then initiate male-typical dispersal behavior.

This model appears to describe the relationship between hormones and energy balance in the population of *S. beldingi* that I studied at Tioga Pass. However, this population resides at high altitude (3040 m), in a habitat with a very short growing season (about 3 months). The brevity of the growing season places major energetic constraints on resident squirrels at Tioga Pass (Morton et al., 1974; Maxwell and Morton, 1975; Morton, 1975), and energetic demands of dispersal behavior may be especially severe in these squirrels. Thus, it is not clear whether my model applies to male mammals in general. However, potential risks and energetic demands are associated with dispersal in many mammals (e.g., Waser, 1996; Smale et al., 1997), and I suggest that the model may describe the physiological underpinnings of dispersal in many mammalian species.

My results also suggest interesting parallels between dispersal and migratory behavior. Hormones directly or indirectly stimulate migratory behavior, and help mobilize energy to support it, in insects, fish, amphibians, and birds (Hoar, 1988; Wingfield et al., 1990; Young et al., 1989; Rankin, 1991; Dingle, 1996). My results indicate that perhaps T has similarly been "captured" during the evolutionary history of *S. beldingi* to function in the mediation of dispersal movements as well as in reproduction. The actual mechanisms by which perinatal T exposure interacts with lipid stores to influence male dispersal behavior in *S. beldingi* are unknown. One possibility is that concentrations of circulating metabolic fuels such as glucose or fatty acids, or metabolic hormones such as insulin or glucagon, are influenced by the size of fat stores, and are detected by endogenous mechanisms that mediate dispersal behavior. Circulating glucose in fact regulates estrous cycles of female hamsters (*Mesocricetus auratus*) via effects on the area prostrema of the hind brain (Schneider and Wade, 1989; Wade and Schneider, 1992;

Schneider and Zhu, 1994; Wade et al., 1996). A second possibility is that leptin, a compound released by adipose tissue (Campfield et al., 1995; Halaas et al., 1995; Pellymounter et al., 1995), plays a role in mediating emigration from the natal area. Leptin regulates feeding and activity in mice via endocrine effects on the central nervous system (Campfield et al., 1995). In *S. beldingi*, the amount of leptin released might be proportional to the overall amount of body fat, and might convey information about the overall size of fat stores to endogenous mechanisms mediating natal dispersal behavior.

Chapter 7

ECOLOGICAL INFLUENCES ON DISPERSAL

INTRODUCTION

Because of the striking sexual dimorphisms observed in dispersal behavior, Dobson (1979) and Holekamp (1984a) proposed that evolutionary explanations of natal dispersal might often differ for males and females. Recent evidence suggests that the ultimate causes of natal dispersal may differ between the sexes among Townsend's voles *(Microtus townsendii*; Lambin, 1994) and various species of mammalian carnivores (e.g. Waser, 1996). However, because dispersal by both sexes is typically rare among mammals, collecting data on sex differences in behavior is often difficult and requires long-term study (e.g., Pusey and Packer, 1987b), fortuitous observations of rare dispersal events (e.g., Mills, 1990; Holekamp et al., 1993), or experimental induction of dispersal (e.g., Dobson, 1979). In a food provisioning experiment examining the timing of dispersal behavior by juvenile male *S. beldingi* (Nunes and Holekamp, 1996), I observed an unexpected change in the frequency of emigration by juvenile female *S. beldingi* from areas receiving extra food. This allowed me to compare the dispersal behavior of male and female *S. beldingi* receiving supplemental food with that of unprovisioned animals to determine whether the ultimate causes of natal dispersal differ between males and females of this species.

Natal dispersal by male *S. beldingi* appears not to be driven by demand for environmental resources (Holekamp, 1986). It is more likely that juvenile male *S. beldingi* disperse to avoid inbreeding (Sherman, 1976; Holekamp, 1984b), or to increase their probability of encountering receptive mates (Holekamp, 1986). On the other hand, juvenile female ground squirrels may disperse to improve their access to environmental resources (Dobson, 1979). If female but not male *S. beldingi* emigrate from the natal area in response to demand for environmental resources, then altering the availability of resources such as food, should affect the probability of dispersal by juvenile females but not by juvenile males.

Female *S. beldingi* require three critical environmental resources for successful reproduction: nest sites, food, and space in which to establish maternal territories. If females emigrate to improve access to nest sites, then they should move from areas containing few burrows to areas containing abundant burrows. Holekamp (1986) was able to rule out the nest demand hypothesis as an explanation for dispersal in both male and female *S. beldingi*, because numbers of immigrants and emigrants in different study areas were unrelated to numbers of available burrows. Here I examined the remaining possibilities that female dispersal improves access to food or space in which to establish territories.

If female *S. beldingi* disperse to improve their access to food, then they should emigrate from areas in which competition for food is intense, and immigrate into areas containing abundant food. Thus, provisioning an area with supplemental food should reduce emigration from that area and increase immigration into it (Arcese, 1989). However, if female *S. beldingi* disperse to improve their access to space for territory establishment, then they should emigrate from areas of high to low population density, regardless of food abundance. Thus, experimentally increasing population density by food provisioning (Boutin, 1990; Dobson, 1995) should increase the probability of dispersal by juvenile female *S. beldingi* from provisioned areas.

METHODS

During the summers of 1993 and 1994, I trapped and tagged 135 juvenile males and 155 juvenile females from 65 complete litters. I provided extra food to adult and yearling females in their maternal territories during pregnancy and lactation, and to offspring of these females in their natal areas for six weeks after weaning. I used unprovisioned young of unprovisioned mothers as controls. I gave extra food to juveniles from 15 litters in 1993 and 18 litters in 1994. I used juveniles from ten litters in 1993 and 22 litters in 1994 as unprovisioned controls. I measured body mass of these juveniles weekly from 4-11 weeks of age, and monitored dispersal status weekly from 6-10 weeks of age. Also in 1993 and 1994, I monitored the density of females that weaned litters and overall density of *S. beldingi*.

During the summer of 1995, I gave extra food to 20 reproductive females, and used 34 reproductive females in unprovisioned areas as controls. To estimate the intensity of resource competition in provisioned areas, I observed the aggressive and vigilant behavior of these females.

RESULTS

I compared body mass between provisioned and unprovisioned juvenile males and females from 4-11 weeks of age. Provisioned juveniles were significantly heavier than unprovisioned juveniles during this interval (Figure 31; ANOVA, $F = 432.984$, $df = 1$, $P <$

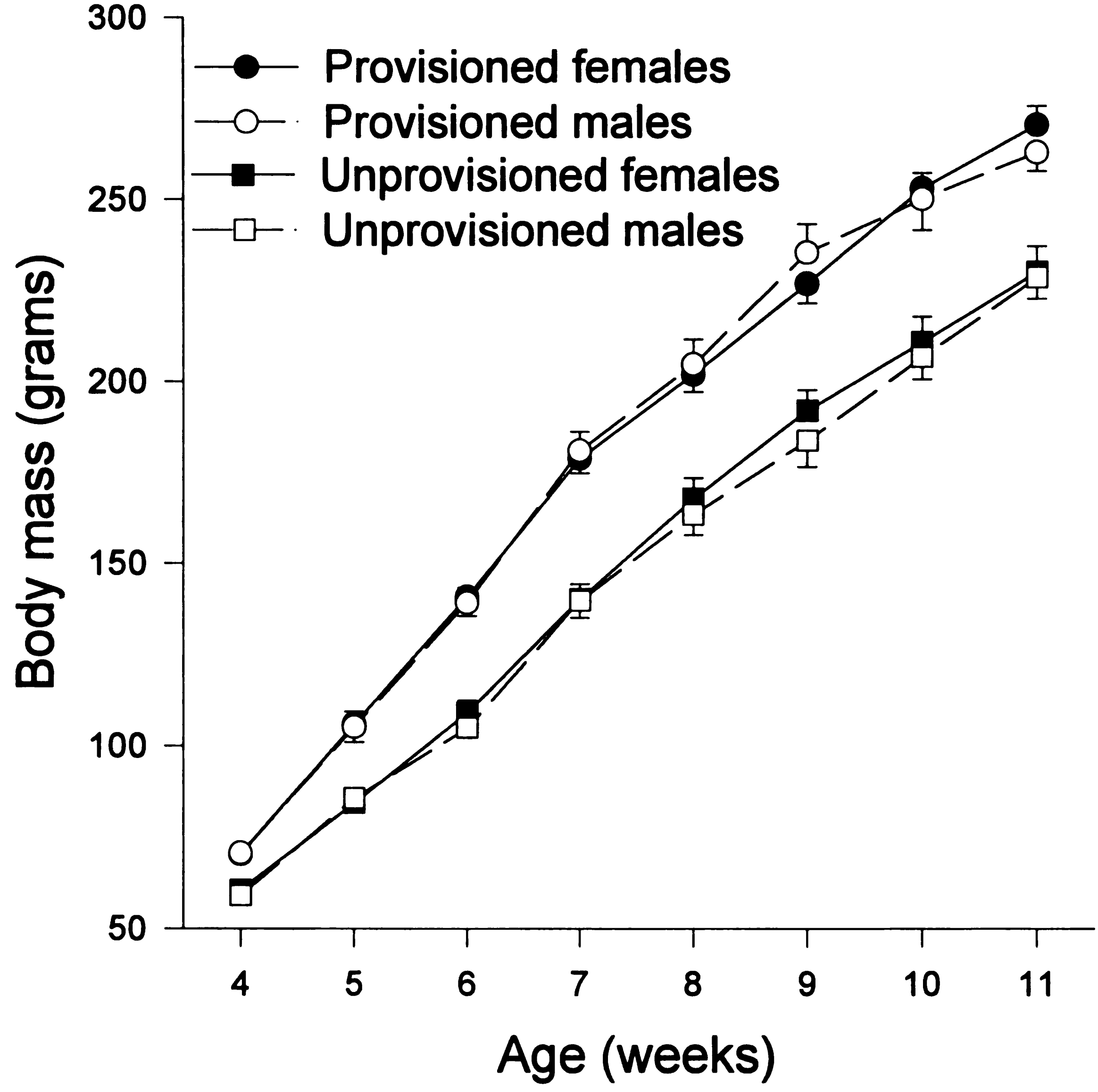

Figure 31 - Body mass of juveniles, 1993-1994. Sample sizes are as follows: provisioned females: 79, 47, 52, 42, 28, 30, 30, 23; provisioned males: 75, 30, 36, 35, 19, 18, 17, 16; unprovisioned females: 60, 30, 37, 38, 35, 34, 35, 29; unprovisioned males: 51, 30, 36, 42, 36, 30, 32, 25.

0.001); however, I observed no sex difference in body mass (Fig. 31; ANOVA, $F = 0.703$, $df = 1$, $P = 0.402$). Thus, provisioning appears to have increased body mass similarly in both males and females.

The overall density of females weaning litters in provisioned areas was approximately twice that in unprovisioned areas (73 provisioned females/hectare vs. 36 unprovisioned females/hectare, respectively). This density difference can be explained in part by the fact that reproductive females in provisioned areas appeared to shift their home ranges closer to feeding boxes.

The median size of litters at weaning was significantly greater for litters of provisioned than unprovisioned females (5.0, $n = 33$ vs. 4.0, $n = 32$, respectively; Kruskal-Wallis test, $U = 303.5$, $P = 0.002$). The median number of juvenile females in each litter at weaning was also greater for litters of provisioned than unprovisioned females (3.0, $n = 33$ vs. 2.0, $n = 32$, respectively; Kruskal-Wallis test, $U = 333.5$, $P = 0.008$).

Prior to the first emergence of juveniles from the natal burrow, during the period in which females were pregnant or nursing young, the mean density of adult *S. beldingi* above ground was significantly higher in provisioned than unprovisioned areas (Figure 32; 84.0/hectare, $n = 135$ observations vs. 44.8/hectare, $n = 187$ observations, respectively, Kruskal-Wallis test, $U = 6134.5$, $P < 0.001$). After the first emergence of juveniles from the natal burrow, mean overall density of *S. beldingi* was also significantly higher in provisioned than unprovisioned areas (Figure 32; 170.5/hectare, $n = 47$ observations vs. 87.1/hectare, $n = 62$ observations, respectively; Kruskal-Wallis test, $U = 659.5$, $P <$ 0.001). Thus, adult and yearling females and their young experienced significantly higher

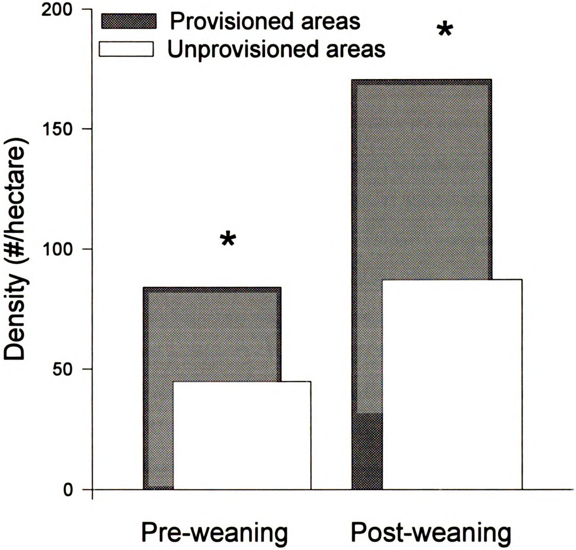

Figure 32 - Density of *S. beldingi*, 1993-1995. Mean densities both prior to and after the first weaning of young are presented. Asterisks indicate a significant difference between provisioned and unprovisioned areas.

densities of both maternal females and all conspecifics in provisioned than unprovisioned areas of the study site.

I evaluated aggressive and vigilant behavior of females that weaned litters during the summer of 1995. Pregnant and lactating females in provisioned areas exhibited overall higher rates of vigilance (Figure 33; Kruskal-Wallis test, $U = 3630.5$, $P < 0.001$), and aggressive behavior (Figure 34; Kruskal-Wallis test, $U = 3342.0$, $P < 0.001$) than did maternal females in unprovisioned areas. Provisioned females had significantly higher rates of vigilance during all four observation periods (Figure 33; Kruskal-Wallis tests, early gestation: $U = 90.0$, $P < 0.001$; late gestation: $U = 420.5$, $P < 0.001$; early lactation: $U = 307.0$, $P = 0.001$; late lactation: $U = 237.0$, $P = 0.006$). Provisioned females also had somewhat higher rates of aggression during gestation (Figure 34; Kruskal-Wallis tests, early gestation: U = 217.5, P = 0.033; late gestation: U = 257.5, P = 0.055), and significantly higher rates of aggression during the first half of lactation (Figure 34; Kruskal-Wallis test, U = 377.5, P = 0.004) than did unprovisioned females.

Provisioning influenced the timing of dispersal in both sexes, but altered the probability of dispersal only by females. A significantly larger cumulative percentage of provisioned than unprovisioned females had dispersed by 7-10 weeks and 55 weeks of age (Figure 35; 7 weeks: Fisher's exact test, $P = 0.006$; 8 weeks: $\chi^2 = 11.647$, $df = 1$, $P = 0.001$; 9 weeks: $\chi^2 = 8.746$, $df = 1$, $P = 0.004$; 10 weeks: $\chi^2 = 8.142$, $df = 1$, $P = 0.004$; 55 weeks: Fisher's exact test, $P = 0.013$). Cumulative dispersal percentages differed significantly between provisioned females and unprovisioned males only during the 55 week age interval (Figure 35; 55 weeks: Fisher's exact test, $P = 0.006$). However, a significantly larger cumulative percentage of provisioned males than provisioned females

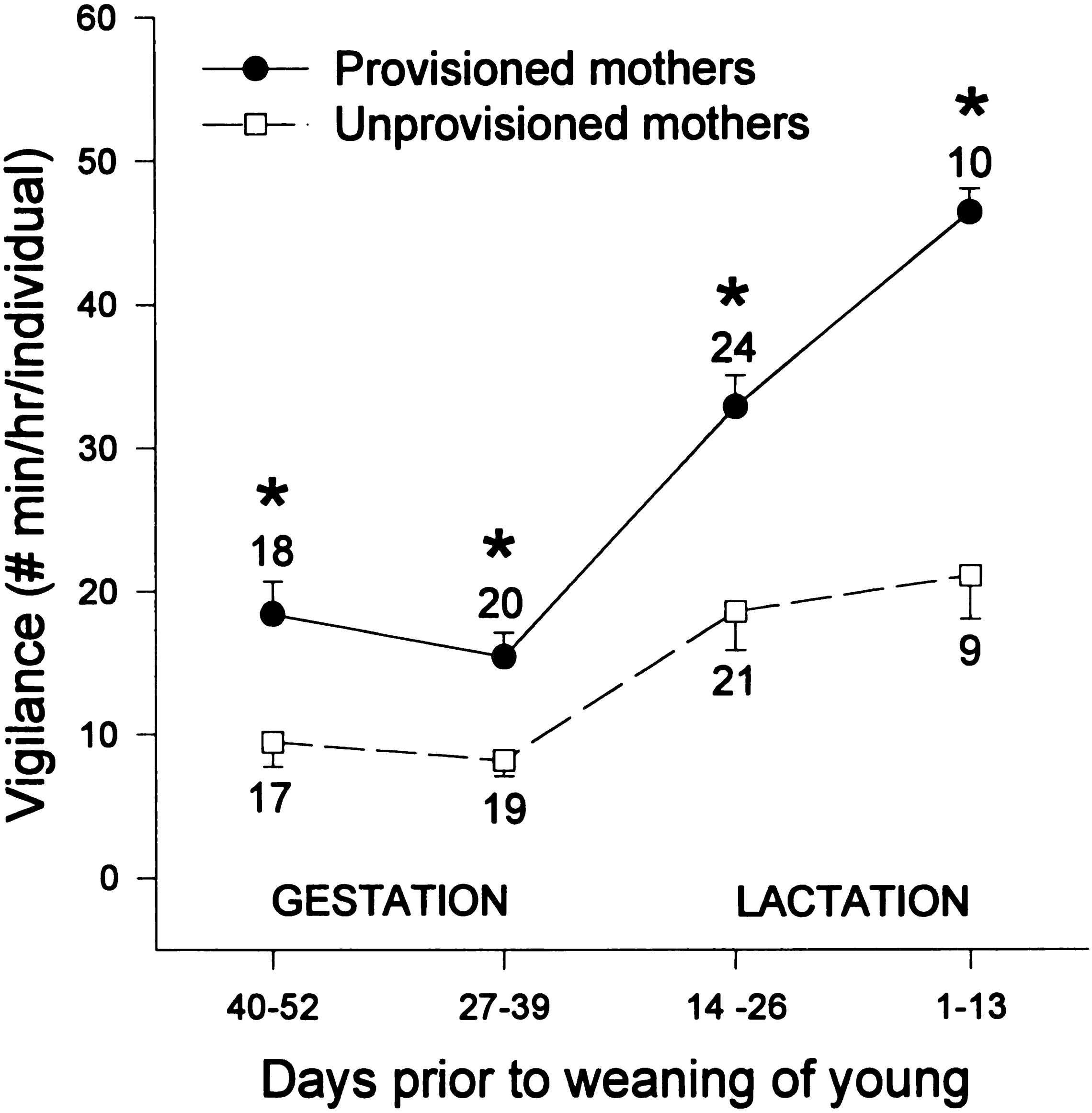

Figure 33 - Vigilance behavior of adult females, 1995. Mean rates of vigilance for females that weaned litters in provisioned and unprovisioned areas are presented. Asterisks indicate a significant difference between females in provisioned and unprovisioned areas. Sample sizes are given on the graph.

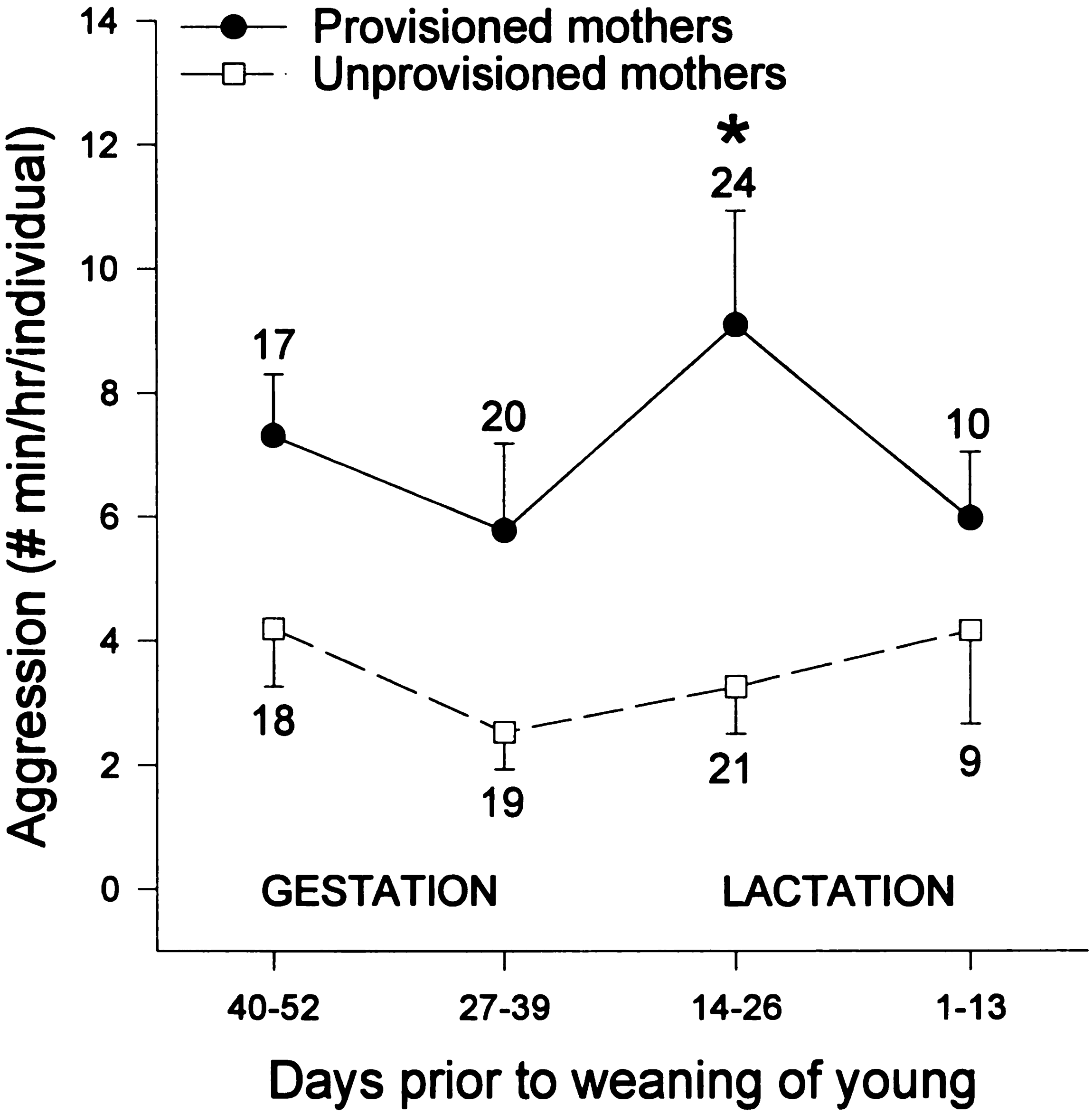

Figure 34 - Aggressive behavior of adult females, 1995. Mean rates of aggression for females that weaned litters in provisioned and unprovisioned areas are presented. Asterisks indicate a significant difference between females in provisioned and unprovisioned areas. Sample sizes are given on the graph.

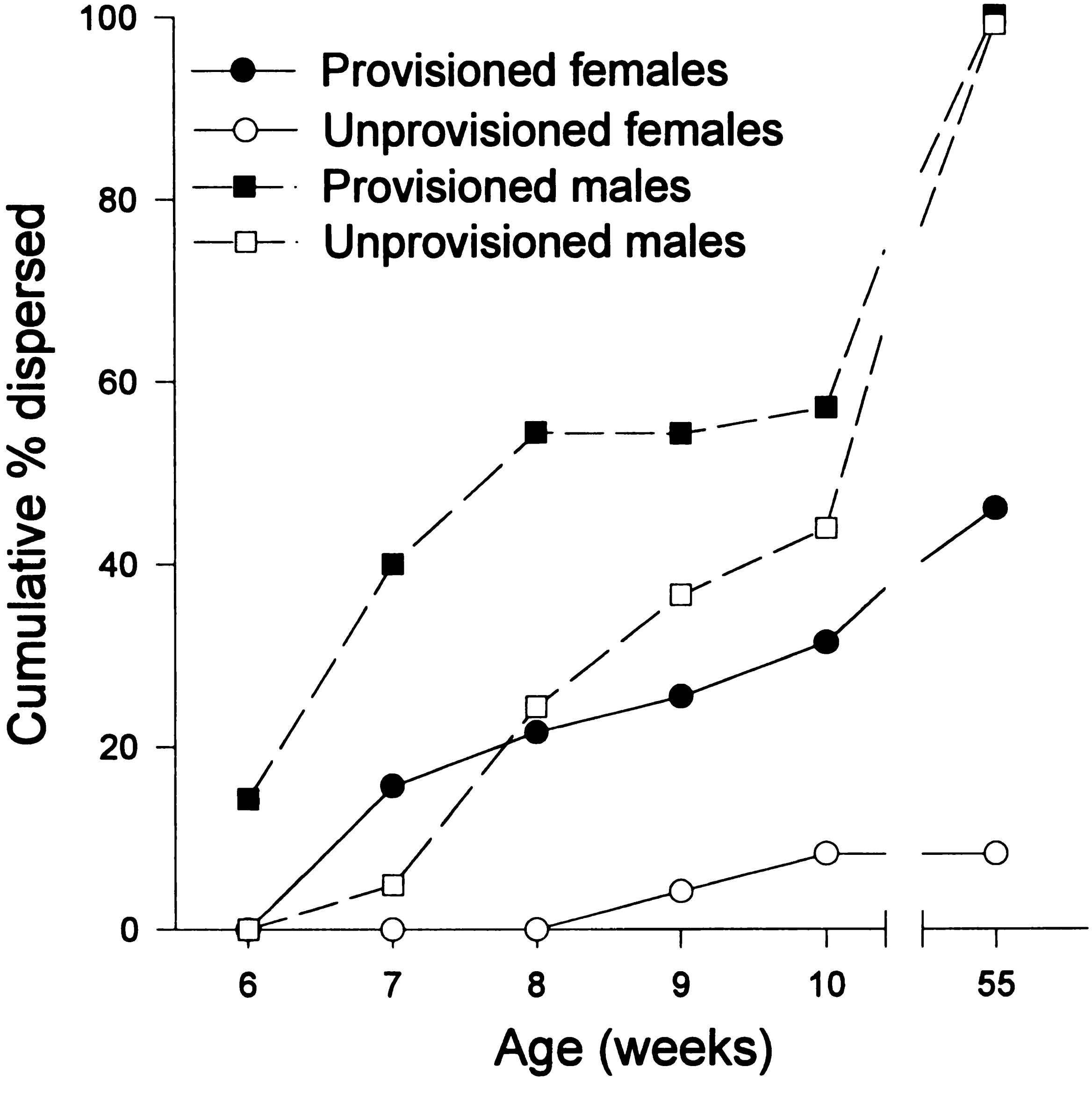

Figure 35 - Dispersal of the 1993 and 1994 cohorts. The cumulative percentages of provisioned and unprovisioned males and females that dispersed as juveniles from 6-10 weeks of age, and as yearling by 55 weeks of age, are presented. Samples of juveniles included 51 provisioned females, 48 unprovisioned females, 35 provisioned males, and 41 unprovisioned males. Samples of yearlings included 13 provisioned females, 24 unprovisioned females, 12 provisioned males, and 11 unprovisioned males.

dispersed by all ages for which we monitored dispersal behavior (Figure 35; 6 weeks: Fisher's exact test, $P = 0.009$; 7 weeks: $\chi^2 = 6.445$, $df = 1$, $P = 0.011$; $\chi^2 = 8.280$, $df = 1$, $P = 0.004$; 9 weeks: $\chi^2 = 7.366$, $df = 1$, $P = 0.007$; 10 weeks: $\chi^2 = 5.664$, $df = 1$, $P = 0.017$; 55 weeks: Fisher's exact test, $P = 0.005$). Thus, cumulative dispersal frequencies of provisioned females during the juvenile summer more closely resembled those of unprovisioned males than those of unprovisioned females. However, 100% of surviving males dispersed before the end of the yearling summer, whereas more than half of provisioned females remained in their natal areas as yearlings.

Of the 21 dispersed juvenile females that I recovered during 1993 and 1994, eight had sisters that also dispersed as juveniles. I observed six of these after dispersal within five meters of a sister that had also dispersed. By contrast, of 38 juvenile males that dispersed during 1993 and 1994, 17 had brothers that also dispersed as juveniles, but I never observed brothers to have overlapping home areas after dispersing. Thus, among same-sex siblings that dispersed, females were significantly more likely to settle near a sister than males were likely to settle near a brother (Fisher's exact test, $P = 0.004$). Of the 38 dispersed juvenile males that I recovered, eight settled in provisioned areas, whereas all 21 dispersed juvenile females settled in areas not receiving extra food. Thus, among dispersed juveniles, females were significantly less likely to settle in provisioned areas than were males (Fisher's exact test, $P = 0.041$). Juvenile females that dispersed apparently avoided settling in provisioned areas.

Two juvenile females from provisioned areas established home areas and sleeping burrows outside their natal areas for > 1 week, and then returned to their natal areas. These two females spent the remainder of the juvenile summer in their natal areas. One disappeared over the winter, and the other later dispersed as a yearling. By contrast, I never observed juvenile males to re-establish themselves in their natal areas after emigrating.

I compared body mass between dispersed and undispersed juvenile females. Overall, females that had dispersed were significantly heavier than were same-aged females still residing in their natal areas overall from 7-11 weeks of age (Figure 36; ANOVA, $F = 4.289$, $df = 1$, $P = 0.039$). The difference in body mass between dispersed and natal females was marginally significant at 10 and 11 weeks of age, after significance levels were adjusted (Figure 36; *t*-tests for independent samples, 10 weeks: $t = 2.245$, $df = 20$, $P = 0.036$; 11 weeks: $t = 2.415$, $df = 35$, $P = 0.021$). Thus, females that dispersed apparently did not have limited access to food in either their natal areas, or in their new home areas.

I evaluated juveniles' body mass relative to time of dispersal. Body mass of provisioned females did not differ significantly from that of unprovisioned females from two weeks prior to dispersal to two weeks after dispersal (*t*-test for independent samples; $t = 1.315$, $df = 37$, $P = 0.197$), and so I combined body mass data from these two groups for statistical analysis. Similarly, provisioned and unprovisioned males did not differ in body mass from two weeks prior to dispersal to two weeks subsequent to dispersal (*t*-test for independent samples, $t = -1.644$, $df = 104$, $P = 0.103$), so I also combined body mass data from these two groups for statistical analysis. Overall, juvenile male *S. beldingi* were significantly heavier than juvenile female *S. beldingi* from two weeks prior to dispersal to two weeks after dispersal (Figure 37; ANOVA, $F = 14.102$, $df = 1$, $P < 0.001$). After significance levels of pairwise comparisons were adjusted, differences in body mass were

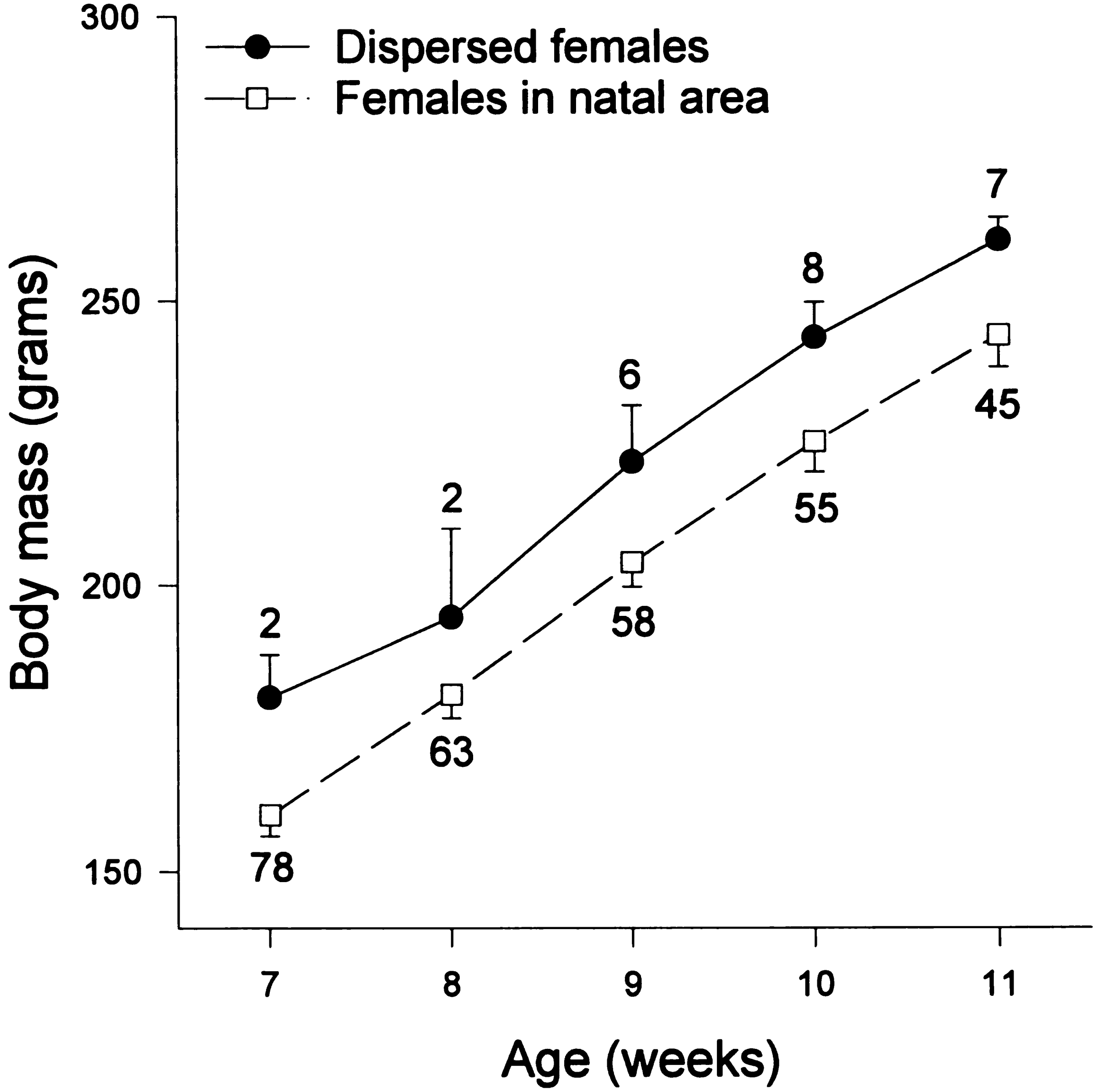

Figure 36 - Body mass of dispersed and natal females, 1993-1994. Sample sizes age given on the graph.

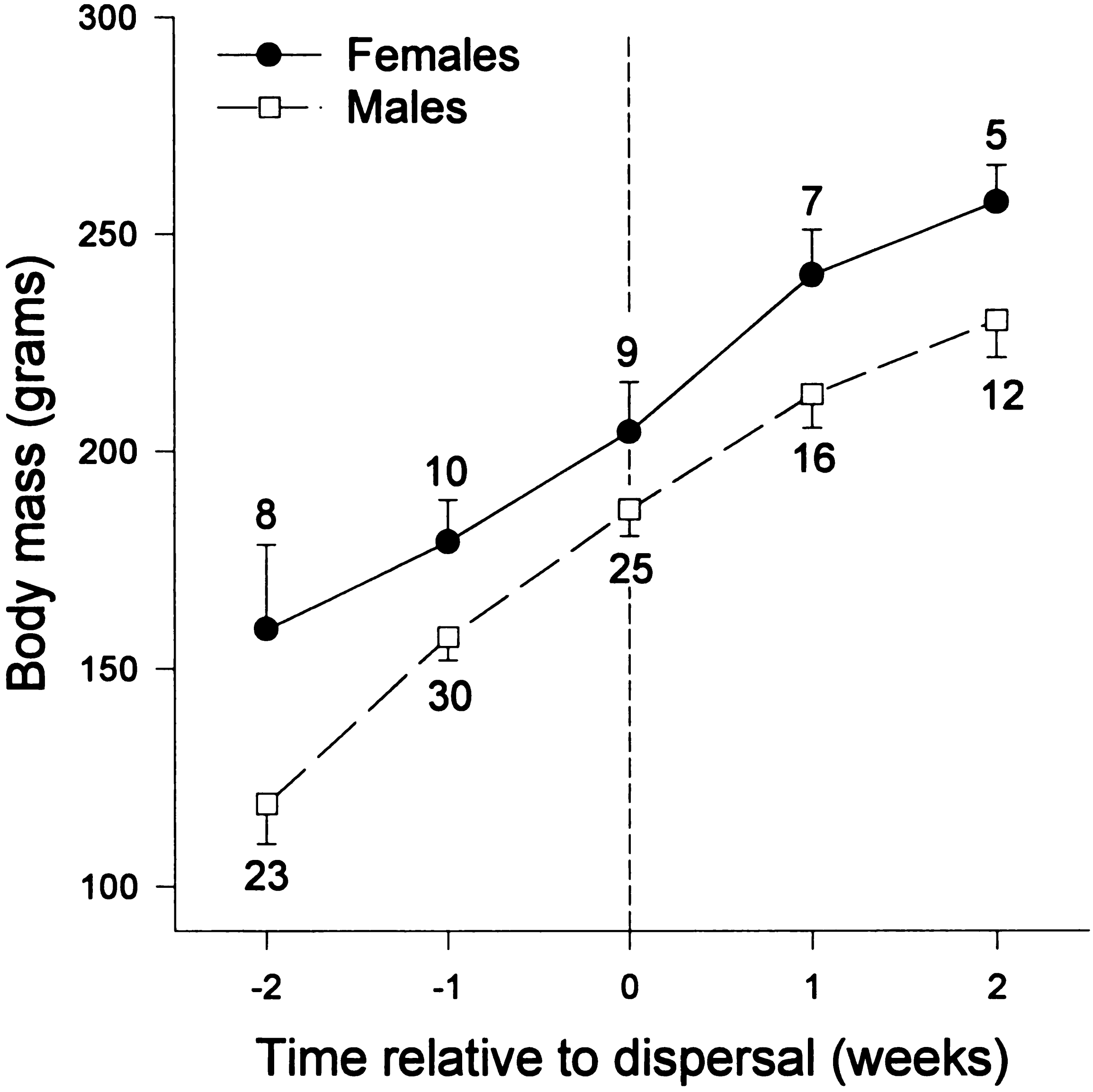

Figure 37 - Body mass of juveniles relative to dispersal, 1993-1994. The vertical dashed line indicates the week of dispersal. Sample sizes are given on the graph.

marginally significant at 1 and 2 weeks prior to dispersal and at 1 and 2 weeks subsequent to dispersal (Fig. 37; *t*-tests for independent samples; -2 weeks: $t = -2.104$, $df = 29$, $P = 0.044$; -1 week: $t = -1.989$, $df = 38$, $P = 0.054$; +1 week: $t = -2.010$, $df = 21$, $P = 0.057$; +2 weeks: $t = -2.293$, $df = 12$, $P = 0.041$).

I also observed sex differences in the ages at which juvenile *S. beldingi* dispersed. The median age of dispersal was significantly younger for provisioned males than for provisioned females (7.0 weeks, $n = 20$ vs. 7.5 weeks, $n = 17$, respectively; Kruskal-Wallis test, $U = 219.5$, $P = 0.044$), and was also significantly younger for unprovisioned males than for unprovisioned females (8.0 weeks, $n = 18$ vs. 9.5 weeks, $n = 4$, respectively; Kruskal-Wallis test, $U = 62.0$, $P < 0.001$) and between provisioned and unprovisioned females (7.5 weeks, $n = 16$ vs. 9.5 weeks, $n = 4$, respectively; Kruskal-Wallis test, $U = 11.0$, $P = 0.038$). Thus, males dispersed at significantly younger ages than did females in both the provisioned and unprovisioned groups, and provisioned animals dispersed at younger ages in both sexes.

DISCUSSION

I observed a sex difference in the dispersal behavior of *S. beldingi* in response to food provisioning. All surviving male *S. beldingi* in both provisioned and unprovisioned groups dispersed by the end of their yearling year. Thus, my results confirm the finding of Holekamp (1984a, 1986) that all male *S. beldingi* eventually emigrate from the natal area, and support an hypothesis that male *S. beldingi* disperse for reasons other than to enhance access to environmental resources. Young male *S. beldingi* disperse probably either to avoid breeding with close kin (Sherman, 1976; Holekamp, 1984b), or to enhance access to receptive mates (Holekamp, 1986). In contrast to young male *S. beldingi*, young females weaned in areas receiving extra food were significantly more likely to emigrate from the natal area than were unprovisioned females. Holekamp (1984b, 1986) observed that about 8% of females ordinarily disperse from the natal area by 55 weeks of age. Similarly, about 8% of unprovisioned females in the 1993 and 1994 cohorts emigrated from the natal area, whereas over 40% of surviving provisioned females eventually dispersed.

Three lines of evidence suggest that competition for food was not the impetus for juvenile female dispersal in this study. First, juvenile females were more likely to emigrate from provisioned areas than unprovisioned areas. Second, no young female *S. beldingi* that dispersed settled in a provisioned area. Finally, dispersed juvenile females were heavier throughout the summer than same-aged females remaining in their natal areas, and thus, females that dispersed were apparently not those least successful at competing for food.

The overall density of *S. beldingi* and the density of female *S. beldingi* weaning litters were higher in provisioned than unprovisioned areas of my study site. Rates of aggression and vigilance were significantly higher among provisioned than unprovisioned mothers. This suggests that competition for non-food resources was greater in provisioned areas, and that young female *S. beldingi* may have dispersed to find areas in which this competition was less intense. Factors other than food availability are proximal stimuli for natal dispersal many female mammals (Wolff, 1993a, 1994). For example, female white-footed mice (*Peromyscus leucopus*) disperse in response to the presence of the opposite-sex parent and the availability of vacant habitat nearby (Wolff, 1992).

I suggest that competition for space may have increased the frequency of dispersal by provisioned juvenile females in 1993 and 1994. Female *S. beldingi* defend maternal territories during gestation and lactation, as do many small mammals (Dobson, 1983; Wolff, 1993b). Infanticide is also common among small mammals, and reproductive females typically defend territories to protect their young from infanticidal conspecifics (e.g., Sherman 1981b, 1982; McClean, 1983; Hoogland, 1985, 1995; Dobson, 1990; Hare, 1991; Vestal, 1991; Wolff, 1993b). Under conditions of high population density, costs to females of defending territories may be especially high, or the likelihood of successfully defending young may be relatively low. Young females might disperse from areas of high density to improve their future chances of obtaining a territory they can successfully defend. Increases in the relative frequency of dispersal in response to increases in conspecific density and aggression are in fact common among small mammals in unsaturated habitats (Christian, 1970; Lidicker, 1975; Gaines and McClenaghan, 1980).

I did not monitor dispersal behavior of adult and yearling female *S. beldingi* from provisioned and unprovisioned areas subsequent to the weaning of their litters. However, one might expect the relative frequency of emigration by these females to be especially high in areas with intense competition for space. For example, female Columbian ground squirrels (*S. columbianus*) and female red squirrels (*Tamiasciurus hudsonicus*) sometimes disperse so that their offspring can inherit their territories (Harris and Murie, 1984; Price and Boutin, 1993). Alternatively, females that have weaned litters might be more behaviorally experienced than juveniles and thus better able to successfully compete for resources or defend young in the future, and therefore be less likely to disperse. For example, primiparous rhesus monkeys (*Macaca mulatta*) are more likely to be targets of aggression than are multiparous mothers, and primiparous female prairie voles *(M. ochragaster)* exhibit less proficient parental care than do multiparous mothers (Hooley and Simpson, 1981; Wang and Novak, 1994).

In food provisioning experiments with *S. beecheyi*, Dobson (1979) observed an increase in immigration of young females into an area receiving extra food. I suggest that the discrepancy between my results and those of Dobson (1979) may be due to differences in provisioning protocols between the two studies. I provided extra food throughout gestation and lactation, and subsequent to weaning of young. Dobson (1979) began provisioning near the time young were weaned, and put food out openly where many individuals could access it simultaneously. Immigration in the study of Dobson (1979) was therefore associated with increased access to food without the cost of facing territorial aggression from maternal females.

Trombulak (1991) provided extra food to reproductive female *S. beldingi* during gestation and lactation, as I did. However, he provisioned individual females randomly throughout his study site and did not give extra food to all females in a provisioned area. He observed that juvenile male but not juvenile female *S. beldingi* began disappearing from their natal areas 2-3 weeks after weaning. Trombulak (1991) was not specifically examining dispersal, so he reported disappearance but not dispersal of young *S. beldingi*. Thus, his results are not directly comparable to mine. However, food provisioning studies of house mice (*Mus musculus*) support the idea that spatial distribution of resources can influence dispersal behavior. *M. musculus* are more likely to have a rigid social structure (Stueck and Barrett, 1978; Noyes et al., 1982) and relatively high dispersal rates (Maly et al., 1985) when extra food is provided at centralized than scattered feeding stations.

Dispersed females in my study were heavier than same-aged females remaining in their natal areas, which suggests that energetic or other costs might be potentially associated with female dispersal (Nunes and Holekamp, 1996; see also Gaines and McClenaghan, 1980; Wauters et al., 1994; Isbell and Van Vuren, 1996). Moreover, a tradeoff might exist between the potential costs and benefits of dispersal (Isbell and Van Vuren, 1996) for young females. Small or average-sized females that reside in areas characterized by high levels of intraspecific competition might do better to remain in these areas until competition declines or until they grow and become more competitive, rather than risk the potential costs of dispersal. Density and aggression might be proximate indicators of competition in future years. Immediate and future benefits of dispersal, such as reduced conspecific density and competition, might outweigh its costs only for larger or fatter than average females able to contend effectively with rigors of the dispersal movement itself.

I observed striking sex differences in the patterns of natal dispersal by *S. beldingi*. Females began dispersing at later ages than did males in both provisioned and unprovisioned groups. Home areas of dispersed sisters, but not of dispersed brothers, sometimes overlapped after emigration from the natal area. Sisters may settle near each other to gain benefits to fitness from nepotistic cooperation in defense of maternal territories (Sherman, 1981a), whereas brothers may avoid settling in the same area to avoid competing with close kin for mates (Sherman, 1976). These trends are similarly apparent in the dispersal patterns of many gregarious animals in which females emigrate en masse via group fission but males disperse alone or with their male siblings (e.g., Chepko-Sade and Sade, 1979; Hanby and Bygott, 1987; Pusey and Packer, 1987b; Dittus, 1988;

Holekamp et al., 1993). In the present study, I also observed that juvenile female, but not juvenile male, *S. beldingi* sometimes abandoned attempts to disperse, and re-established home sites in the natal area, a pattern also observed among females of other gregarious mammals (Smale et al., 1997). Young female *S. beldingi* might return to the natal site when dispersal fails to improve their immediate or potential future ability to compete for resources. Thus, dispersal by juvenile female *S. beldingi* appears to be more labile, and more responsive to social and ecological variables, than does dispersal by juvenile males.

Chapter 8

SUMMARY AND CONCLUSIONS

INFLUENCES OF MASS AND FAT ON DISPERSAL

I examined the influences of body mass and body fat on the timing of natal dispersal behavior in juvenile male Belding's ground squirrels (*Spermophilus beldingi*). I manipulated body mass and body fat of juveniles by provisioning them with food, and I used unprovisioned juveniles as controls. I evaluated the percentage of body mass composed of fat using the non-destructive method of measuring electrical conductivity of the body. Provisioned males were significantly heavier than unprovisioned males throughout the juvenile summer, and dispersed at younger ages. Body mass of juvenile males at dispersal was constant regardless of the age at which individuals dispersed. Although body mass differed between provisioned and unprovisioned males when evaluated with respect to age, it did not differ when considered relative to time of dispersal. These results indicate that juvenile male *S. beldingi* postpone dispersal until they have attained a threshold body mass. A greater proportion of provisioned than unprovisioned males dispersed in the 6-8 week age intervals, when provisioned males were fatter than their unprovisioned counterparts. However, at ages 9-10 weeks of age there was no significant difference between provisioned and unprovisioned males in either body fat or the proportion dispersed. Moreover, dispersing juvenile males were fatter than were same-aged juvenile males still residing in their natal areas. This close association between body fat and dispersal suggests that emigrating from the natal area has potential energetic costs, and that young males may delay dispersal until their energy reserves, as reflected in body fat, are adequate to meet these energetic demands.

ENDOGENOUS TIMING MECHANISMS

I assessed the relationship between dispersal behavior, body mass and body fat of juvenile male *S. beldingi* over three consecutive years, one of which had a substantially delayed active season due to heavy winter and spring snowfall. Body fat was evaluated using the non-destructive method of measuring electrical conductivity of the body. When the active season began late, acquisition of body mass and body fat were accelerated, and dispersal behavior was inhibited. This suggests that ontogenetic trends in patterns of mass and fat gain in natal dispersal behavior by juvenile male *S. beldingi* are influenced by a seasonal time-keeping mechanism. Energy allocation was heirarchically organized in *S. beldingi*, with pre-hibernation fattening taking precedence over dispersal.

My results suggest that physiological signals reflecting body fat interact with an endogenous timing mechansim in *S. beldingi* to regulate the dispersal behavior of juvenile males. For example metabolic fuels such as glucose or fatty acids, or metabolic hormones such as glucagon, insulin, or leptin, might be influenced by the size of fat reserves, and might convey information about body fat to time-keeping and other mechanisms in the central nervous system which influence natal dispersal behavior.

ENDOCRINE MEDIATION OF DISPERSAL

I tested competing hypotheses suggesting that (1) testosterone (T) activates the natal dispersal behavior of juvenile male *S. beldingi* during ontogeny, and (2) that during neonatal life T organizes tissues which later mediate the natal dispersal behavior typical of juvenile male *S. beldingi*, as well as the responsiveness of masculine natal dispersal behavior to energetic variables such as body fat. I collected blood samples from free-living juvenile *S. beldingi* during the primary dispersal period to measure concentrations of circualting T. I also concurrently manipulated perinatal T exposure and body fat of free-living *S. beldingi*. I treated newborn females with T, and used oil-treated females as controls. Within T- and oil-treated groups, I manipulated body fat of females and their brothers by food provisioning, and used unprovisioned females and their brothers as controls. Plasma T concentrations of juveniles were undetectable, suggesting that T does not activate natal dispersal behavior during ontogeny. However, treatment of neonatal females with T induced masculine dispersal behavior, the timing of which varied with the amount of energy stored as fat. Thus, whereas energy reserves influenced when dispersal would occur, the probability of dispersal was determined by early T exposure.

ECOLOGICAL FACTORS INFLUENCING DISPERSAL

I examined the effects of food provisioning on the natal dispersal behavior of juvenile male and female *S. beldingi*. I provided extra food to adult and yearling females in their maternal territories during pregnancy and lactation, and to offspring of these females in their natal areas for 6 weeks after weaning. I used unprovisioned young of unprovisioned mothers as controls. Provisioning influenced the probability of emigration from the natal area by female but not male *S. beldingi*. All surviving male *S. beldingi* dispersed by 55 weeks of age, regardless of whether they and their mothers received extra food. By contrast, I observed a significant trend, beginning 3 weeks after weaning and continuing through the yearling year, for a greater proportion of provisioned than unprovisioned female *S. beldingi* to emigrate from the natal area. Competition for food did not appear to influence natal dispersal of females. However, overall population density, density of females weaning litters, and rates of aggression and vigilance among these females, were higher in provisioned than unprovisioned areas, suggesting that competition for non-food resources was unusually intense in provisioned areas. It appears that juvenile female, but not juvenile male, *S. beldingi* may emigrate from the natal site to increase access to areas with low densities of conspecifics. Together with findings of other researchers, these results suggest that spatial and temporal distributions of environmental resources are important influences on the dispersal behavior of female ground squirrels.

DIRECTIONS FOR FUTURE RESEARCH

My results have generated many interesting questions, which could feasibly be addressed in future research using *S. beldingi* or other species as a model system.

First, my results revealed a close association between body mass and the timing of natal dispersal in *S. beldingi*. Juvenile males tend to remain in their natal areas until they reach a threshold body mass. This indicates that in *S. beldingi*, and possibly in other species, a requisite amount of physical and behavioral development may need to occur before an individual disperses. For example, before dispersing an individual may need to grow to a large enough size to deal with social resistance from conspecifics in its new home area, or develop adequate predator evasion skills. However, the exact developmental prerequisites for dispersal remain to be determined.

I also observed a close association between the timing of natal dispersal and energy stored as fat in *S. beldingi*. In particular, after achieving a threshold body mass, juvenile males appear to delay dispersal until they have acquired a threshold amount of body fat appropriate for the particular time of the season. This association between dispersal and body fat suggests that energetic costs are potentially associated with dispersal in *S. beldingi* and possibly other species, and that animals have evolved physiological mechanisms to deal with the energetic rigors of dispersing. However, the actual energetic demands of dispersal have not yet been be documented for any species.

Emigration from the natal area occurs at very specific stages of the ontogenetic development of animals. Most male *S. beldingi* disperse as juveniles, and under ordinary circumstances, all disperse before becoming reproductively mature at two years of age (Holekamp 1984, 1986). The energetic costs of dispersal seem especially great for juvenile *S. beldingi*, which typically have limited time in which to both disperse and fatten for hibernation. A question that arises, then, is what survival or reproductive benefits are accrued by male *S. beldingi* that disperse as juveniles, versus those that disperse as yearlings? More generally, what are the ultimate factors that determine when during ontogeny an individual emigrates from its natal area?

The specific mechanisms by which body fat influences dispersal behavior are unknown. My work suggests that fat-related physiological signals inform systems that promote dispersal behavior about the size of body fat reserves. Metabolic fuels such as glucose, or metabolic hormones such as leptin, are possible candidates for such signals. However, whether either of these plays a role in mediating dispersal remains to be determined.

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