ASPECTS OF REPRODUCTION IN THE FOUR-STRIPED FIELD MOUSE *RHABDOMYS PUMILIO.*

THESIS

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(with abbreviated captions)

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ABSTRACT

Rhabdomys pumilio, in the Eastern Cape of South Africa, does not use short day length as an inhibitory cue for reproductive activity, and, despite previous records indicating that these mice are strictly seasonal in their reproduction, litters have been found during the winters of some years, both in the Eastern Cape and in the Western Cape. This led me to believe that the reproductive activity of *Rhabdomys pumilio* is more opportunistic and that the cue or cues used to control reproduction are less predictable and, or more variable than the photoperiod cue used by many seasonally reproducing rodents.

Two experiments were conducted, investigating the influence of low ambient temperature (15^0C) and reduced food availability on the reproductive activity of both male and female four striped field mice. Mice were maintained in one of four conditions (food restricted at 15^0C, food restricted at 26^0C, *ad lib*. food at 15^0C, and *ad lib*. food at 26^0C) for 4 (males) and 8 weeks (females) (photoperiod 12L:12D, humidity 40%).

Results indicated that the males reduced their reproductive activity slightly when exposed to either low temperature or low food availability and that maximum inhibition of reproduction occurred when mice were exposed to both low temperature and low food availability. However, female reproductive activity was inhibited when exposed to low food availability, irrespective of the temperature. Both sexes of mice showed varying abilities to resist fat loss and, in the males, the size of the fat store had a significant effect on reproduction. This varying ability to resist fat loss could be related to levels of activity and in the females (where activity was quantified), high activity scores were significantly associated with reproductive inhibition.

These results support the hypothesis that reproduction in *Rhabdomys pumilio* is opportunistic and controlled by the availability of energy. I propose that the females will be more sensitive to reproductive inhibition due to their far greater post-fertilization responsibilities, where the reproductive activity of the females is rapidly inhibited by a reduction in food availability, while

the males are less readily inhibited by low food availability or low temperature, unless the change in the controlling factors is severe enough, or prolonged, at which stage their reproductive activity will cease.

The significance of opportunistic reproduction in the seasonal but unpredictable climate of the study area is discussed.

CHAPTER ONE
INTRODUCTION: THE CONTROL OF REPRODUCTION IN RODENTS

The life histories of rodent species are highly variable in relation to the timing of their reproductive activity (Zucker, *et al.*, 1980; Neal, 1986). This variation can be between species or can be found between populations of a single species, and includes aseasonal, continuous reproduction at one extreme, and strongly seasonal reproduction at the other extreme, where reproductive activity is restricted to a short breeding season. Between these two extremes is a range of species in which the extent of seasonal inhibition of reproductive activity varies from slight to absolute (Bronson & Perrigo, 1987).

Due to their small size, rodents are highly susceptible to their environment and the timing of their reproduction is usually shaped by complex interactions between a range of environmental factors (including climatic variability, food availability, latitude and altitude), physiological factors (including longevity, diet and body size), and social factors (including predation pressure and social interactions) (Harvey & Zammuto, 1985; Partridge & Harvey, 1985; Bronson, 1989; Read & Harvey, 1989; Promislow & Harvey, 1990; Bronson & Heideman, 1994). It is these factors, and their influence on reproduction, that forms the basis of many studies investigating the control of reproduction in rodents.

The majority of these studies have investigated the environmental and physiological influences on the timing of reproduction, and have worked on rodents that have shown strict seasonal reproduction (eg. *Micromys minutus* - Harris, 1979; *Perognathus formosus* - Kenagy & Bartholomew, 1981; *Rattus fuscipes* - Irby *et al.*, 1984; *Mus musculus* - Hamilton & Bronson, 1985; *Peromyscus maniculatus* - Nelson *et al.*, 1992; *Saccostomus campestris* - Bernard & Hall, 1995). This strict seasonality in reproductive activity is common in the temperate latitudes of America and Europe where seasonal changes in climate are often and marked (Bronson, 1989; Bronson & Heideman, 1994). In such situations the most commonly used cue for the timing of rodent reproduction is photoperiod as it is the only entirely predictable environmental variable

(Bronson, 1989) (eg. *Clethrionomys glarelus* and *Apodemus sylvaticus* - Clarke, 1985; *Peromyscus maniculatus* - Nelson *et al.*, 1992). However, the use of photoperiod is not always reliable and tends not to be used by mammals at lower latitudes (eg. *Pteropus scapulatus* - O'Brien *et al.*, 1993; *Saccostomus campestris* - Bernard & Hall, 1995; *Peromyscus mexicanus* - Duquette & Millar, 1995; *Rhabdomys pumilio* - Jackson & Bernard, 1999). At lower latitudes the climate is often less seasonal and less predictable, and below 10^0 latitude noticeable changes in photoperiod from summer to winter do not occur (Bronson, 1989; Bronson & Heideman, 1994). In these situations more variable factors can be used by rodents to control their breeding and more opportunistic reproductive activity can be obtained.

For small mammals, such as rodents, with short lifespans, opportunistic breeding would be the favoured method of reproductive activity (Bronson, 1989). This type of breeding would allow small mammals, including rodents, to breed whenever conditions are beneficial for such an activity irrespective of the climatic season. For species or populations of rodents that are more opportunistic in their breeding activity, less predictable factors can be used to control their reproduction. Such factors include food availability, food quality and ambient temperature. There are secondary factors such as rainfall and secondary plant compounds that can also influence reproductive activity and can act as cues to help prepare the animals for favourable periods for reproduction, however they can also be used for preparation for unfavourable conditions.

Most of the studies conducted on the less predictable factors that may control reproduction have focused on food availability and on food quality. The effects of food availability have been studied in a variety of ways, either by providing animals with food supplementation during periods of normal food shortage, resulting in an increase in reproductive activity (eg. *Microtus montanus* - Pinter & Negus, 1965; *Clethrionomys glareolus* - Alibhai, 1985; *Peromyscus difficilis* - Galindo-Leal & Krebs, 1998) or by decreasing the availability of food, resulting in a decrease in reproductive activity (eg. *Mus musculus* - Hamilton & Bronson, 1985; *Arvicola terrestris* - Bazhan *et al.*, 1996). Those studies conducted on food quality have shown a variety of results, where some rodents rely on green vegetation and their source of water for the timing of reproduction (*Dipodomys merriami* - Bradley & Mauer, 1971), while others combine the

influences of long photoperiod and high temperature with those of high food quality (*Rattus fuscipes* - Irby *et al.*, 1984).

Related to food quality is the use of secondary plant compounds to time reproductive activity. The most commonly studied of these plant compounds is 6-methoxybenzoxazolinone (6-MBOA) and a number of rodents have been shown to use this compound as a short term cue to control their reproduction, including *Gerbillus harwoodii* and *Mastomys coucha* (Alibhai, 1986; Linn, 1991 - cited by White & Bernard, 1999), *Microtus pinetorum* (Schadler, *et al.*, 1988), *Rattus norvegicus* (Butterstein, *et al.*, 1985; Vaughan, *et al.*, 1988), *Dipodomys ordii* (Rowsemitt & O'Connor, 1989), and *Mus musculus* (Nelson & Shiber, 1990).

The influence of temperature on reproductive activity appears to be purely an energy related factor and plays its role by affecting the energetic costs of thermoregulation (Bronson & Perrigo, 1987; Bronson, 1989). Generally, rodents that are influenced by temperature will reduce their reproductive activity when exposed to either low ambient temperatures, or when they are exposed to temperatures that are above their thermoneutral zone (Bronson, 1989). In both instances available energy is used for thermoregulation while bypassing reproductive needs, causing breeding activity to be reduced or inhibited. The influence of temperature on reproduction is usually linked with other factors (eg. *Apodemus sylvaticus* and *Rattus fuscipes* respond to an interaction between photoperiod and temperature - Clarke, 1985 and Irby *et al.*, 1984), and is, therefore, usually studied in conjunction with other environmental factors.

It is also possible that the influence of temperature (either high or low) is related to the stress that the animal experiences at that temperature, and if this stress was prolonged, it could result in a reduction in the rate of ovulation or a reduction in the fertility of the animal (Daley *et al.*, 1999).

Rainfall is often thought to have an influence on reproduction, especially in conjunction with temperature levels. The importance of rainfall is usually related to the availability of food and is therefore thought to be used as a short-term cue (Perrin, 1980). When combined with high temperatures, seasonal rainfall can influence both seasonal changes vegetation growth and in

insect abundance (Bernard, 1989), which is important to consider in insectivorous rodents and relates back to both food availability and food quality. An example of the interaction between rainfall and temperature having an affect on breeding is provided by Harris (1979) where it was found that the reproductive activity of *Micromys minutus* was inhibited by wet and cold conditions.

The variation in breeding strategies between different species of rodents and between different populations of the same species is problematic when trying to understand their reproductive biology. However, an understanding of the control of reproduction of one species or population can allow scientists to make predictions about similar species or populations living in similar regions and exposed to similar environmental factors. Such information may be used to predict how a problem rodent species will respond to climatic change. However, since the majority of studies on reproductive biology of rodents have been conducted in the northern temperate regions, little of this information can be used to predict the breeding strategies of the more tropical and sub-tropical rodents. For this reason the present study was established to investigate the role of environmental variables in controlling small mammal reproduction at low latitudes and in unpredictable environments, using the four-striped field mouse (*Rhabdomys pumilio*) as the research model and the Eastern Cape of South Africa as the study area.

CHAPTER TWO
RHABDOMYS PUMILIO, THE FOUR STRIPED FIELD MOUSE, IN THE EASTERN CAPE OF SOUTH AFRICA

2.1 EXPERIMENTAL ANIMAL:

Rhabdomys pumilio, commonly known as the four striped field mouse, is a small, diurnal rodent that is characterised by four black stripes running from the back of its head to the base of its tail (Fig. 2.1). It has a mean adult body mass ranging from 30-40g, with no sexual dimorphism (De Graaf 1981; David & Jarvis, 1985; Yom-Tov, 1993; Jackson & Bernard, 1999), is highly active and has a characteristic habit of losing the skin off its tail if caught by the tail (*pers. obs.*).

The four striped field mouse occurs through most of Africa, south of the Sahara (De Graaf, 1981; David & Jarvis, 1985) and has been studied in Malawi (Hanney, 1965), Kenya (Taylor & Green, 1976), Botswana (Smithers, 1971) and in South Africa (eg. Brooks, 1974; Perrin, 1980; Henschel *et al.*, 1982; David & Jarvis, 1985; Jackson & Bernard, 1999) (Fig. 2.2). This mouse is found mainly in grasslands where cover is thick, although it can occur elsewhere as long as there are areas of thick grass or bush available for protection. It feeds mainly on seeds but also eats insects and green vegetable matter (Perrin, 1980; David & Jarvis, 1985). It is also one of the earlier colonisers of recently disturbed (burnt or cleared) areas (van Hensbergen *et al.*, 1992; G.N. Bronner - Potchefstroom University, *pers. comm.*).

This rodent is considered to be of economical and medical importance. Due to its pioneering abilities and its dietary habits, the four striped field mouse is often attracted to cultivated lands, where it is known to cause considerable damage to young trees, especially to conifers in commercial plantations in South Africa (Davis, 1942) and to grain crops. It is also known to carry a number of viruses and parasites (Brooks, 1974) and has been reported to carry the plague bacillus *Pasteuralla pestis* as well as tick-bite fever, and to be a host to nematodes, cestodes and ticks, to name a few. Although it is not yet an economic nor a medical pest in South Africa, the

four striped field mouse is a pest species in other regions of Africa and forms the focus of a number of studies being conducted in Africa (see Leirs, 1999).

Figure 2.1: Typical four striped field mouse, *Rhabdomys pumilio*, showing the characteristic features of the species. Scale = Lifesize.

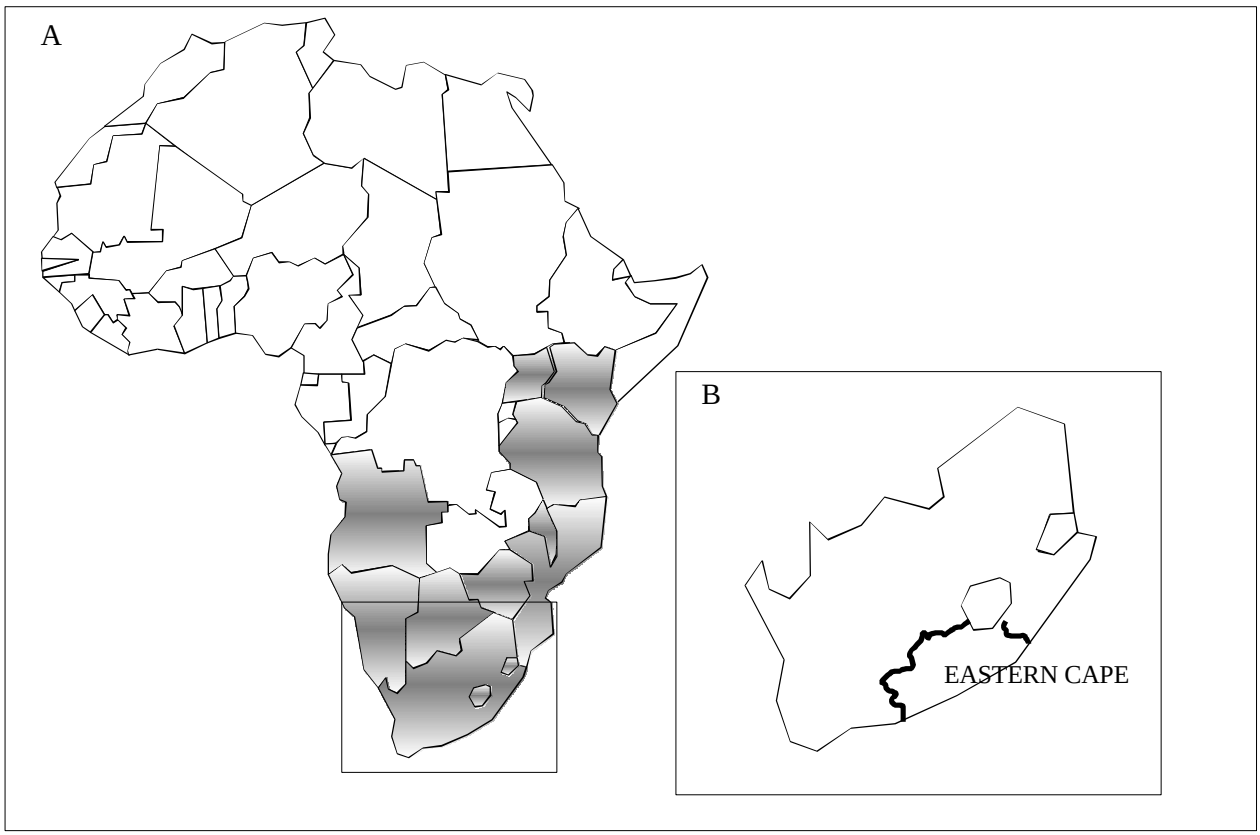

Figure 2.2: (A) Map of Africa showing the distribution of *Rhabdomys pumilio* (shaded area). (B) Map of South Africa indicating where the Eastern Cape (the study area) is situated**.**

Generally it is agreed that *Rhabdomys pumilio* produces large litters (range: 2-9) over a short period of time, with a short gestation period (.25 days) and an early weaning age (14 days) (Dewsbury & Dawson, 1979; David & Jarvis, 1985; Perrin, 1986; Jackson, *pers. obs.*). However there appears to be some confusion as to how many litters individual mice can produce in a season (range 3-7 in the wild, 14 in captivity) and to the timing of the reproduction.

Reproduction of the four striped field mouse has been reported as being seasonal in the wild where they cease their reproductive activity during the winter months (Hanney 1965; Brooks, 1974; Perrin, 1980; Henschel *et al.*, 1982; David & Jarvis, 1985). However, in Botswana *Rhabdomys pumilio* breeds during two parts of the year (January-February and June-July) (Smithers, 1971) and in Kenya they show opportunistic tendencies, breeding when conditions are favourable (Taylor & Green, 1976). Continuous reproduction has also been shown to occur in the laboratory by Dewsbury and Dawson (1979), although these authors also mention that reproduction is seasonal in the wild. It is suggested by Bronson and Heideman (1994) that the pattern of reproduction within a species can change with latitude, where animals at lower latitudes tend to breed continuously and those at higher latitudes breed seasonally. The variation seen in the reproductive activities of *Rhabdomys pumilio* reflect this, where the mice closer to the equator (Kenya) breed continuously and the further south (Botswana to South Africa) the species occurs, so their reproductive activity becomes more seasonal.

In South Africa it appears that the reproduction of *Rhabdomys pumilio* is strictly seasonal, however, most of the studies in this country were short term except for Brooks' (1974) study and that of David and Jarvis (1985). Although Brooks mentions that the four striped field mouse breeds seasonally in the northern parts of South Africa and David and Jarvis claim that these mice are strictly seasonal in their reproduction, the latter study (conducted over five years) reported a small number of reproductively active males and females in the supposed non-breeding season.

Similarly, in a recent study in the Eastern Cape of South Africa, a pregnant female and reproductively active males were collected during the winter of 1997 (Jackson & Bernard, 1999). Although the sample sizes were small, these findings indicate that *Rhabdomys pumilio* is not

strictly a seasonal breeder but is probably an opportunistic species that exhibits seasonal reproduction in response to climatic or dietary changes during the year and has the potential to breed throughout the year if conditions are favourable.

This variation in reproductive activity through the year for different areas raises the question as to what controls the seasonality of reproduction in these mice. Previous studies conducted on rodents to establish controlling factors of their reproduction have indicated that one of the most important controlling factors is photoperiod (See Chapter 1), however short day length acting alone does not inhibit spermatogenesis in the four striped field mouse (Jackson & Bernard, 1999). Other factors that have been proposed include rainfall, temperature, food composition and food availability and quality (Dewsbury & Dawson, 1979; Perrin, 1980; David & Jarvis, 1985; R.M.White- University of Transkei, *pers. comm.*) but despite these suggestions, little laboratory work has been conducted to determine which of these factors, either alone or in combination with others, is used by the four striped field mouse to time reproduction.

Secondary plant compounds, such as 6-methoxybenzoxazolinone (6-MBOA), are used by some small mammals (eg: *Gerbillus harwoodii*, *Mastomys coucha*, and *Microtus townsendii*) to time reproduction, but previous studies have shown that 6-MBOA does not stimulate or inhibit reproductive activity of *Rhabdomys pumilio* (White, 1999). Perrin's (1980) suggestion that rainfall is possibly the controlling factor for the four striped field mouse's reproduction can be discounted. Although no laboratory studies have been conducted on the influences of rainfall, David and Jarvis (1985) studied the reproduction of *Rhabdomys pumilio* in the Western Cape ($34^0$00'S, $18^0$35'E) where a Mediterranean rainfall pattern occurs (winter rainfall) yet reproduction occurs mainly during the summer months when rainfall is at its lowest.

Although rainfall may not be the main stimulating factor for reproduction in these mice, it may act indirectly by affecting food availability and/or food quality. These two factors (food quality and quantity) and temperature or a combination of these factors are the main remaining possibilities that may influence reproduction in the four striped field mouse.

Although still a possibility, food quality seems to be a less plausible factor as these animals are mainly granivorous and it is unlikely for seeds to lose their quality, as would grasses or leaves, from the start of the growing season to the end. Rather, one would expect the availability of seeds to become reduced as the season progressed. A change in seed abundance could force the mice to alter their diet to other food sources and this could result in a change in food quality.

Besides the possibility of food availability having an effect on the reproduction of these mice, climatic conditions could also play a role. The climate of an area can have an effect on both the food available to the mice and on the mice themselves, in terms of thermoregulation. Besides rainfall, the other climatic factor that can be considered important in both of these aspects is temperature, which can vary with altitude and latitude and therefore cause animals in different areas to reproduce at different times and for different lengths of time.

2.2 THE CLIMATE OF THE EASTERN CAPE:

In the Eastern Cape of South Africa (the study area - Fig. 2.2B) the climate is strongly seasonal. Summer occurs from October to April with mean temperatures ranging from 11-26^{0}C (min/max), with winter occurring from May to June where mean temperatures range from 5 to 20^{0}C (min/max) (Fig. 2.3A). The rainfall of this region is also strongly seasonal with the wet season occurring in the summer and the dry season coinciding with the winter months (Fig. 2.3B).

However, due to the Eastern Cape laying on a climatic transition zone between a winter rainfall area (Western Cape) and summer rainfall area (Kwazulu Natal), the climate in this area is highly unpredictable. In some years (eg. 1997 and 1999) heavy rainfalls can be experienced during the winter months where almost double the mean rainfall for these months can occur (eg. May, June, & July) (Fig. 2.3). In addition to unpredictable rainfall in some years, the temperatures can also vary, although the temperatures in the region tend to remain fairly stable. One example of this variation can be seen in July 1999 where the mean minimum temperature is considerably higher compared to the mean minimum temperatures for 1986-1996 and the minima for 1997 and 1998.

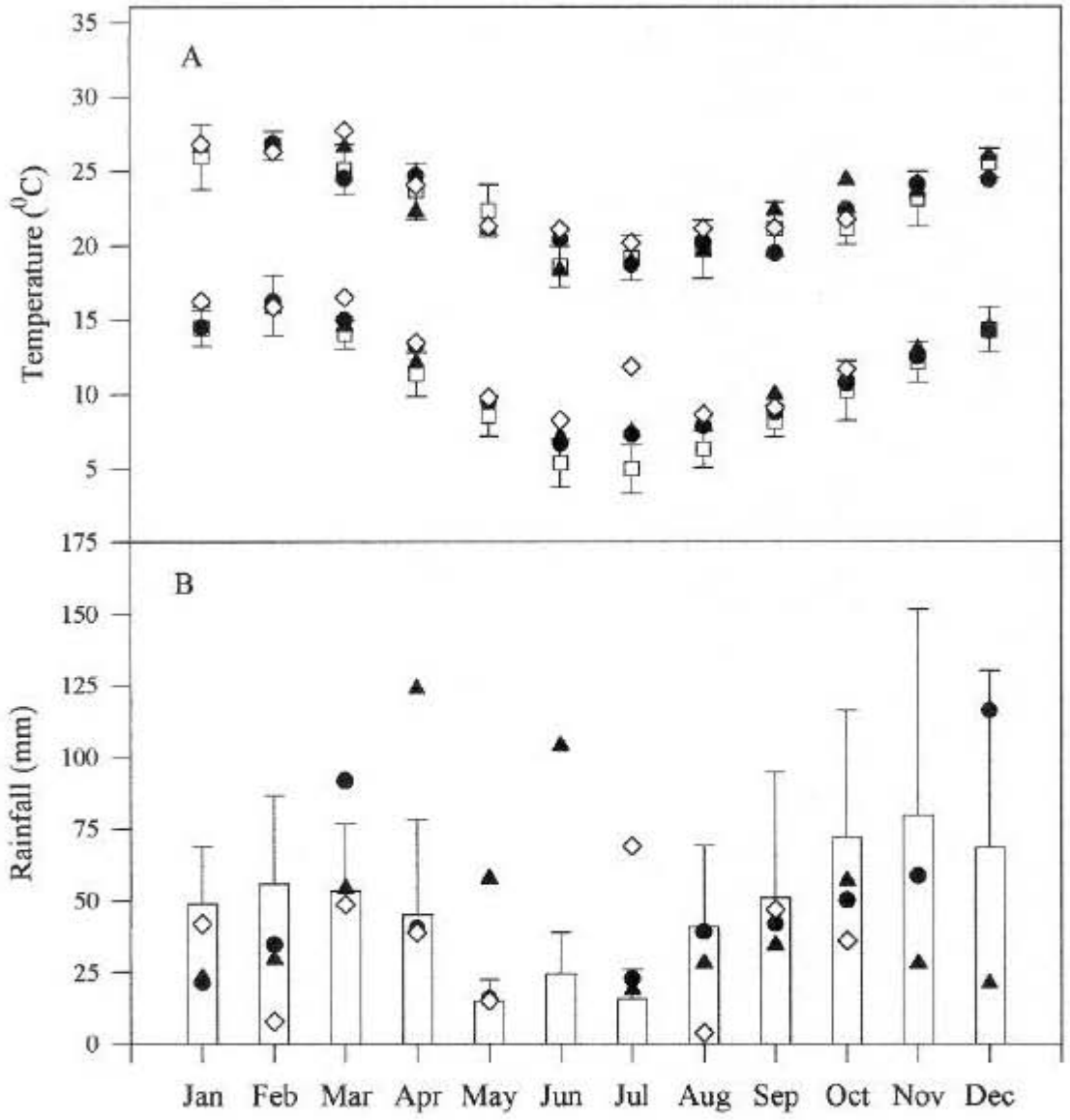

Figure 2.3: A comparison of temperature (A) and rainfall (B) patterns for 1997, 1998 and 1999, with mean values for the previous ten years (1987-1996) for Grahamstown in the Eastern Cape of South Africa. Histogram bar/□ = mean values for years 1987-1996, ▲ = values for 1997, ● = values for 1998, and ◇ = values for 1999.

Due to the seasonal and unpredictable climate of the Eastern Cape, it is likely that *Rhabdomys pumilio* in this region will exhibit opportunistic reproduction. During most years these mice will reproduce in the summer, while in some years of unpredicted favourable conditions in winter, reproduction may be continuous.

Determining what these favourable conditions are and how they can control reproduction in this species forms the focus of this study. Taking into consideration the arguments discussed earlier in this chapter, the effects of low temperature and low food availability on the control of the reproductive activity of *Rhabdomys pumilio* were examined.

2.3 THE STUDY:

This study was conducted over two years where experiments using both male and female *Rhabdomys pumilio* were run. The use of both sexes to test the variables was due to reports indicating that males and females of the same species may respond differently to environmental cues (Bronson & Perrigo, 1987, Cittadino *et al.*, 1994).

The housing and handling of the mice and the running of both experiments were approved by the Rhodes University Ethical Standards Committee.

CHAPTER THREE
EFFECTS OF LOW TEMPERATURE AND REDUCED FOOD QUANTITY ON THE REPRODUCTIVE ACTIVITY OF MALE *RHABDOMYS PUMILIO*

The aim of this experiment was to examine how low temperatures, low food availability and a combination of these two factors affected the reproductive activity of male four-striped field mice with regard to spermatogenesis, sperm storage, secretory activity of the accessory glands and the size of the Leydig cells.

3.1 MATERIALS AND METHODS:

Rhabdomys pumilio were trapped, using Sherman traps baited with peanut butter and oats, in Thomas Baines Nature Reserve in the Eastern Cape of R.S.A (lat $33^0$18'S, long $26^0$32'E), throughout February 1998. Over a period of 30 days, 40 males were brought into the laboratory, placed singly in similar sized cages (41x26x15cm) in controlled environment rooms and were each provided with shredded paper for bedding, a toilet roll for shelter, and water and food (Rabbit pellets, Epol) *ad libitum*.

3.1.1 *Acclimation:*

The mice were divided into two groups of twenty males each so that both groups contained individuals of a similar size. The first group was placed into a controlled environment (CE) room set at 15^0C, while the other group was placed into a second CE room set at 26^0C. In both rooms the photoperiod was 12L:12D and humidity about 40%. The animals were fed pre-weighed food, consisting of rabbit pellets, *ad libitum* and were supplied with water *ad libitum*. The mice were weighed every second day during the week (Monday, Wednesday and Friday), before feeding, to monitor weight gain or loss and to establish mean body weights for the mice for use during the experiment. The cages were cleaned at the beginning of each week (every Monday) prior to feeding the mice.

The acclimation period ran for two weeks and at the end of the second week all left-over food in the cages was collected and used to calculate the daily food intake for each mouse using equation 1:

$$\textbf{Daily food intake (g/animal)} = \frac{\textbf{Food provided for week (g/animal) - food remaining (g/animal)}}{\textbf{7 Days}} \quad (1)$$

3.1.2 *Experimental design:*

The experiment was designed to run for six weeks, however two animals under food restriction and low temperature died in the fourth week and therefore the experiment was terminated at the end of week four.

In each CE room, the mice were divided evenly in terms of body mass, hind-foot length and the position of their testes in the scrotal sacs, into two groups of ten animals each. The one group was allocated as the control group and these mice were maintained on an *ad libitum* diet of rabbit pellets which were pre-weighed before being fed to the mice. The other ten mice were provided with a food-restricted diet. Although previous studies on food-restriction have used a 30% reduction in food availability (Hamilton and Bronson, 1985), this reduction in the four-striped field mouse resulted in ten deaths within the first week of the experiment. For this reason the daily food intake of the experimental mice was restricted by 10%.

Mice were weighed every second day (as in the acclimation period) and their reproductive status was noted as being scrotal, non-scrotal or moving scrotal, depending on the position of the testes in the scrotal sacs and their visibility within the sac. Any mice in which body weight dropped below 70% of the mean body weight (calculated during the last week of acclimation) were provided with additional food until their body weight increased to above this level, at which stage their diets were returned to the original 10% restriction.

At the end of the experimental period (week 4), the faeces and remaining uneaten food were collected and stored at -10^0C and used to calculate daily food consumption (equation 1) and to calculate the energy intake and assimilation of each animal for that week (see 3.1.4).

3.1.3 *Sacrifice:*

At the end of the experimental period the mice were sacrificed by administration of 0.2ml of Euthanaze. They were weighed and then dissected rapidly and the testes, epididymides and the accessory gland complex (composed of the seminal vesicles, coagulatory glands and the prostate gland) removed. These reproductive organs were weighed (to the nearest 0.001g) and placed in Bouin's fixative.

A fat index (0-3) was determined subjectively by looking at the amounts of fat around the testes, kidneys and under the skin and providing a score for each animal (0 = no fat, 1 = fat around either the testes, kidneys or under the skin, 2 = fat around two of these points, 3 = fat around the testes, kidneys and under the skin).

A number of animals died at various stages of the experiment and the final sample size was 30 mice, fourteen from the warm room conditions (7 control and 7 food-restricted) and sixteen from the cold room conditions (9 control and 7 from the food-restricted group).

3.1.4 *Energetics:*

At the time of analysis, sub-samples of food and faeces were dried in an oven at 60^0C to constant dry mass. These samples were then crushed to form a powder, which was then placed into a bomb calorimeter (Energy instrumentation - MC1000). Each sample was run twice to determine the mean energetic content. These values were then used to calculate daily energy intake, output and assimilation using equations 2, 3 and 4.

Daily energy intake (kJ/animal) = Daily food intake (g/animal) x Energy of food eaten (kJ/g) (2)

Daily energy output (kJ/animal) = **Daily faeces produced (g/animal) x Energy of faeces (kJ/g)** (3)

Daily energy assimilated (kJ/animal) = **Energy intake (kJ/animal) - Energy output (kJ/animal)** (4)

The digestive efficiency for each mouse was also calculated, using equation 5:

$$\textbf{Digestive efficiency (\%)} = \frac{\textbf{Energy assimilated (kJ/animal)}}{\textbf{Energy intake (kJ/animal)}} \times 100 \quad (5)$$

3.1.5 ***Histological analysis:***

The organs that were placed in Bouin's fixative were embedded following routine procedures. The wax blocks were then sectioned using a Leica microtome and two ribbons of sections from different regions of the tissue, per reproductive organ, were mounted on microscope slides. These sections were then stained with Mallorys trichome and examined under a light microscope.

The spermatogenic activity of the testes was calculated as follows. One hundred seminiferous tubules per animal were examined and scored as being spermatogenically active if spermatozoa or spermatids were present (1) or inactive (0) if there were only spermatogonia, primary spermatocytes and sertoli cells present. For each animal the score was expressed as a percentage and for each experimental group the mean value was calculated. Animals were considered spermatogenically inactive if less that 25% of the tubules examined were spermatogenically active and active if over 70% of the tubules examined showed spermatogenic activity.

The number of spermatids in each of the active tubules per animal was also noted and spermatids and spermatozoa were counted if less than fifty were present in the tubule. If there were more than fifty spermatids and spermatozoa in the tubule then their number was estimated to the nearest fifty (100 or 150). If there appeared to be more than 200 spermatids present in the tubule then that tubule was recorded as having over 200 spermatids (>200).

Twenty sections through the cauda epididymis were examined per animal and the amount of sperm present in the sections was given a subjective score ranging from 0 to 3:

0 = no sperm in storage

1 = little sperm in storage (<⅓ full)

2 = tubule half full of sperm

3 = large amount of sperm in storage (>⅔ full)

The mean score per animal was then expressed as a percentage of the maximum (3).

The seminal vesicles were scored in a similar way to that used for the epididymides, however in this case it was the volume of stored secretory material that was noted.

The Leydig cells of the animals were also examined by measuring the diameter of the nucleus of these cells using a light microscope and an optical micrometer. Ten to twenty cells per animal were recorded, with two measurements per nucleus being taken at right angles to each other.

3.1.6 *Statistical analyses:*

Mean hind food length and body weights of the mice in the four experimental groups before the experimental period started were not significantly different ($P > 0.05$), and therefore changes in weight of the reproductive organs during this study were directly related to the conditions to which the males were exposed and not to changes in body size. For this reason, absolute values have been used throughout the study.

Statistical analyses were conducted using both SigmaStat (Jandel Scientific, 1994) and Statgraphics (Manugistics, 1992). Groups were compared using multiple analyses of variance (MANOVA), multiple analyses of covariance (MANCOVA), student t-tests and the non-parametric equivalents where applicable. Individuals were compared with a repeated measures t-test. Differences were considered significant at $P<0.05$ and data is represented as mean values ± 1SD throughout.

3.2 RESULTS:

At capture 30 of the males had scrotal testes while ten males had testes that were moving between a scrotal and a non-scrotal position. These mice were divided equally between the four experimental groups. At the end of the experiment 38% of the mice in the 26^0C, food-restricted (26FR) group, 86% of the mice in the 26^0C, *ad libitum* (26AL) group, 22% of the mice in the 15^0C, *ad libitum* group (15AL) and none of the mice in the 15^0C, food-restricted group (15FR) were scrotal (Table 3.1).

The body masses of the animals in the four groups changed from the beginning of the acclimation period to the end of the experimental period. Both groups of mice under food restriction lost weight significantly ($P< 0.001$). The control mice all showed small, statistically non-significant increases in their body weight (Fig. 3.1)

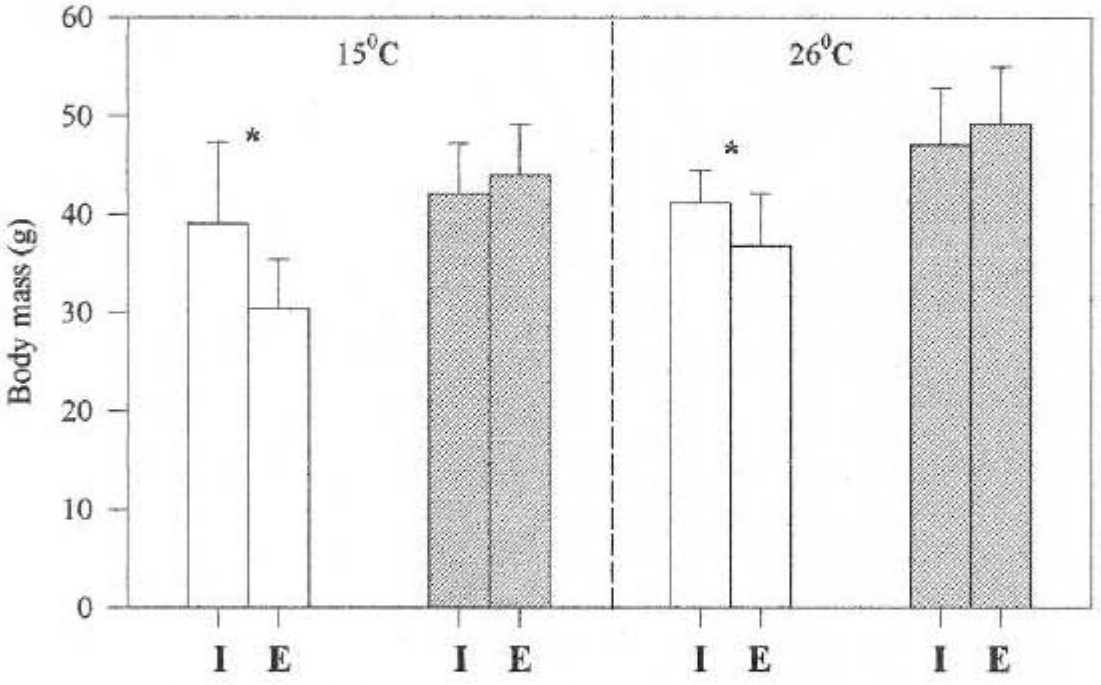

Figure 3.1: Changes in body mass from the start of the acclimation period (I) to the end of the experiment (E) for male *Rhabdomys pumilio*.
□ food-restricted ▨ control diet. * significantly different.

Table 3.1: Changes in the position of the testes in the scrotal sacs of male *Rhabdomys pumilio* from the start of the acclimation period to the end of the experimental period. Data are the percentage of animals per group that were scrotal, partly scrotal or non-scrotal.

Group	Scrotal		Partly/ moving scrotal		Non-scrotal	
	Start	**End**	**Start**	**End**	**Start**	**End**
26^{0}C AL	90%	86%	10%	14%	0%	0%
26^{0}C FR	80%	38%	20%	24%	0%	38%
15^{0}C AL	70%	22%	30%	56%	0%	22%
15^{0}C FR	80%	0%	20%	0%	0%	100%

3.2.1 ***Dissection data:***

At dissection, the mean body mass of the mice on food restriction was lower than that of the control mice within the temperature groups, and across the temperatures the mice at 26^{0}C were heavier irrespective of their diets (Fig. 3.2 A). Comparison of the mean body masses of the mice in the four groups at the end of the experiment, using a Manova, has shown that although temperature and food acting individually had significant effects on the mean body mass of mice ($P< 0.01$ & $P<0.001$, respectively), there was no significant interaction between these two factors ($P> 0.05$) (Table 3.2).

When considering the masses of the testes, epididymides and accessory glands, it is evident that the males from the control diets had the heavier organs compared to the mice on food restriction at the same temperature, and that the mice from the warm conditions had the heavier organs compared to the mice in the cold room (Fig. 3.2: B, C & D). Statistically, these differences were only significant between food groups where the control mice had significantly larger reproductive organs than the mice on food restriction ($P< 0.001$) (Table 3.2). Temperature had a significant effect on the testes only ($P< 0.01$) and at no time was there a significant interaction between these two factors ($P> 0.05$) (Table 3.2).

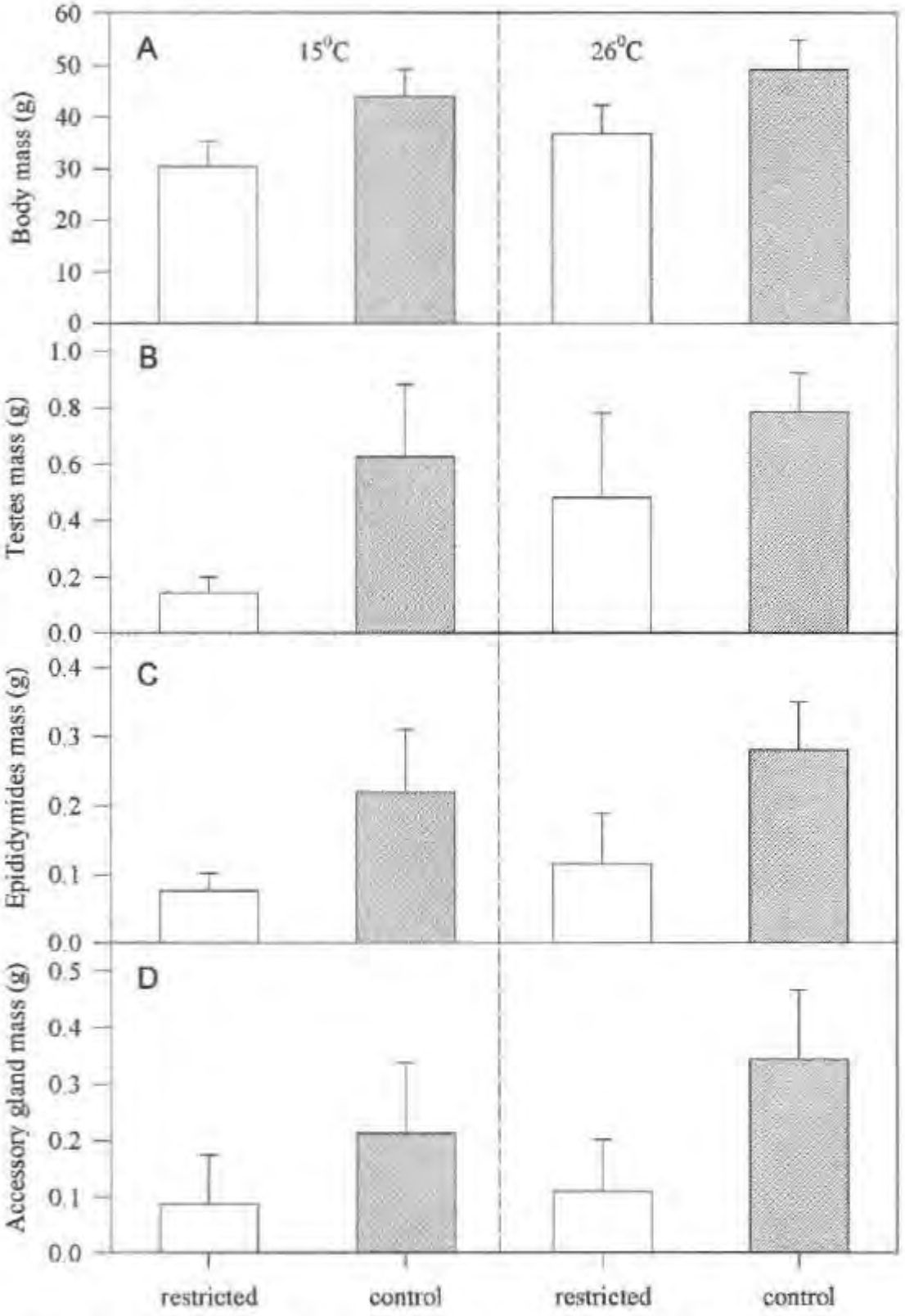

Figure 3.2: Dissection data for male *Rhabdomys pumilio* which have been exposed to one of two temperatures and one of two diets, ☐ food-restricted, ▨ control diet.

Table 3.2: Results of the statistical analysis (MANOVA) of the effect of temperature, food and an interaction between temperature and food on the reproductive activity of male *Rhabdomys pumilio*.

Dependent variable	Temperature		Food		Food and temperature	
	P value	F value	P value	F value	P value	F value
Body mass	0.0066*	8.724	<0.0001*	44.1073	0.7816	0.0785
Testes mass	0.0037*	10.145	<0.0001*	33.316	0.1376	2.347
Epididymides mass	0.069	3.599	<0.0001*	34.626	0.6795	0.18
Accessory gland mass	0.0655	3.699	0.0002*	19.624	0.1902	1.809

* significant difference at P<0.05 level.

During the dissection it was apparent that the mice within each of the four groups had responded differently to the experimental conditions and possessed different amounts of body fat. In order to see if the ability of the mice to resist fat loss had an effect on their reproduction, the animals in each of the four experimental groups were subdivided according to their fat scores (Fig. 3.3).

In Figure 3.3 it appears that the amount of fat that mice were able to store had an effect on the size of the reproductive organs. The general trend being that the heavier organs occurred in the fatter mice and the mice with no body fat had the smallest organs. To test the effect of fat, fat scores of either 0 (no fat) or 1 (with fat) were included as a dummy-covariable in a MANCOVA to assess the effects of temperature and food on the masses of the reproductive organs. This dummy-covariable had a significant effect as a covariate on all reproductive organs and on the body mass of the animals (Table 3.3).

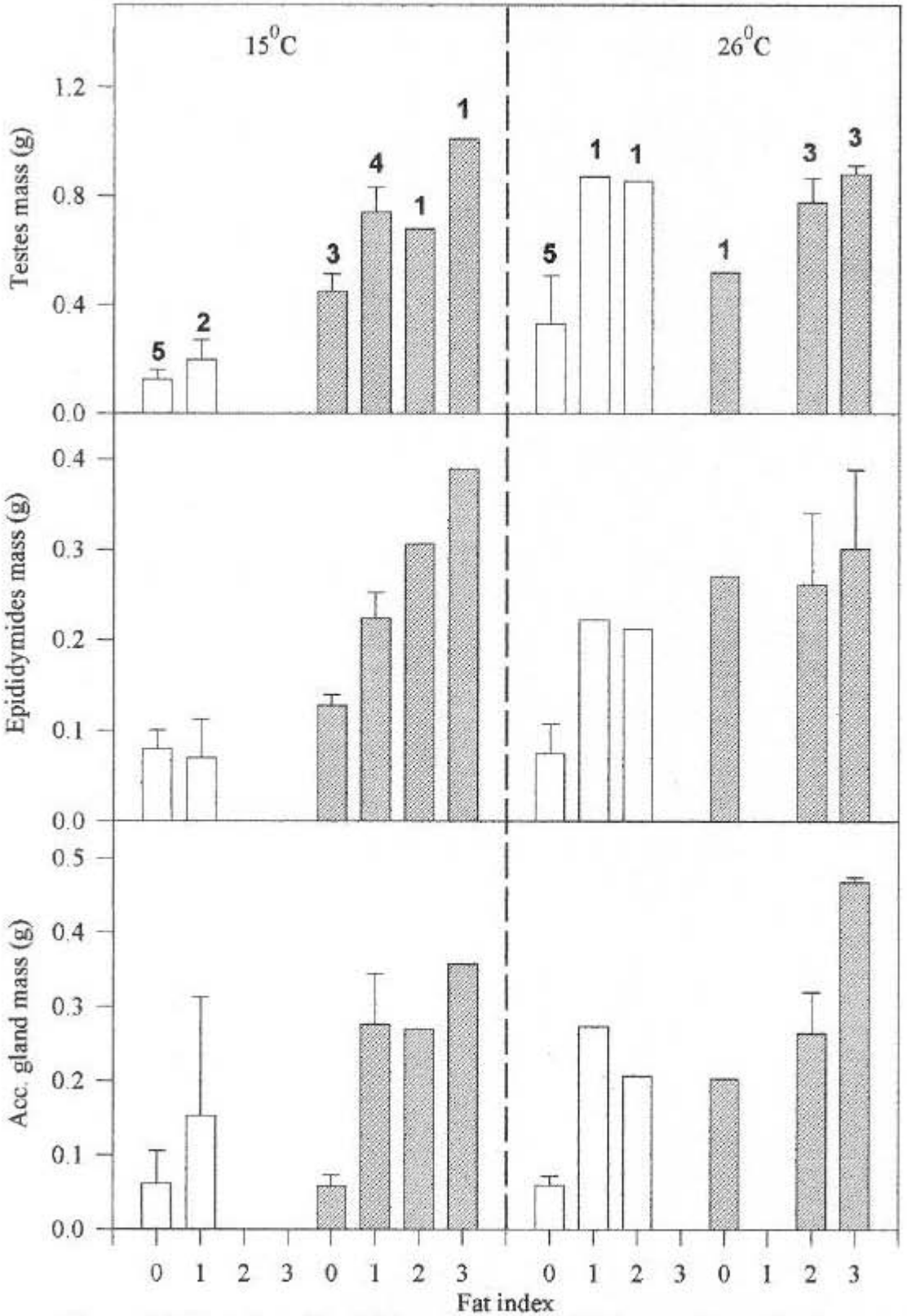

Figure 3.3: Data from Fig. 3.2. have been subdivided according to the fat score of the animal. The general trend of decreasing organ mass with decreasing fat score can be seen. Numbers above the bars indicate the sample size within that fat score.
□ food-restricted ▨ control diet.

Table 3.3: Results of the statistical analysis (MANCOVA), with fat as the covariate, of the effect of temperature and food on reproductive activity of male *Rhabdomys pumilio* once the effects of fat have been removed.

Dependent variable	**Covariate (fat)**	**Temperature**		**Food**		**Interaction**	
		P value	**F value**	**P value**	**F value**	**P value**	**F value**
Body mass	0.0239*****	0.007*****	8.619	0.000*****	27.102	0.5849	0.316
Testes mass	<0.001*****	<0.001*****	16.709	0.0001*****	23.259	0.008*****	8.257
Epididymides mass	0.0032*****	0.0751	3.448	0.0001*****	20.263	0.89	0.020
Accessory gland mass	<0.0001*****	0.0377*****	4.815	0.0053*****	9.331	0.1871	1.839
Fat storage	no test	0.0566	3.982	0.0009*****	14.207	0.1327	2.409

* significant difference at P<0.05 level.

With the removal of the effects of fat, temperature now had a significant effect on the accessory gland mass (P< 0.05) and the interaction between temperature and food had a significant effect on the size of the testes, where the mice from the cold room on a restricted diet had significantly smaller testes compared to all the other mice (P< 0.01) (Table 3.3, and see Table 3.2).

In addition to examining the effect of fat on the size of the reproductive organs, it is now also possible to see whether temperature, food or an interaction between these two factors had an effect on the amount of fat that was stored by the animals. From the results we can see that mice that received the restricted diet had significantly less fat stores than did the control mice, and that neither temperature nor an interaction between food and temperature had a significant effect on the amount of fat being stored by the mice (Table 3.3)

3.2.2 *Histology:*

The males on the control diets, at both temperatures, were all spermatogenically active and the epididymides were full of sperm. They also had high scores for accessory gland activity although the range of scores was greater in the mice from the cold room. 86% of the mice from the warm room that were exposed to a food-restricted diet were also spermatogenically active, however scores for sperm storage and the activity of the accessory glands were lower and ranged from 20-80% and 40-100% respectively (Table 3.4). For the mice exposed to cold room conditions and to a food restricted diet, all aspects of reproductive activity were reduced considerably with 86% of the mice being spermatogenically inactive, 40% of the sections through the epididymides storing sperm and the accessory glands ranging from being inactive to fully active (Table 3.4, Plate 3.1 & 3.2).

Table 3.4: A summary of the reproductive status of male *Rhabdomys pumilio* at the time of dissection, after being exposed to one of two diets and one of two temperatures. The percent of reproductively active males refers to the number of mice within the groups with more than 70% of their seminiferous tubules producing spermatozoa or spermatids; spermatogenic activity refers to the mean percentage of tubules with spermatozoa or spermatids; epididymal sperm storage and the accessory gland activity refer to the mean percentage scores for those groups (± 1SD).

Condition	% reproductively active	Spermatogenic activity (%)	Epididymal sperm storage (%)	Accessory gland activity (%)
26 AL	100	94.0 ± 2.52	95.1 ± 4.26	95.1 ± 8.30
26 FR	86	79.0 ± 32.67	54.3 ± 28.8	78.6 ± 28.47
15 AL	100	94.0 ± 3.00	78.6 ± 31.34	75.0 ± 38.87
15 FR	14	16.0 ± 25.97	17.8 ± 21.83	49.8 ± 40.91

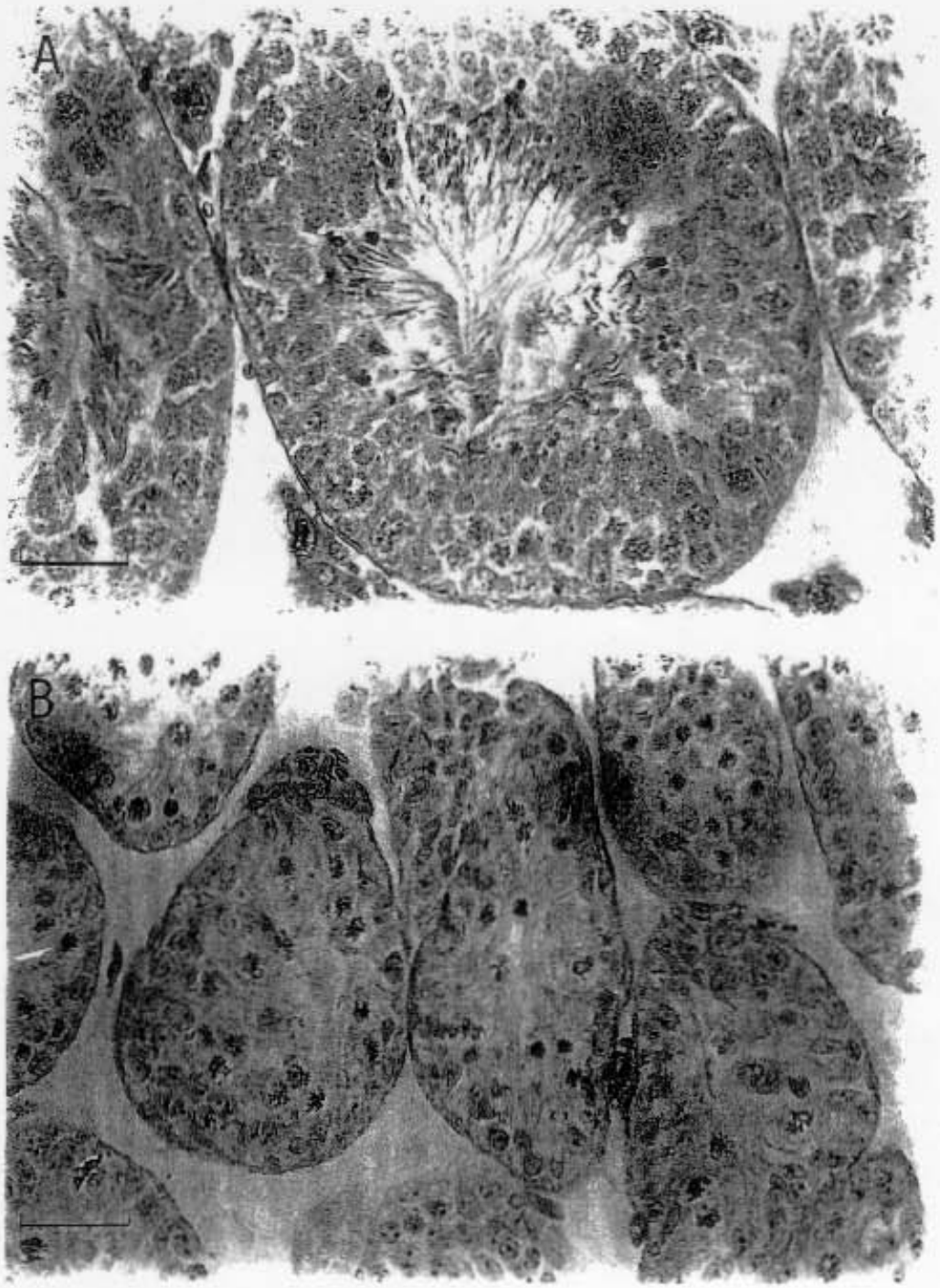

Plate 3.1: C/S through seminiferous tubules of *Rhabdomys pumilio* that had been exposed to 26^{0}C and either to an *ad lib.* diet (A) or to a food-restricted diet (B). Note the presence of large numbers of spermatozoa in A and that there is still cell division occurring in the seminiferous tubules of B although no spermatids or spermatozoa are present. Scale bars represent 0.025mm (A & B).

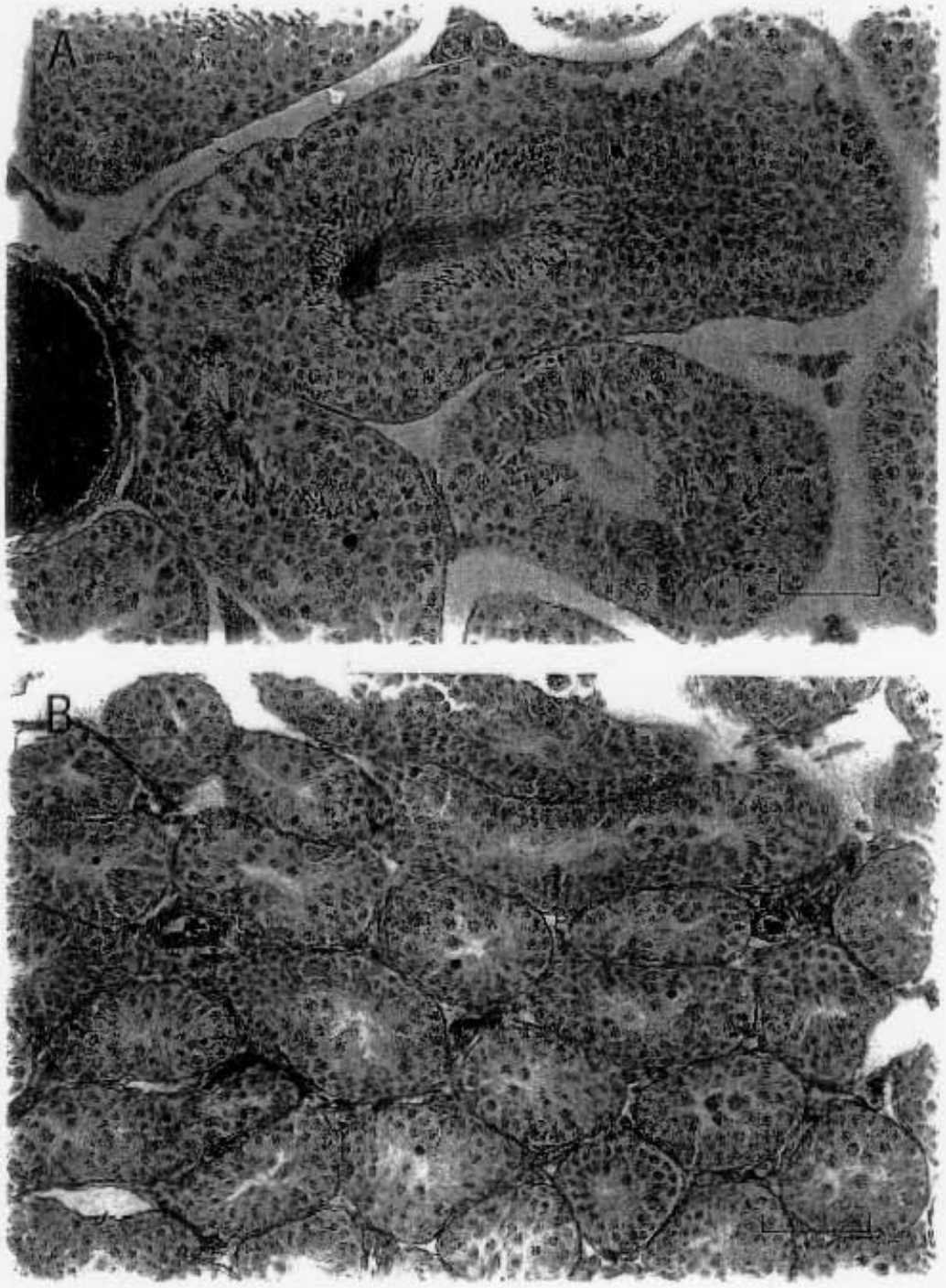

Plate 3.2: C/S through seminiferous tubules of *Rhabdomys pumilio* that had been exposed to 15°C and either to an *ad lib.* diet (A) or to a food-restricted diet (B). Note the lack of spermatozoa and the smaller seminiferous tubules in B compared to those tubules in A. Scale bars represent 0.05mm (A & B).

A Manova run on these data (Table 3.5) indicated that low temperatures, food restriction and an interaction between these two factors had significant effects on the spermatogenic activity of *Rhabdomys pumilio*, where mice exposed to 15^0C and a food-restricted diet had significantly lower spermatogenic activity compared to the other groups. Both the temperature and food availability, acting separately, had a significant effect on the amount of sperm being stored in the epididymides where mice either under low temperature or under food-restriction had lower amounts of sperm being stored within the cauda epididymis than mice with high food availability or exposed to high temperatures. There was no significant interaction between the low temperatures and the low food quantity. Neither temperature nor food had a significant effect on the production and storage of secretion in the accessory glands nor was there a significant interaction between these two factors, although temperature had the greatest effect (Table 3.5).

Table 3.5: Results of the statistical analysis (MANOVA) of the effect of temperature and food on the reproductive activities of the reproductive organs of male *Rhabdomys pumilio*.

Dependent variable	Temperature		Food		Food and temperature	
	P value	F value	P value	F value	P value	F value
Spermatogenic activity	0.0002*	18.1	<0.0001*	39.5	0.0002*	18.1
Epididymal sperm storage	0.009*	8.14	<0.0001*	29.87	0.2954	1.14
Accessory gland activity	0.054	4.1	0.0966	2.99	0.7249	0.127
Nucleus size of Leydig cells	<0.001*	11.66	<0.0001*	88.1	0.7213	0.127

* significant difference at P<0.05 level.

The size of the nucleus in the Leydig cells was significantly affected by both temperature and food acting separately ($P<0.0001$), however there was no significant interaction between these two variables. Mice exposed to low ambient temperatures or to low food availability had significantly smaller Leydig cells compared to the mice maintained under the warm ambient temperature and those mice maintained on a control diet (Table 3.5 & Fig. 3.4).

To further analyse the relationship between body fat scores and spermatogenic activity, the number of spermatogenically active tubules in an animal was plotted against the number of spermatids within the tubules and the points colour coded to indicate the fat index (Fig. 3.5). It is evident from the graph that all but one of the 15FR mice had the lowest numbers of spermatogenically active tubules, the lowest number of sperm per active tubule and fat indices of 0 or 1. In comparison, all the mice that were exposed to a control diet had high spermatogenic activity and high numbers of sperm per active tubule (>100 sperm/tubule). Although the fat indices of these control mice range from 0 to 3, there appears to be little interaction between the fat score and the number of spermatids present in the tubules. The control mice that have fat scores ranging from 1 to 3 all tend to have high spermatid counts irrespective of the fat score, however, the non-fat mice from the 15AL group tend only to have spermatid counts ranging from 100 to 150 spermatids/tubule. Of interest are the mice from the 26FR group. These mice ranged in their spermatogenic activity from being spermatogenically inactive (<20 of the tubules being active) with low general sperm counts to being spermatogenically active (>85 of the tubules being active) with high sperm counts (>100 spermatids/tubule).

There were two mice from the whole experimental group that had high spermatogenic activities (>70 of the tubules being active) but had low general sperm counts (<50 spermatids/tubule). These included a mouse from the 15FR group which had a fat index of 1 and a mouse from the 26FR group which had no fat in storage. No animals fell in the range of 25-70% spermatogenic activity.

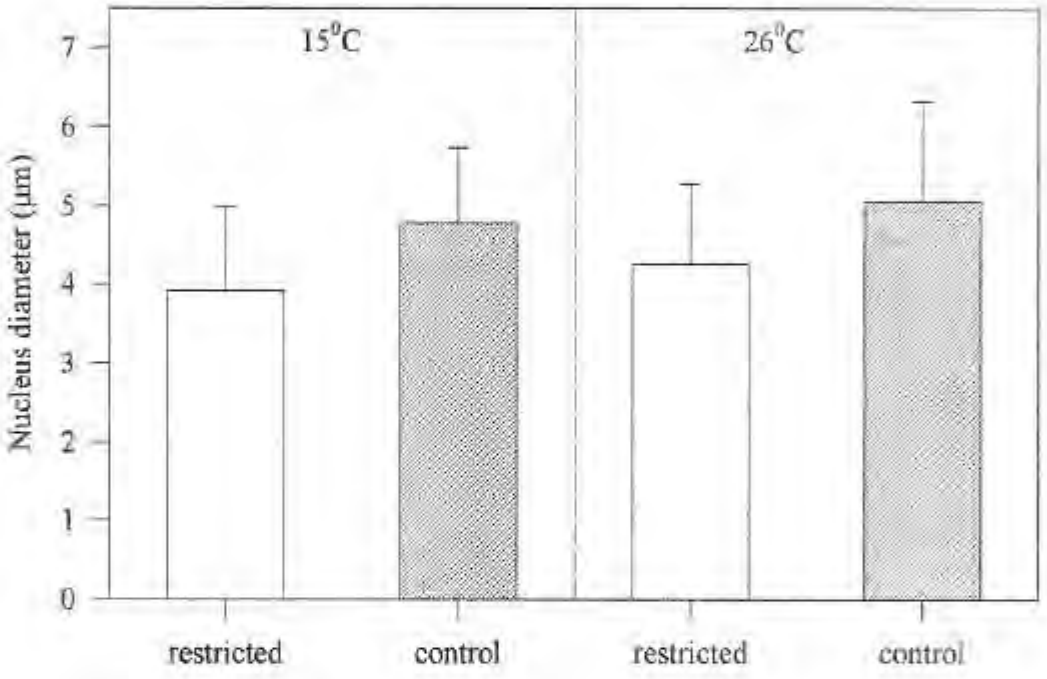

Figure 3.4: Mean diameter of the nucleus of Leydig cells in male *Rhabdomys pumilio* that were exposed to one of two temperatures and one of two diets. ☐ food restricted ▨ control diet.

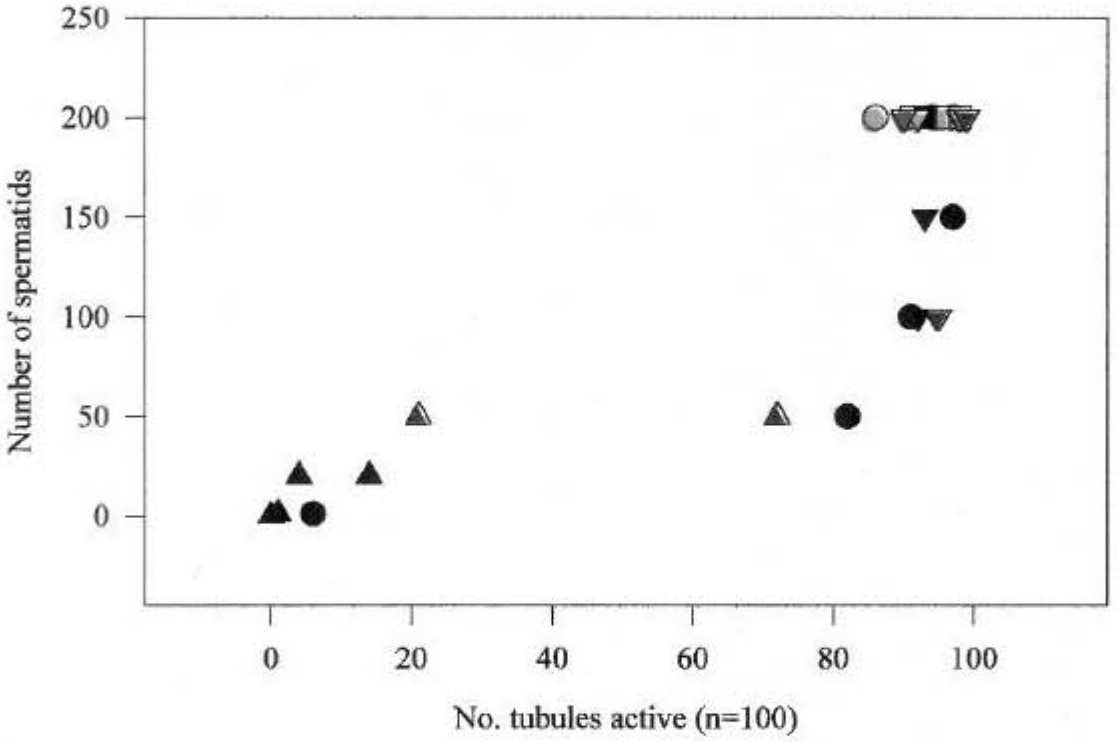

Figure 3.5: The relationship between the number of sperm found in active tubules, the percentage of active tubules and the fat index of each animal. Black = no fat, red = fat 1, green = fat 2 & blue = fat 3. ● = 26 restricted, ■ = 26 control, ▲ = 15 restricted & ▼ = 15 control.

Comparison of the mean testis mass of the mice from the different groups and their mean spermatogenic activity shows that the mice exposed to food-restriction in the cold room had the smallest testes and the lowest spermatogenic activities, while the mice from the control groups had the largest testes and the highest spermatogenic activities (Table 3.6).

Table 3.6: Comparison of the mean testes mass and the mean spermatogenic activity of the testes of male *Rhabdomys pumilio*.

Experimental group	Mean Testis mass (g)	Mean Spermatogenic activity (%)
15 FR	0.145 ± 0.054	16 ± 25.97
15 AL	0.626 ± 0.259	94 ± 3.00
26 FR	0.482 ± 0.298	79 ± 32.67
26 AL	0.784 ± 0.139	94 ± 2.52

3.2.3 *Energetics:*

As previously mentioned, the body masses of the control mice increased during the experiment irrespective of the temperature while those of the mice under a food-restricted diet decreased. The amount of food consumed also changed during the experiment. Besides the intended decrease in food consumption for the mice under food-restriction other changes occurred during the experiment which were not intended. Food consumption increased by about 5% for the 15AL mice, decreased by about 10% for the 26AL mice (not intended), decreased by the intended 10% for the 26FR mice and decreased by 20% for the 15FR mice, 10% more than was intended.

Using equations 2-5 (see Materials and Methods), it is possible to convert the data obtained in this section to energy taken in by the mice (energy intake), the energy released (faecal energy), the amount of energy used (energy assimilated) and a measure of digestive efficiency (Figs 3.6 and 3.7).

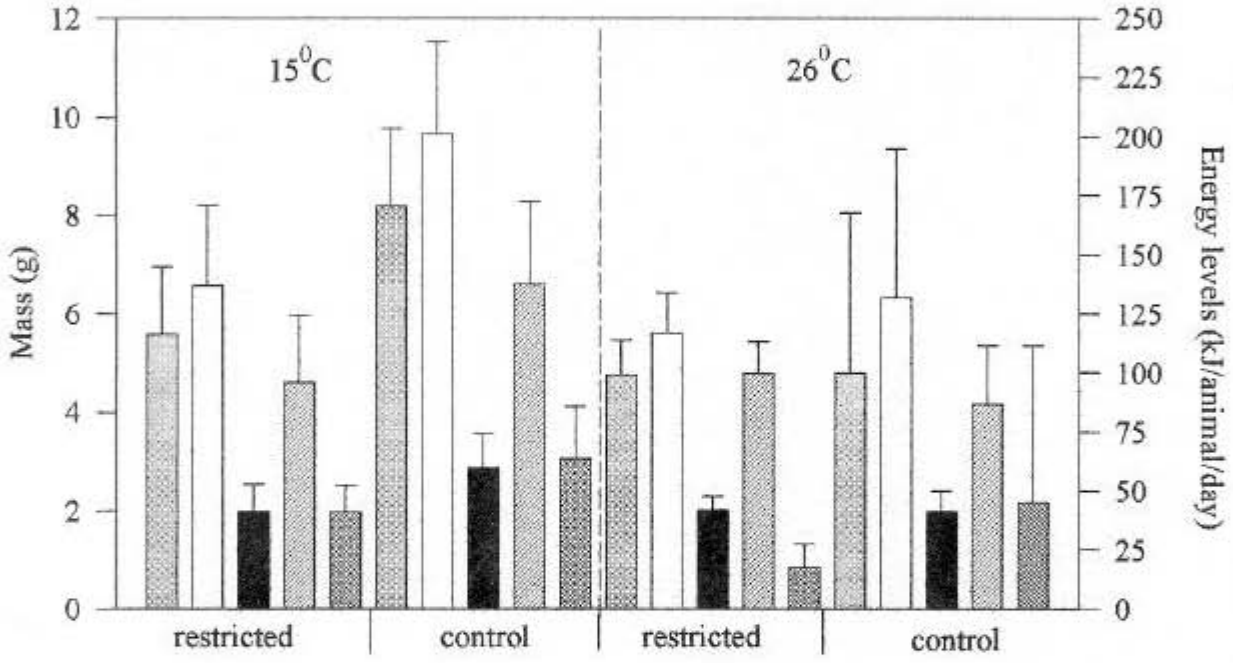

Figure 3.6: Mean daily energetics of male *Rhabdomys pumilio*, exposed to different temperatures and to different diets, for the final week of the experiment: food intake (g)
faeces produced (g)
energy input (kJ/animal/day)
energy output (kJ/animal/day)
energy assimilated (kJ/animal/day)

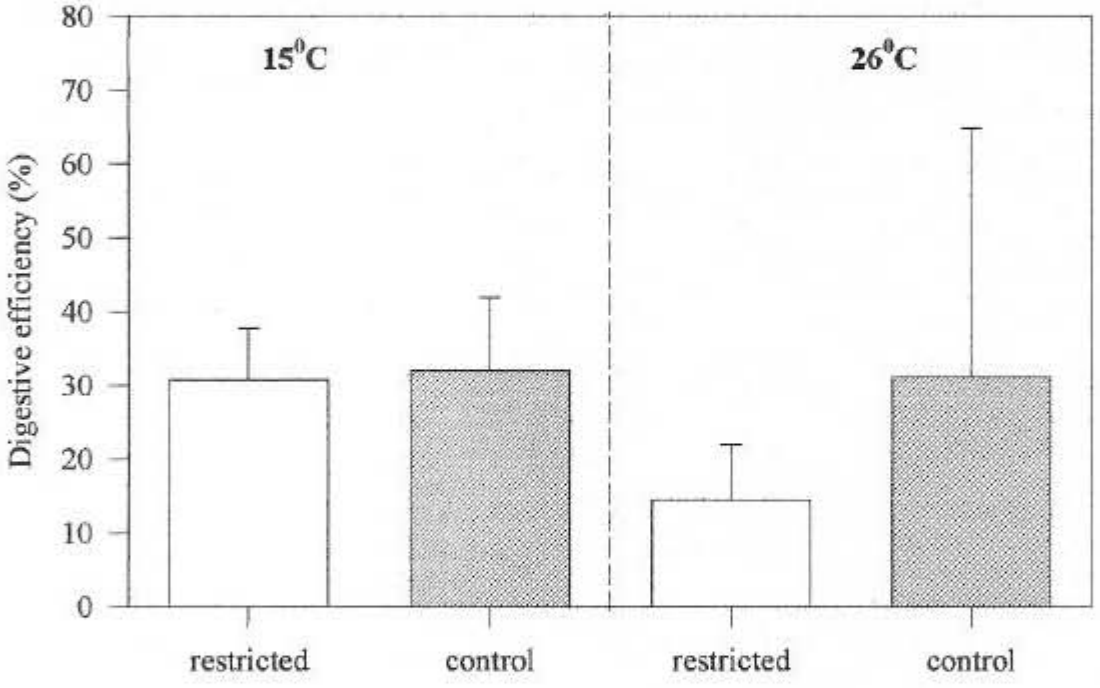

Figure 3.7: The digestive efficiency of male *Rhabdomys pumilio* exposed to one of two temperatures and one of two diets. ☐ food-restricted ▨ control diet.

A Manova run on these data indicated that mice at 15^0C ate significantly more food than the mice at 26^0C ($P<0.05$) and that mice on a food restricted diet ate significantly less food than the control mice ($P<0.05$). However, there was no significant interaction between these two factors (Table 3.7). These results also apply to the energy intake of the mice as the amount of energy taken in is directly related to the amount of food eaten.

Table 3.7: Results of the statistical analysis (MANOVA) of the effects of food and temperature on the energy intake, energy output, energy assimilation and the digestive efficiency of male *Rhabdomys pumilio*.

Dependent variable	Temperature (P value)	Food (P value)	Interaction (P value)
Energy input	0.0148*****	0.0298*****	0.1614
Food eaten (g/animal/day)	0.0148*****	0.0298*****	0.1614
Energy output	0.0408*****	0.2022	0.0208*****
Faeces produced (g/animal/day)	0.0614	0.0556	0.0472*****
Energy assimilated (kJ/animal/day)	0.1675	0.1042	0.8634
Digestive efficiency (%)	0.1588	0.9988	0.9148

***** significant difference, $P< 0.05$

Faecal production (g) was not significantly affected by temperature nor by diet, however a significant interaction between these two factors did occur ($P<0.05$). However, faecal energy (kJ/animal/day) was significantly affected by temperature, where mice from the warm room had lower faecal energy. The faecal energy of the mice from the 15AL group was significantly greater ($P<0.05$) than the other groups, where a significant interaction between low temperature and *ad lib.* food occurred. Although the amount of energy assimilated by the animals in the cold room

was higher than the mice in the warm room and was higher for the animals with an unrestricted diet (control mice), these differences were not significant ($P>0.05$ for both). The digestive efficiency of the mice in the cold room was slightly greater than that of the mice in the warm room (Fig. 3.7) however, neither temperature nor food had a significant effect on the digestive efficiency nor was there a significant interaction between these two factors.

To summarise, at the start of the experiment the mice were almost certainly reproductively active as they were caught during the breeding season (summer) and they were mostly scrotal. By the end of the experiment, mice exposed to food-restriction had significantly smaller reproductive organs, and those mice that were exposed to typical South African winter temperatures (15^0C) showed a significant decrease in testes size. Body fat, which was significantly affected by the amount of food the mice were given, had a significant influence on the size of the reproductive organs, and, once the effects of fat were removed, mice exposed to the low temperature had significantly smaller accessory glands in addition to the smaller testes and those mice that were exposed to both low temperature and low food availability had the smallest testes. Spermatogenic activity and the amount of sperm being stored in the epididymides were also significantly reduced for the mice exposed to the low temperature compared to those animals in the warm rooms.

Although the males from the 15FR group had the smallest and least active reproductive organs compared to the other three groups of mice, the interaction between low temperature and low food availability was only statistically significant for testis mass when fat was included as a covariate. The only other interaction between food and temperature that was significant occurred when the energy output of the mice was tested, where mice exposed to high food availability but low temperatures (15AL) had significantly higher energy outputs compared to the rest of the mice.

CHAPTER FOUR
THE EFFECTS OF TEMPERATURE AND FOOD DEPRIVATION ON REPRODUCTIVE ACTIVITY OF FEMALE *RHABDOMYS PUMILIO*

The aim of this experiment was to examine how low ambient temperature, low food availability and a combination of these two factors affected the reproductive activity of female four-striped field mice.

4.1 MATERIALS AND METHODS:

Female *Rhabdomys pumilio* were trapped in Thomas Baines Game Reserve ($33^0$18'S $26^0$32'E) and on Brentwood farm 50kms south-west of Grahamstown (towards Port Elizabeth) ($33^0$29'30"S $26^0$09'15"E) from late January to early March, 1999. As females were brought into the laboratory, they were placed singly into similar sized cages (41x26x15cm) in CE rooms set at 26^0C (photoperiod 14L:10D, humidity 40%). The mice were each provided with shredded paper and a small square of blanket for bedding, and a toilet roll for cover. Food, consisting of rabbit pellets (Epol), and water were supplied *ad libitum*.

Because these females were trapped during the breeding season they were monitored for 23 days (the gestation period of the four-striped field mouse - Dewsbury and Dawson, 1979) to see if they were pregnant. After it had been established that all the females were non-pregnant and all litters had been weaned from their mothers, a three week acclimation period was started.

4.1.1 *Acclimation:*

Because four-striped field mice can become infested with tapeworm (*pers. obs.*), the females were treated for tapeworm using a LINTEX solution. A tablet (0.65g) of LINTEX (Bayers; active ingredient being Niclosamide) was dissolved in 100 ml of water and 5ml (0.0065g/ml) of this was force-fed to each mouse using a disposable pipette. The females were then divided into two groups and one group (n=20) was placed into a CE room set at 15^0C, while the second group (n=18) was maintained in the warm room (26^0C). In both CE rooms the photoperiod was altered

to 12L:12D and the humidity was kept at 40%. Mice were fed pre-weighed rabbit pellets *ad libitum* and water was always available. During the acclimation period mice were weighed every Monday, Wednesday and Friday, however their reproductive status (perforate or non-perforate vaginal opening) was only noted once during this time to reduce handling time and stress levels. The cages were cleaned every Monday prior to feeding, however nests were just shaken to remove excess food and faeces and then returned to the cage.

During the last week of acclimation (week 0) the daily food intake for each mouse was calculated (equation 1) and the faeces were collected for the calculation of energy assimilation (equations 2-5) (for all equations see Materials and Methods, chapter 3, 3.1.1 and 3.1.4).

4.1.2 *Experimental design:*

Within each CE room the mice were divided into two groups so that there was no significant difference in the mean body weight of the mice in the four experimental groups. Mice in the warm room were divided evenly, resulting in two groups of nine mice each. In the cold room, the group which was to receive a food-restricted diet had two extra mice (n=11) while the control group consisted of nine mice. This division was used to allow for some deaths but to keep sample sizes high enough for statistical comparisons.

The two groups per CE room were allocated to two diets, one being food-restricted where mice were fed their daily food intakes, as calculated during week 0, less 10% and the other group being the control group where mice were supplied with, pre-weighed, food *ad libitum.* The mice were fed daily and were weighed every second day during the week (Mondays, Wednesdays and Fridays), and cages were cleaned every Monday prior to feeding. The females' reproductive status was recorded during the sixth week of the experiment and then again at the time of dissection, where it was noted whether they were perforate or non-perforate. Vaginal smears of the females were not taken as a concurrent study on these mice indicated that the females do not show a regular oestrous cycle (G.M. Tinney- Rhodes University and R.M. White, *pers. comm.*).

In the final week of the experiment (week eight) the faeces and remaining uneaten food were collected and stored at -10^{0}C. These were used to calculate daily food intake (energy intake), energy output, energy assimilation and digestive efficiency (see 4.1.6) for week eight.

4.1.3 ***Physical activity:***

The physical activity of the mice was recorded on a random basis once a day, during daylight hours, through the experiment. Observations were random in terms of the time at which they were taken and in relation to which mouse group was observed first. Mice were recorded as being either in their nest or out of it and, if out, then whether they were eating, sitting or actively moving about (running, jumping or scratching around). These records were converted to a percentage of the total number of observations during the experiment and were used as an index of the level of activity.

4.1.4 ***Sacrifice and collection of Tissue:***

At the end of the experimental period the mice were sacrificed by administration of 0.2ml of Euthanaze. The females were then weighed and the reproductive tract (uterine horns, oviducts and ovaries) was removed and fixed in Bouins fixative. A subjective fat index (0-3) was also determined for each animal, (see Materials and Methods, chapter 3, 3.1.3) depending on the presence of fat under the skin, around the uterine horns and ovaries, and around the kidneys.

After fixation of the reproductive organs, fat was removed from around the ovaries and uterine horns. The organs were then weighed as a whole and then separately, the uterine horns and oviducts together and then the ovaries. Before histological analysis, the ovaries were examined under a dissecting microscope to determine their possible activity, in terms of the presence and number of corpora lutea which were visible as lobes on the ovary.

The final sample size in each group was ten mice in the food-restricted group at 15^0 C (15FR), eight mice in the 15^{0}C, control group (15AL), nine mice in the 26^{0}C, food-restricted group (26FR) and eight mice in the 26^{0}C, control group (26AL).

4.1.5 ***Histological analysis:***

After fixation in Bouin's fixative, the uterine horns and ovaries were embedded, sectioned and stained following routine procedures (see Chapter 3, 3.1.5).

Four sets of serial sections through the centre of the ovary were cut per animal. These were examined under the light microscope, where the numbers of secondary follicles, Graafian follicles and corpora lutea were noted. The greatest diameter of the corpora lutea was measured using an optical micrometer, and the extent of the vascularisation was noted and given a subjective score ranging from 0 to 3 (0 represents no vascularisation, 1= few small areas of vascularisation, 2= larger areas of vascularisation, and 3= large areas vascularised and central cavity full of blood).

Transverse sections through one uterine horn were cut and two slides of serial sections were prepared. For each animal, two measurements were taken per section on opposite sides of the uterine horn, and these data were used to generate a mean value for each animal. The measurements made were the total thickness of the uterine wall, the thickness of the endometrium and the thickness of the myometrium. The number of uterine glands in one section through the uterus of each animal was counted and the extent of vascularisation was give a subjective score from 1 to 3 where 1 represents little to no vascularisation present, 2 represents vascularisation around the uterus but with small vessels and 3 represents large amounts of vascularisation and large blood vessels in the uterine wall.

4.1.6 ***Energetics:***

The left over food and the faeces that were collected during weeks 0 and 8 were dried in an oven at 60^0C and the energy content calculated by bomb calorimetry. This information was then used to calculate the energy input, output, energy assimilated and the digestive efficiency for each animal using equations 2 to 5 (Chapter 3, 3.1.4).

4.1.7 ***Statistics:***

Groups were compared using MANOVA's, MANCOVA's or the non-parametric equivalent using both Statgraphics (Manugistics, 1992) and SigmaStat (Jandel Scientific, 1994) computer

programs. Individuals were compared with a repeated measures t-test. Differences were considered significant when $P< 0.05$ and data is represented as mean values ± 1SD throughout.

4.2 RESULTS

At acclimation, prior to exposure to different temperatures and diets, the four groups of mice were not significantly different from each other in terms of their body mass ($P>0.05$) nor was there any significant difference between their sizes based on hind-foot lengths ($P>0.05$). For this reason absolute data have been used throughout the comparisons, as any changes or differences in reproductive organ size will be due to changes in reproductive activity alone and not to differences in body size.

Both groups of animals exposed to food restriction showed a significant decrease in body mass by the end of the experiment while those on the control diets had all increased their masses (Fig. 4.1.).

At the start of the experiment all mice were perforate and non-pregnant. By the end of the experiment all the mice under food-restriction at 15^0C (15FR), 89% of the mice under food-restriction at 26^0C (26FR), 63% of the mice on the control diet at 15^0C (15AL) and 33% of the mice on the control diet at 26^0C (26AL) were non-perforate (Table 4.1).

4.2.1 *Dissection data:*

At dissection, the mean body mass of the mice in the groups on a food-restricted diet were significantly lower than the mice in the control groups ($P< 0.0001$), irrespective of temperature (Table 4.2 & Fig. 4.2), however, neither temperature nor an interaction between low food availability and low temperature had a significant effect on the mass of the mice ($P>0.05$).

When looking at the reproductive organs of the females as a whole (uterus, oviducts and ovaries) the pattern produced was similar when the organs were examined individually and therefore only the weights of the individual organs will be compared. Food had a significant effect on the mass of the uterine horns, where, irrespective of temperature, the mice exposed to food-restriction had

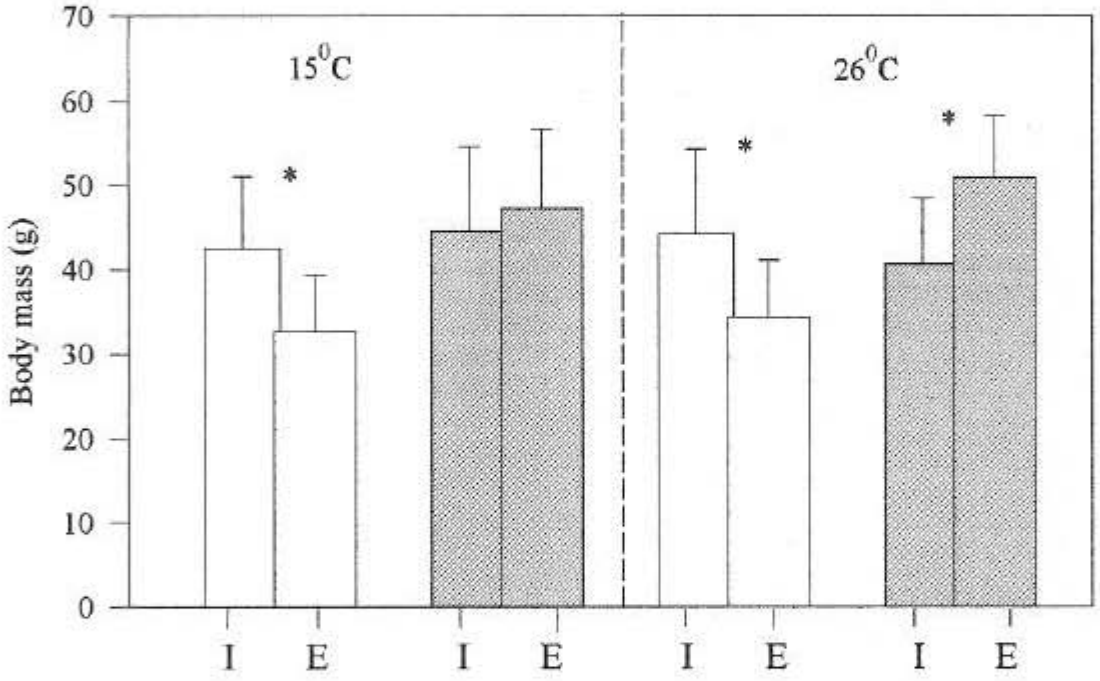

Figure 4.1: Changes in body mass of female *Rhabdomys pumilio* from the start of the acclimation period (I) to the end of the experimental period (E), after exposure to different temperatures and diets. * significant difference. ☐ food-restricted and ▨ control diet.

significantly lighter uterine horns compared to the control groups within the same temperature ($P<0.001$). Temperature differences and the interaction between food and temperature, however, had no significant effect on the masses of the uterine horns ($P>0.05$ for both) (Fig. 4.2, Table 4.2).

Table 4.1. Changes in reproductive activity of female *Rhabdomys pumilio* based on external examination of the vaginal opening. Note the general decline in the numbers of perforate specimens as the experiment progressed from week 0 to week 8.

Group	**%age Perforate**			**%age Non-Perforate**		
	Week 0 Start	**Week 6 Middle**	**Week 8 End**	**Week 0 Start**	**Week 6 Middle**	**Week 8 End**
26AL	100	89	67	0	11	33
26FR	100	11	11	0	89	89
15AL	100	37	37	0	63	63
15FR	100	10	0	0	90	100

Table 4.2. Results of the statistical analysis (MANOVA) of the effect of temperature and food on the body mass and the mass of the reproductive organs of female *Rhabdomys pumilio*.

Dependent variable	Temperature		Food		Food and temperature	
	P value	F value	P value	F value	P value	F value
Body mass	0.4118	0.72	0.0001*****	34.681	0.855	0.035
Uterine horn mass	0.1162	2.611	0.0008*****	13.646	0.1143	2.64
Ovary mass	0.0733	3.436	0.0019*****	11.553	0.3403	0.98

***** significant difference at $P<0.05$.

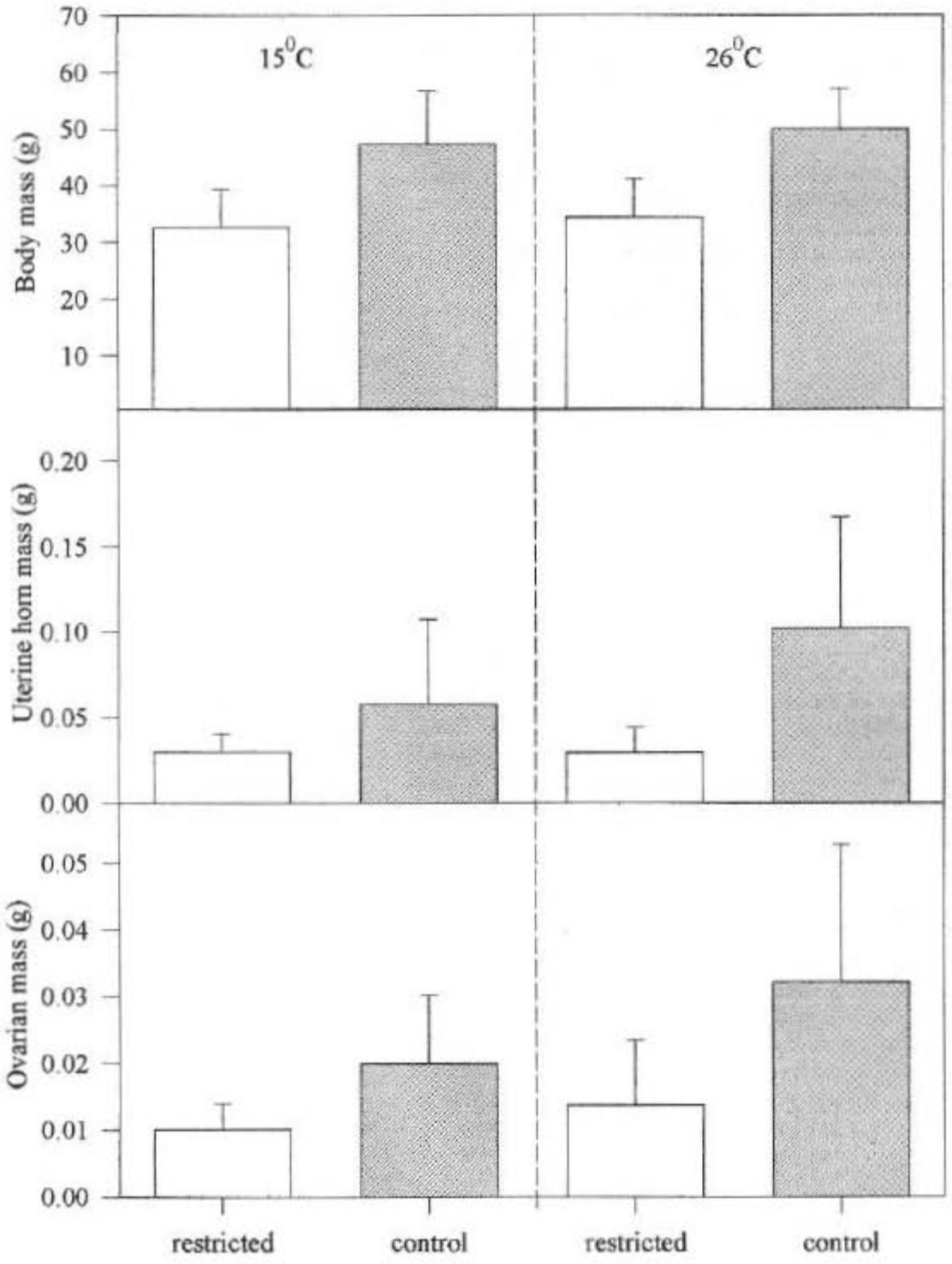

Figure 4.2: Dissection data for female *Rhabdomys pumilio* which have been exposed to one of two temperatures and one of two diets, ☐ food-restricted and ▒ control diet.

A similar pattern was obtained when the mean ovarian masses for the four groups were compared. Mice exposed to a food-restricted diet had significantly lighter ovaries compared to the control mice ($P<0.01$), irrespective of the temperature. The effect of temperature acting alone and the interaction between food and temperature on the ovary masses were not significant ($P>0.05$ for both) (Fig. 4.2, Table 4.2).

During the dissection it was apparent that the females in the four groups had responded differently to their treatment and possessed different amounts of body fat. When the fat index of each mouse was taken into consideration, it become evident that there was a slight trend where animals with little to no fat had smaller reproductive organs compared to those mice with fat indices of two or three (Fig. 4.3). However, the inclusion of fat in a MANCOVA indicated that fat only had a significant effect as a covariate on body mass and that with the effects of fat removed, food continued to have a significant effect on the body mass and the mass of the organs (Table 4.3).

Table 4.3. Results of the statistical analysis (MANCOVA) of the effect of temperature and food on the dissection data for female *Rhabdomys pumilio* once any effects of fat and activity have been removed.

		Body mass	Uterine horn mass	Ovary mass
Covariate:	**Fat**	0.0005*****	0.2799	0.5978
Main effects:	Temperature:	0.5103	0.1394	0.0856
	Food:	0.0023*****	0.0288*****	0.0252*****
Interaction:		0.9508	0.1329	0.3665
Covariate:	**Activity**	0.0004*****	0.0315*****	0.0345*****
Main effects:	Temperature:	0.1483	0.0588	0.0352*****
	Food:	0.0001*****	0.0008*****	0.0019*****
Interaction:		0.2073	0.0308*****	0.1195

***** significant difference at $P<0.05$

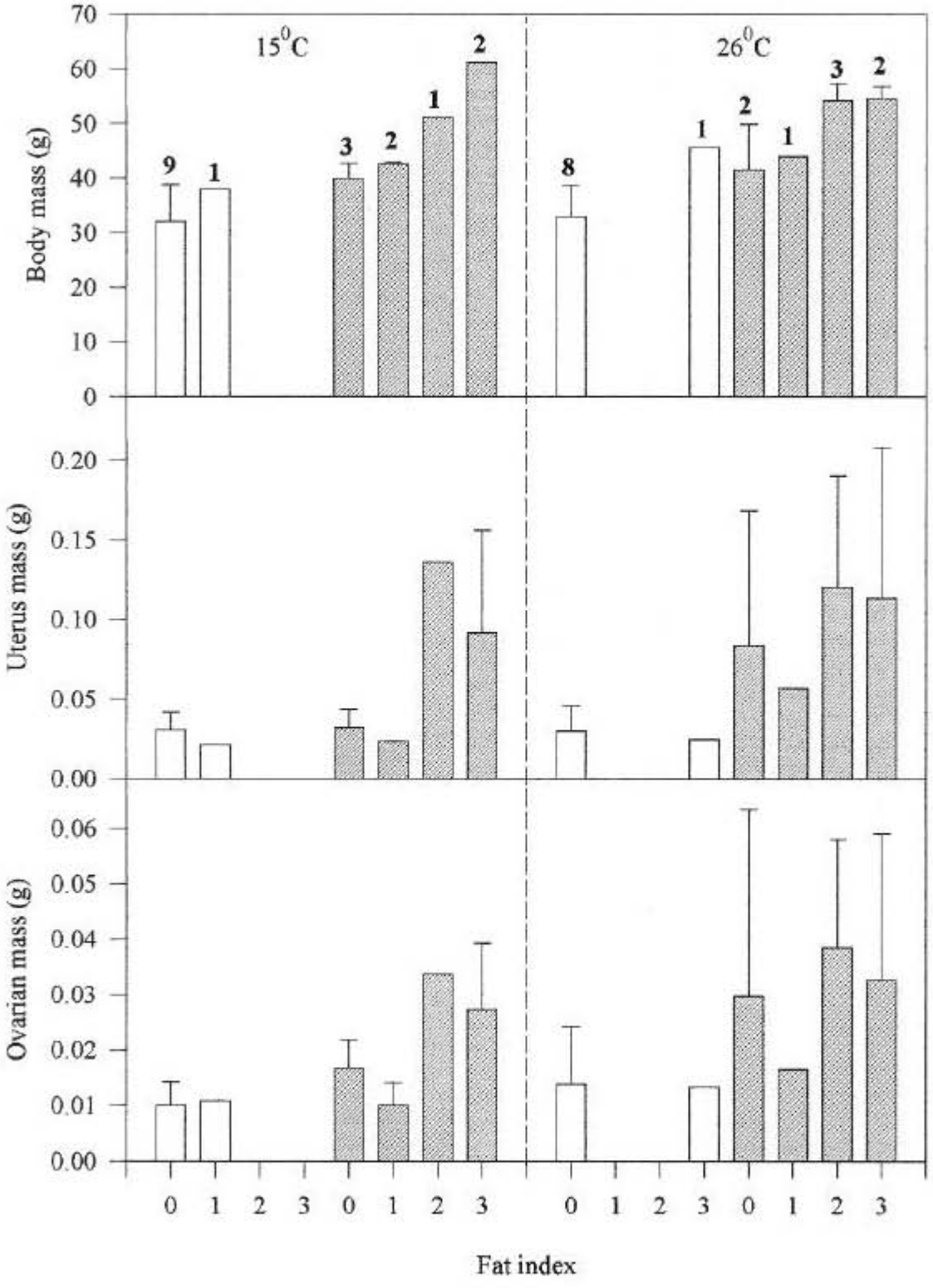

Figure 4.3: Data from Fig. 4.2. have been subdivided according to the fat scores of the animals. Note the slight trend of increasing mass with increasing fat score. (Numbers above the bars indicate the sample size within the division), ☐ food-restricted and ▨ control diet.

Although fat had no significant effect on the mass of the reproductive organs in the females, it was decided to investigate whether the level of activity of the females had an influence on the amount of fat they were able to store. In all cases, there was a very weak negative correlation between time spent out of the nest, in various forms of activity and the fat index ($r^2<0.06$ for all) (Fig. 4.4).

To see whether the level of physical activity had any influence on the body mass and on the mass of the reproductive organs of the females, animals were divided into five groups depending on the percentage of observations in which they were fully active. The time spent fully active by all mice had a slight negative effect in all four experimental groups where the more time spent physically active resulted in lighter animals and in lighter uterine horns and ovaries, compared to the inactive mice (Fig. 4.5). An analysis of the effect of activity on the size of the reproductive organs (MANCOVA) revealed that activity had a significant effect on both the body mass of the mice and on the mass of the uterine horns and the ovaries ($P<0.05$ in all cases) (Table 4.3). Once the effects of activity were removed the mass of the uterus was significantly affected by food ($P<0.001$) and by the interaction between food and temperature ($P<0.05$) and the mass of the ovaries were significantly affected by both temperature and food, acting alone ($P<0.05$ and $P<0.002$, respectively) (Table 4.3).

External examination of the ovaries revealed the presence of a number of protruding structures which the histology confirmed were corpora lutea. The number of corpora lutea visible on the ovaries was significantly affected by the diet of the animals, where mice under food-restriction had fewer corpora lutea on their ovaries than did the mice from the control diets ($P<0.05$) (Fig. 4.6 & Table 4.4). As a result of the above, the gross appearance of the ovaries varied, with the ovaries of the mice from the food-restricted groups being smoother and less lobulated compared to the control mice.

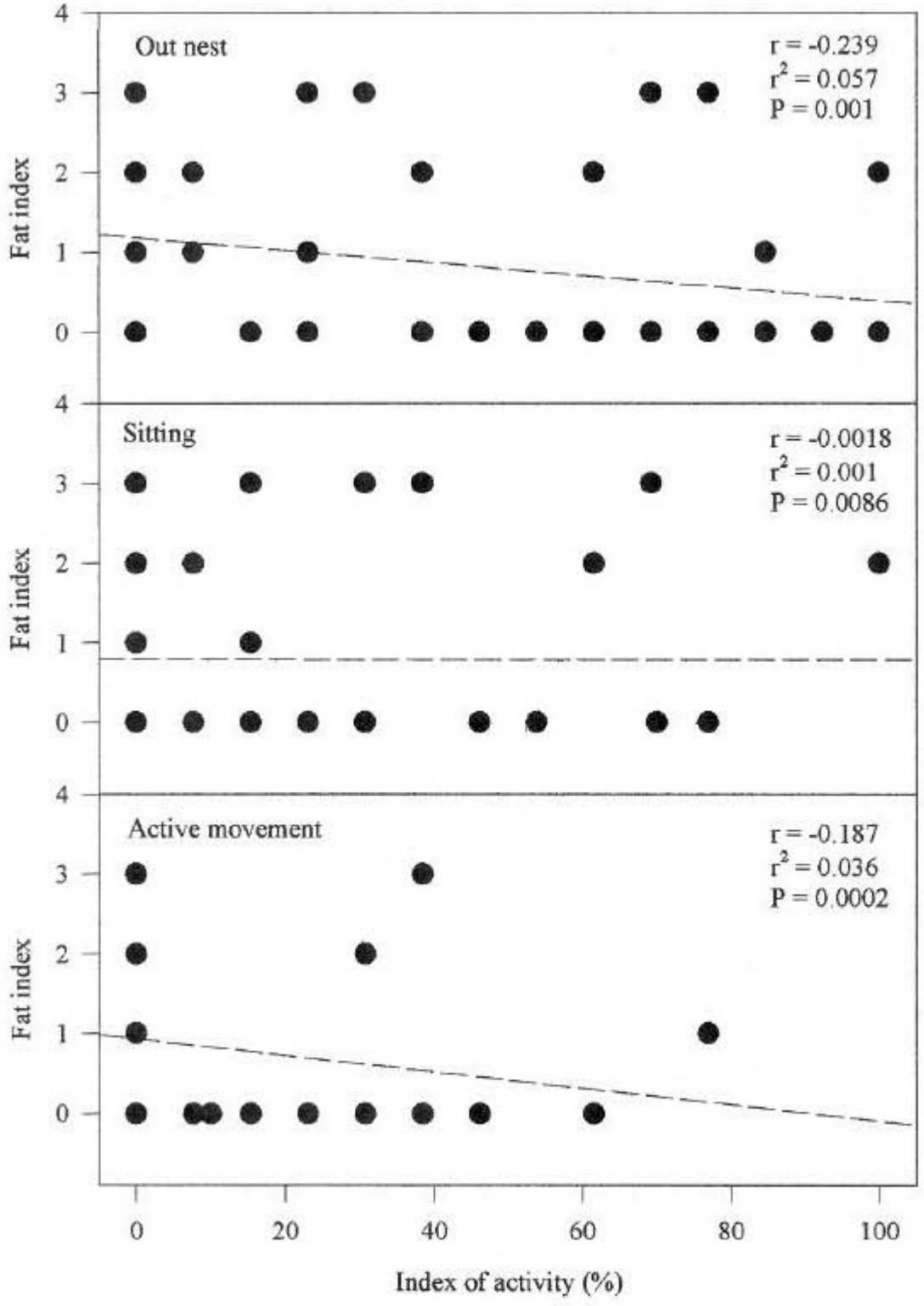

Figure 4.4: Regressions of index of activity with fat scores for female *Rhabdomys pumilio.*

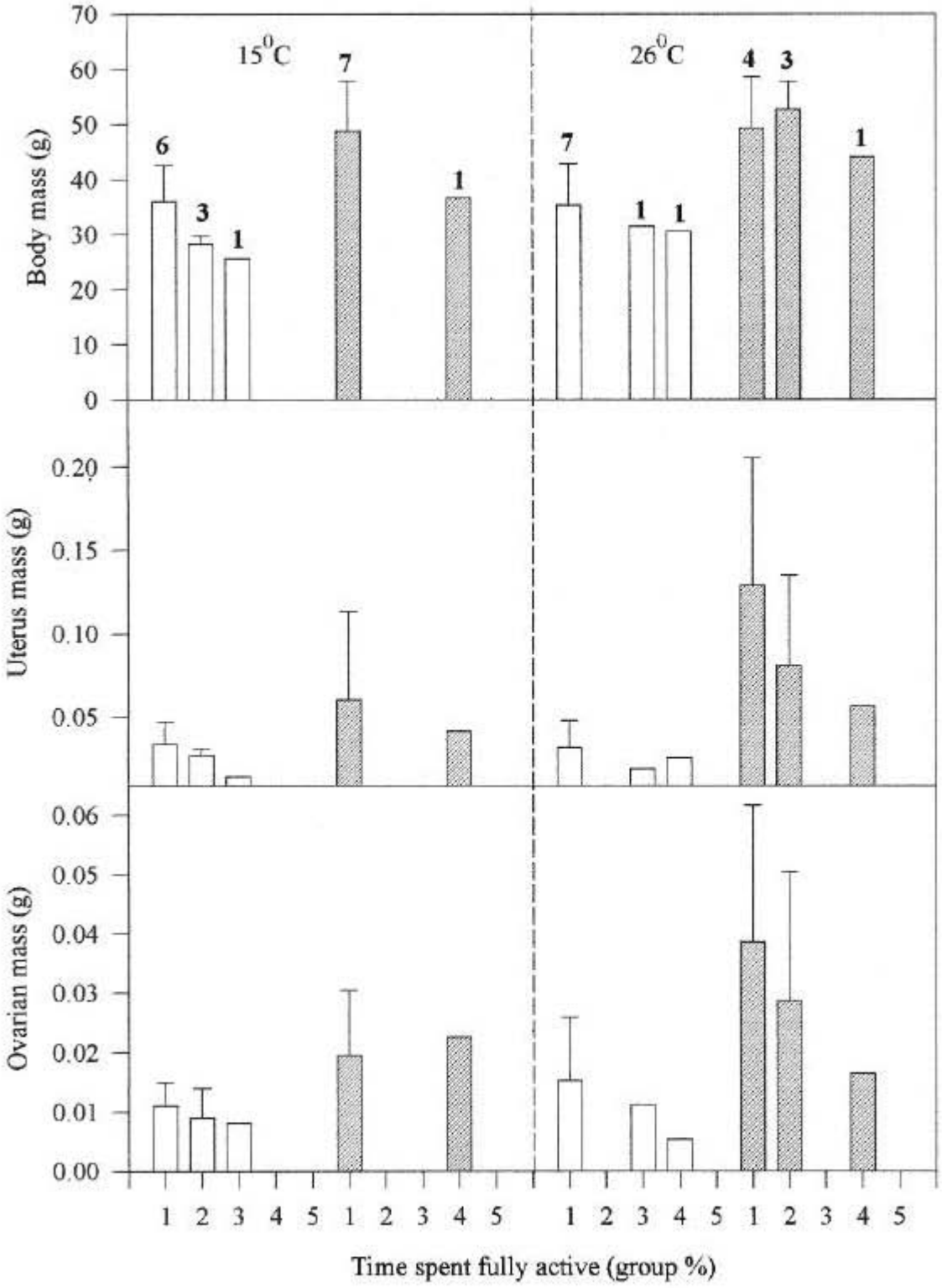

Figure 4.5: Influence of level of physical activity on the size of uterine horns and ovaries, for female *Rhabdomys pumilio* exposed one of two diets and one of two temperatures. Groups: 1= 0-20%, 2= 21-40%, 3= 41-60%, 4= 61-80%, 5= 81-100%.
□ food-restricted and ▨ control diet.

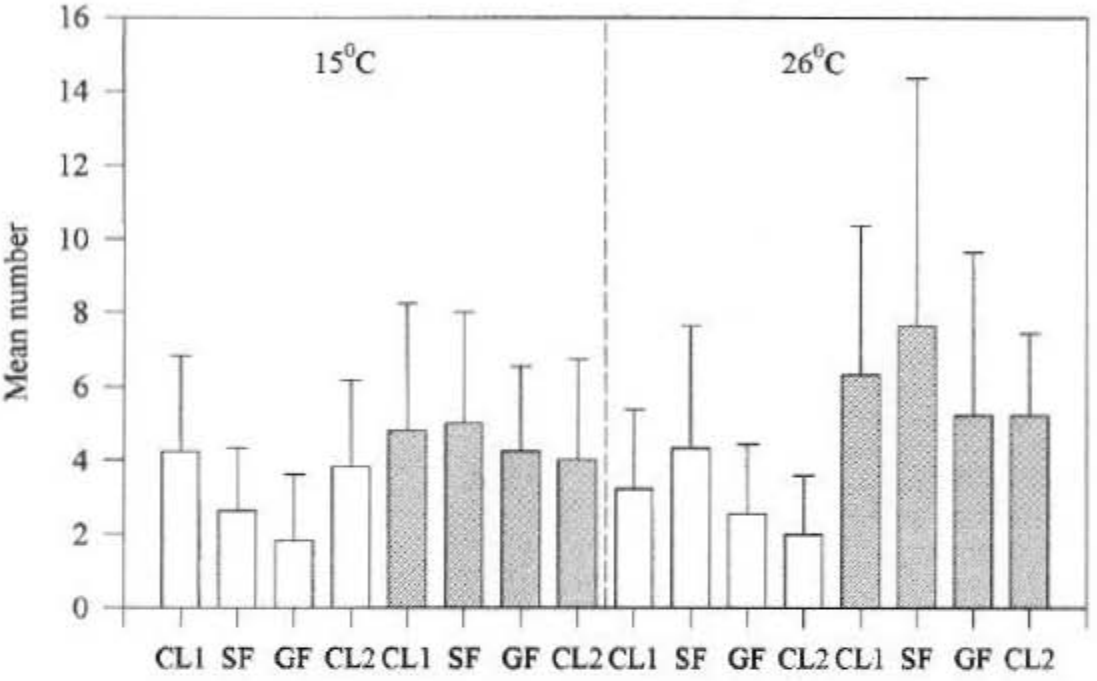

Figure 4.6: Mean number of corpora lutea from external examination (CL1) and the mean number of secondary follicles (SF), Graafian follicles (GF) and corpora lutea (CL2) from histological analysis of ovaries of female *Rhabdomys pumilio*. ☐ food-restricted and ▒ control diet.

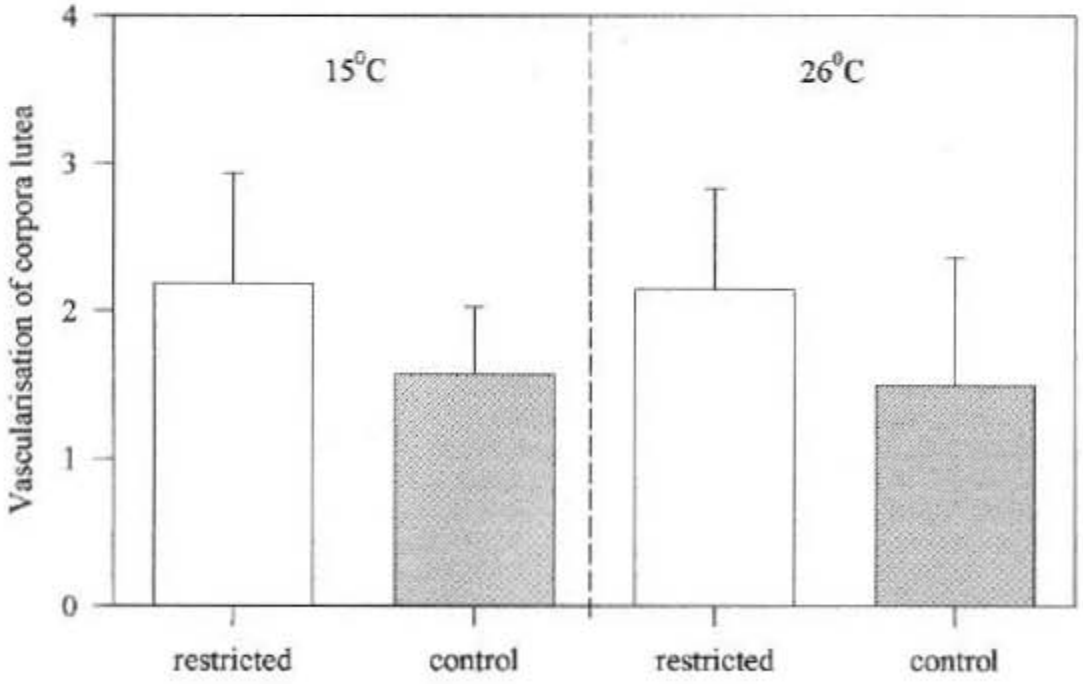

Figure 4.7: Vascularisation of the corpora lutea of female *Rhabdomys pumilio* exposed to one of two temperatures and one of two diets. ☐ food-restricted and ▒ control diet.

Table 4.4. Statistical analysis of the effects of temperature and reduced diet on the number of corpora lutea (CL) visible on outer surface of ovaries of female *Rhabdomys pumilio* and on the numbers of secondary follicles (SF), Graafian follicles (GF), and corpora lutea (CL) and the vascularisation (Vasc.) of the CL, noted during histological analysis on the same ovaries.

Dependent variable	Temperature		Food		Food and temperature	
	P value	F value	P value	F value	P value	F value
External view of CL	0.7409	0.11	0.016*****	6.12	0.0909	2.946
Histology:						
SF	0.1116	2.67	0.04*****	4.56	0.7187	0.132
GF	0.3576	0.87	0.009*****	7.74	0.8988	0.016
CL	0.6917	0.16	0.029*****	5.22	0.0493*****	4.164
Vasc. of CL	0.8286	0.05	0.019*****	6.15	0.9492	0.004

***** significant difference at $P<0.05$.

4.2.2. *Histology:*

Follicular development within the ovaries of the food restricted mice was significantly inhibited compared to the mice under the control diets, where the number of secondary follicles, Graafian follicles and corpora lutea were significantly lower in the ovaries of the food-restricted animals ($P< 0.05$). However, there was no significant influence of temperature on the number of follicles and corpora lutea within the ovaries ($P> 0.05$) and the only significant interaction between food and temperature was on the number of corpora lutea ($P< 0.05$) (Fig. 4.6 & Table 4.4). The vascularisation of the corpora lutea was also significantly affected by diet ($P< 0.02$) where the food-restricted mice exhibited higher vascularisation of their corpora lutea compared to the control mice irrespective of temperature. Neither temperature nor an interaction between food and temperature had an effect on the vascularisation of the corpora lutea (Fig. 4.7 & Table 4.4).

The histological appearance of the ovaries of an animal from each experimental group is shown in Plates 4.1 to 4.4.

Although the corpora lutea appeared larger in the ovaries of the food-restricted mice this was because the ovaries were smaller in this group and measurements indicated that there was no significant difference in the diameters of the corpora lutea between the experimental groups ($P>0.05$).

The mean measurements of the uterus, including the total thickness of the uterine wall, the thickness of the myometrium and the thickness of the endometrium, for the four groups all yielded similar results. In all three cases the mean thicknesses were significantly lower in the food restricted groups compared to the control groups irrespective of the temperature (Manova, $P<0.001$ for all) (Fig. 4.8, Table 4.5). Temperature acting alone and the interaction between low food and low temperature had no significant statistical effect on the dimensions of the uterus ($P>0.05$).

The extent of the vascularisation of the uterine wall was not significantly affected by either temperature or food acting alone, or by an interaction between these two factors ($P>0.05$) (Fig. 4.9). However, temperature had the greatest effect ($P=0.09$) (Table 4.6). The number of glands present in the uterine wall was significantly affected by temperature ($P<0.05$), where animals that were exposed to 26^0C had a larger number of glands compared to the mice in the cold room, however neither food nor an interaction between food and temperature had a significant effect on the number of glands present in the endometrium (Fig. 4.9, Table 4.6). The histological appearance of the uterine horn of an animal from each experimental group is shown in Plates 4.5 and 4.6.

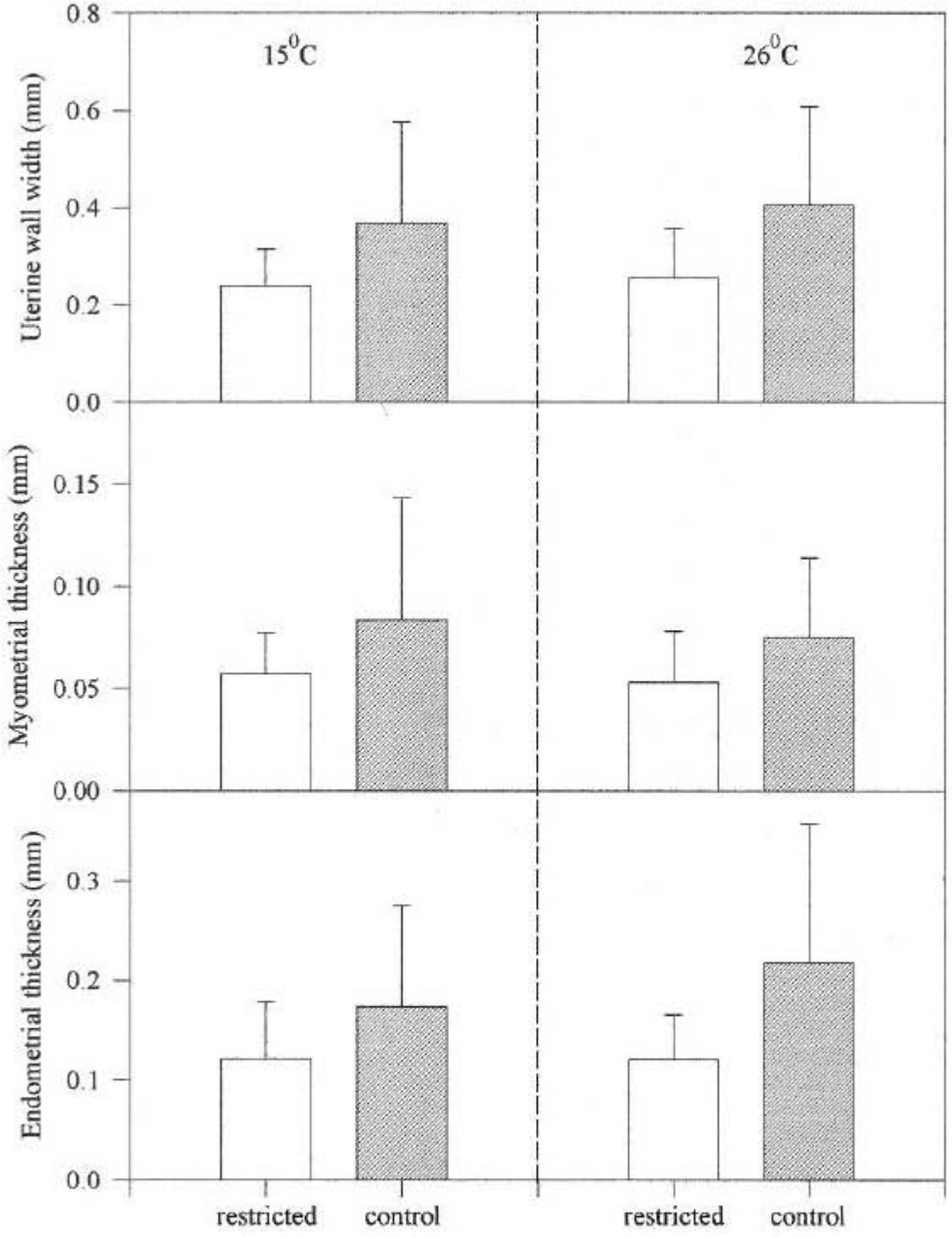

Figure 4.8 : Width of uterine wall, myometrium and endometrium taken from female *Rhabdomys pumilio* that were exposed to one of two temperatures and one of two diets (food restricted or control). ☐ food-restricted and ▨ control diet.

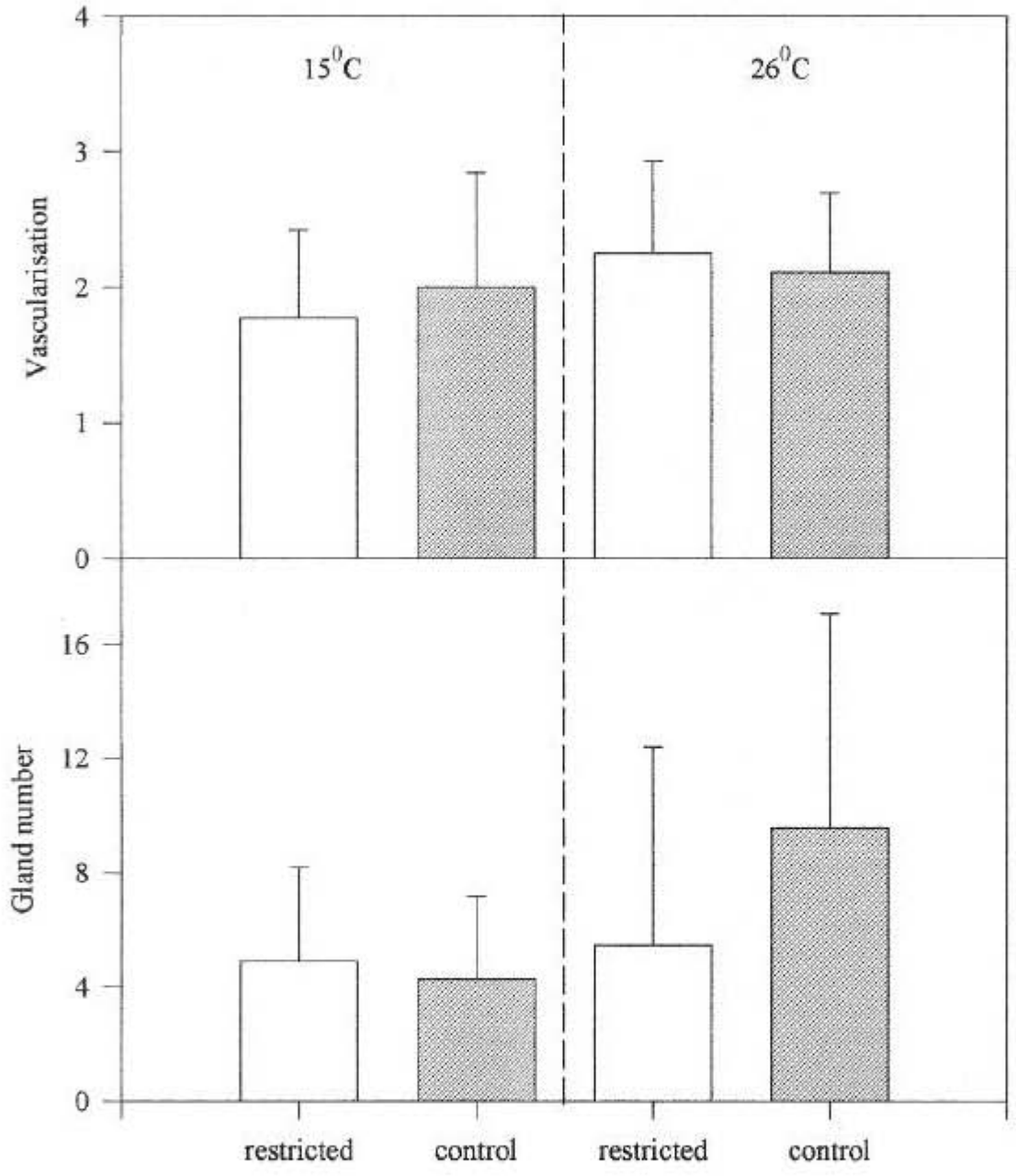

Figure 4.9: Mean vascularisation (as a subjective score) and mean number of glands within the endometrium of the uterine wall for female *Rhabdomys pumilio* exposed to different experimental conditions. ☐ food-restricted and ▨ control diet.

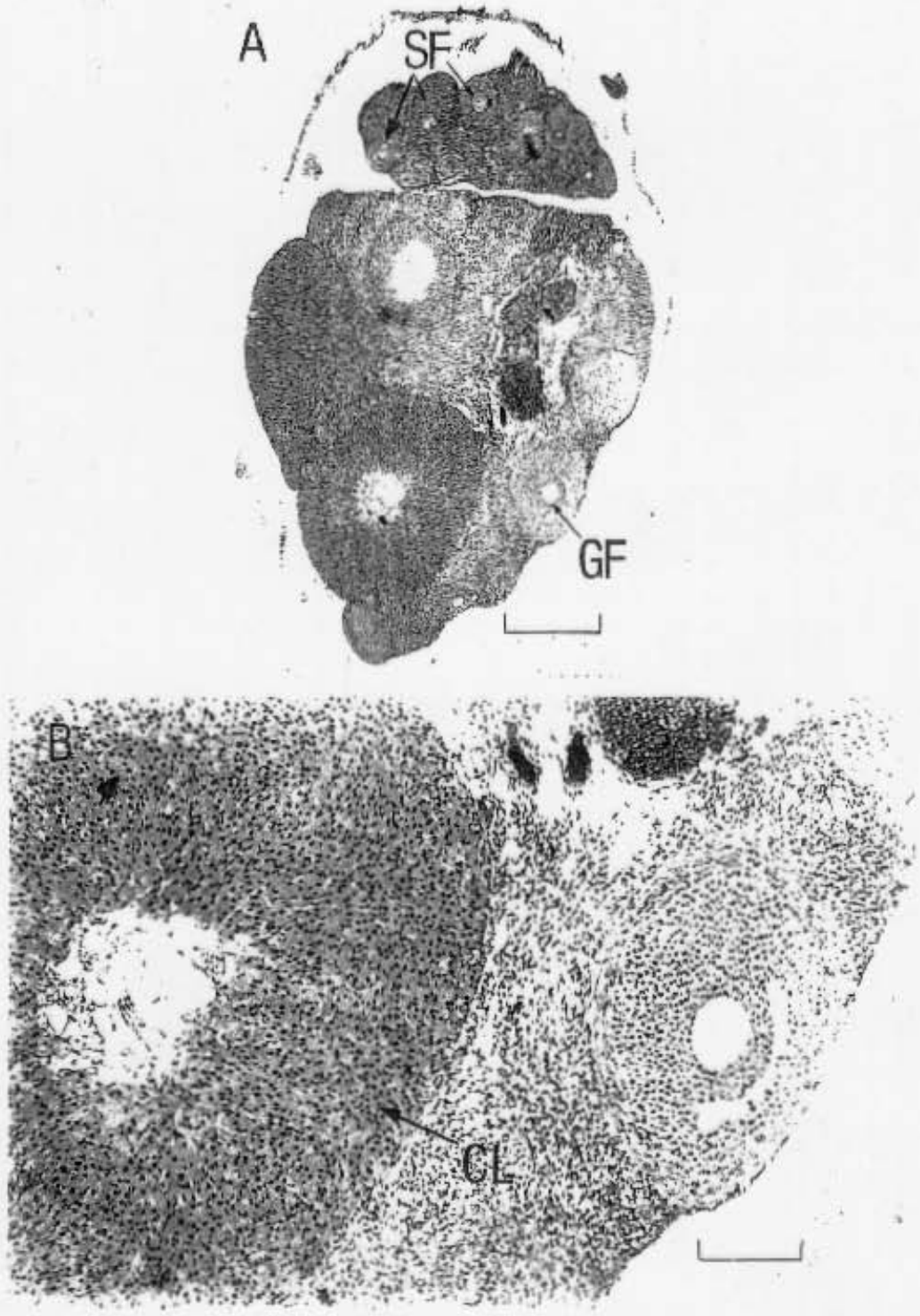

Plate 4.1: C/S through an ovary of *Rhabdomys pumilio* that had been exposed to 26°C and on an *ad lib.* diet. Note the newly developing corpus luteum (CL) with little vascularisation, the numerous secondary follicles (SF) and the large Graafian follicle (GF). Scale bars represent 0.3mm (A) and 0.1mm (B).

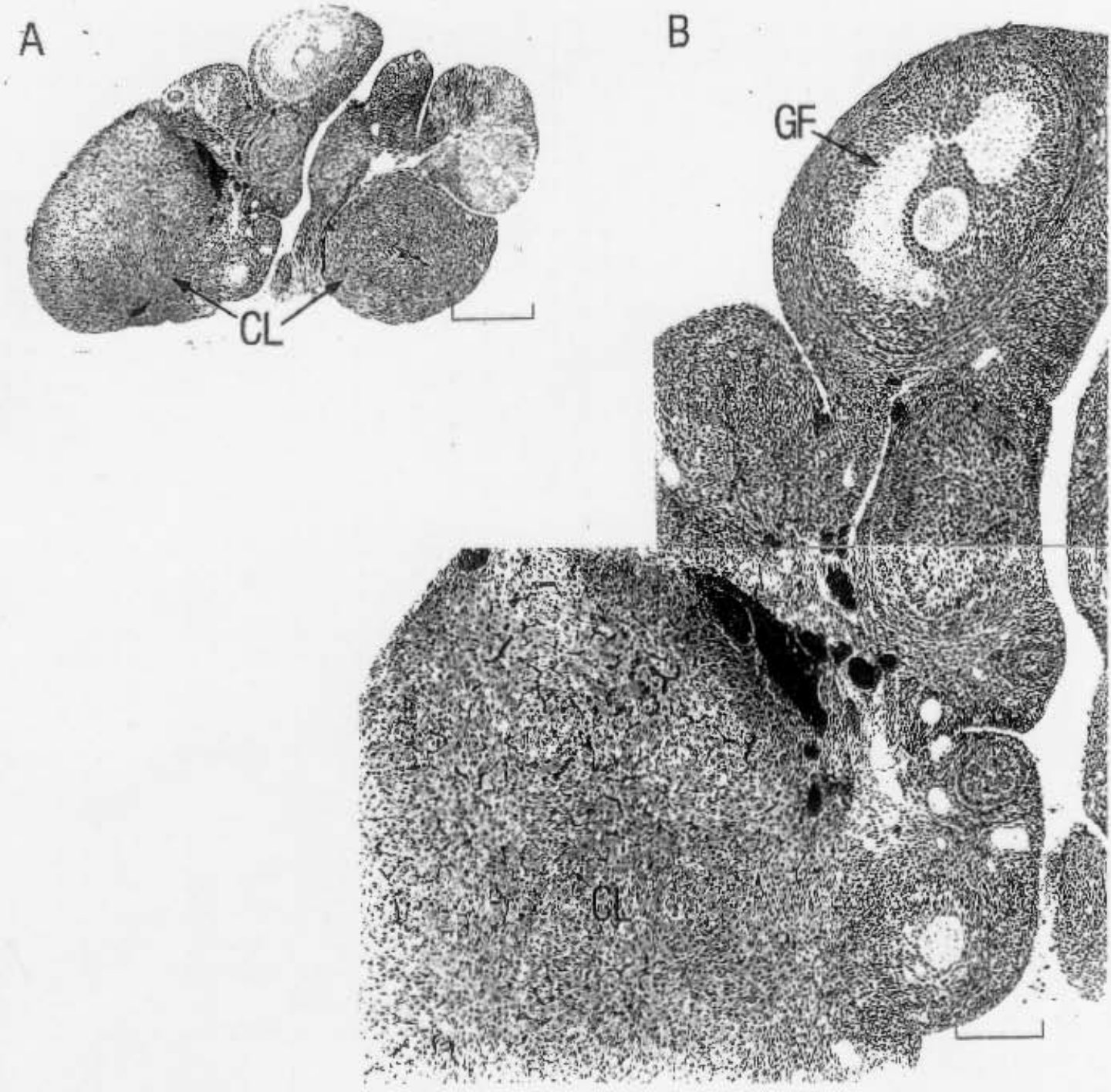

Plate 4.2: C/S through an ovary of *Rhabdomys pumilio* that had been exposed to 26°C and a food-restricted diet. Note the large Graafian follicle (GF) and that the corpora lutea (CL) are highly vascularised. Scale bars represent 0.3mm (A) and 0.1mm (B).

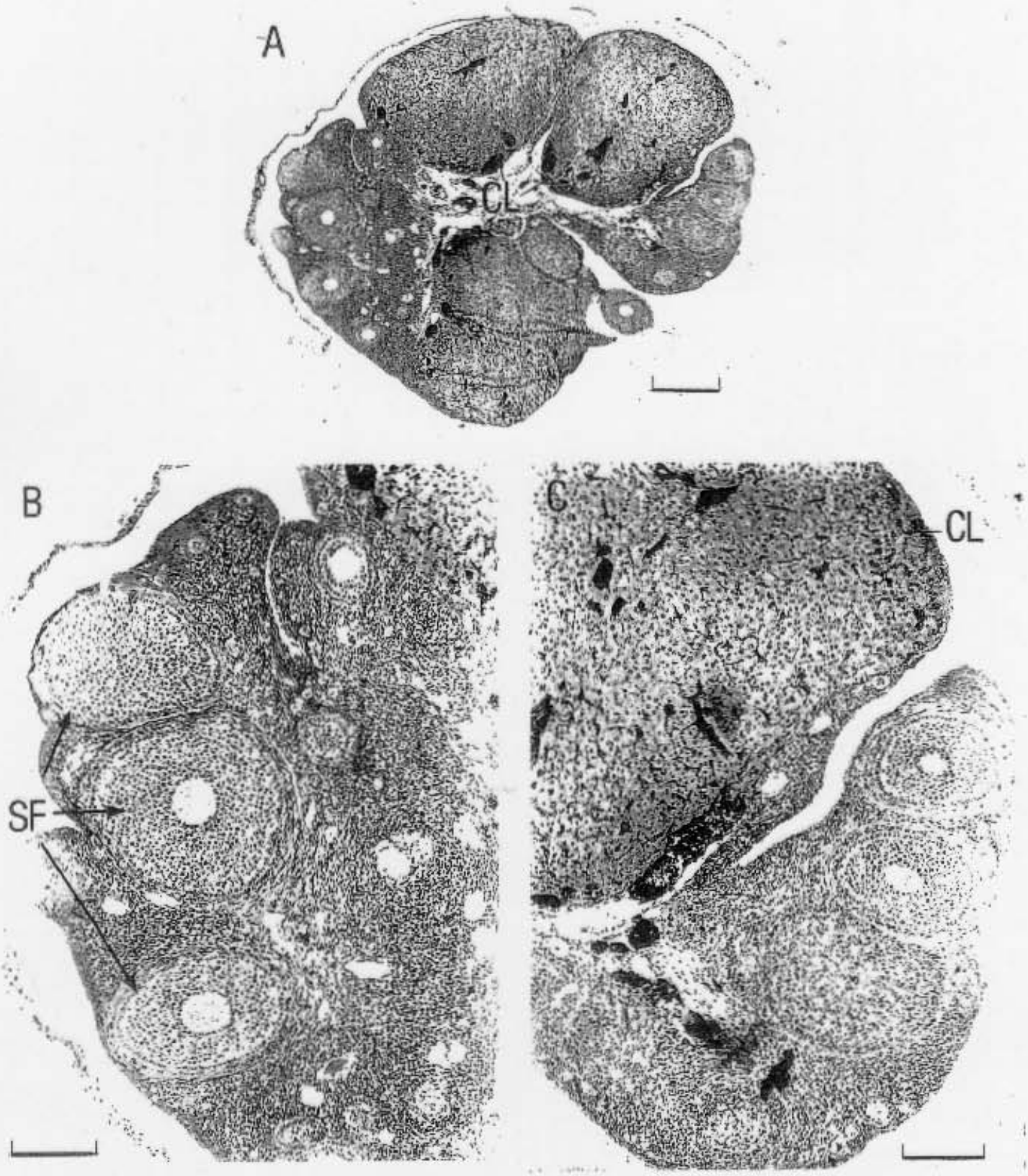

Plate 4.3: C/S through an ovary of a female *Rhabdomys pumilio* that was exposed to 15°C and to an *ad lib.* diet. Note the highly vascularised corpora lutea (CL) and the large secondary follicles (SF). Scale bars represent 0.3mm (A) and 0.1mm (B & C).

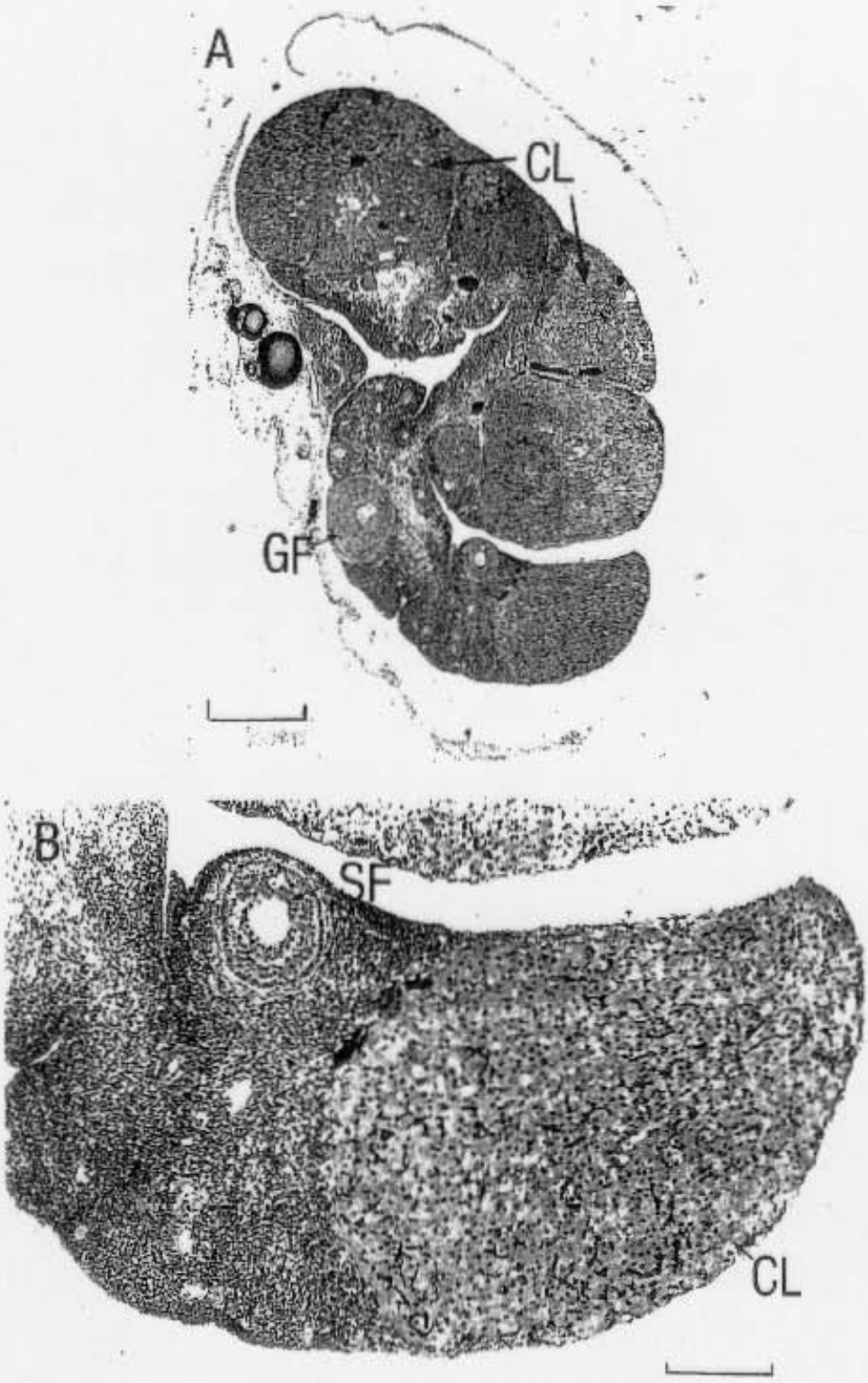

Plate 4.4: C/S through an ovary of *Rhabdomys pumilio* that had been exposed to 15°C and a food-restricted diet. Note the highly vascularised corpora lutea (CL) and the small number of Graafian follicles (GF) and secondary follicles (SF). Scale bars represent 0.3mm (A) and 0.1mm (B).

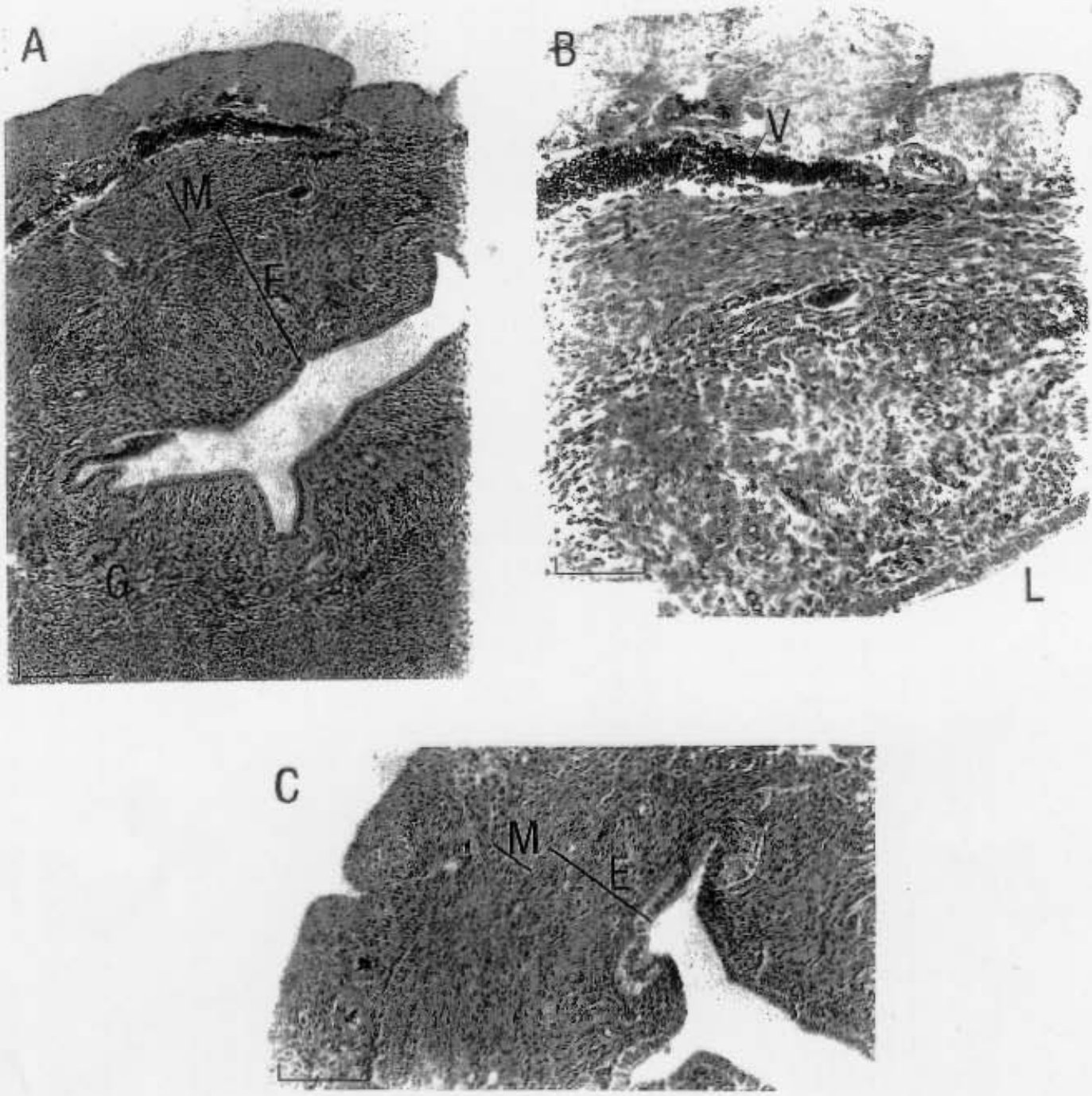

Plate 4.5: C/S through the uterine horns of female *Rhabdomys pumilio* that had been exposed to 26°C and either to an *ad lib.* diet (A and B) or to a food-restricted diet (C), showing a reduction in the vascularisation (V) of the uterus, the thickness of the endometrium (E) and the myometrium (M), and a reduction in the number of glands (G) in C compared to A and B. (L = Lumen.) Scale bars represent 0.1mm (A) and 0.05mm (B & C).

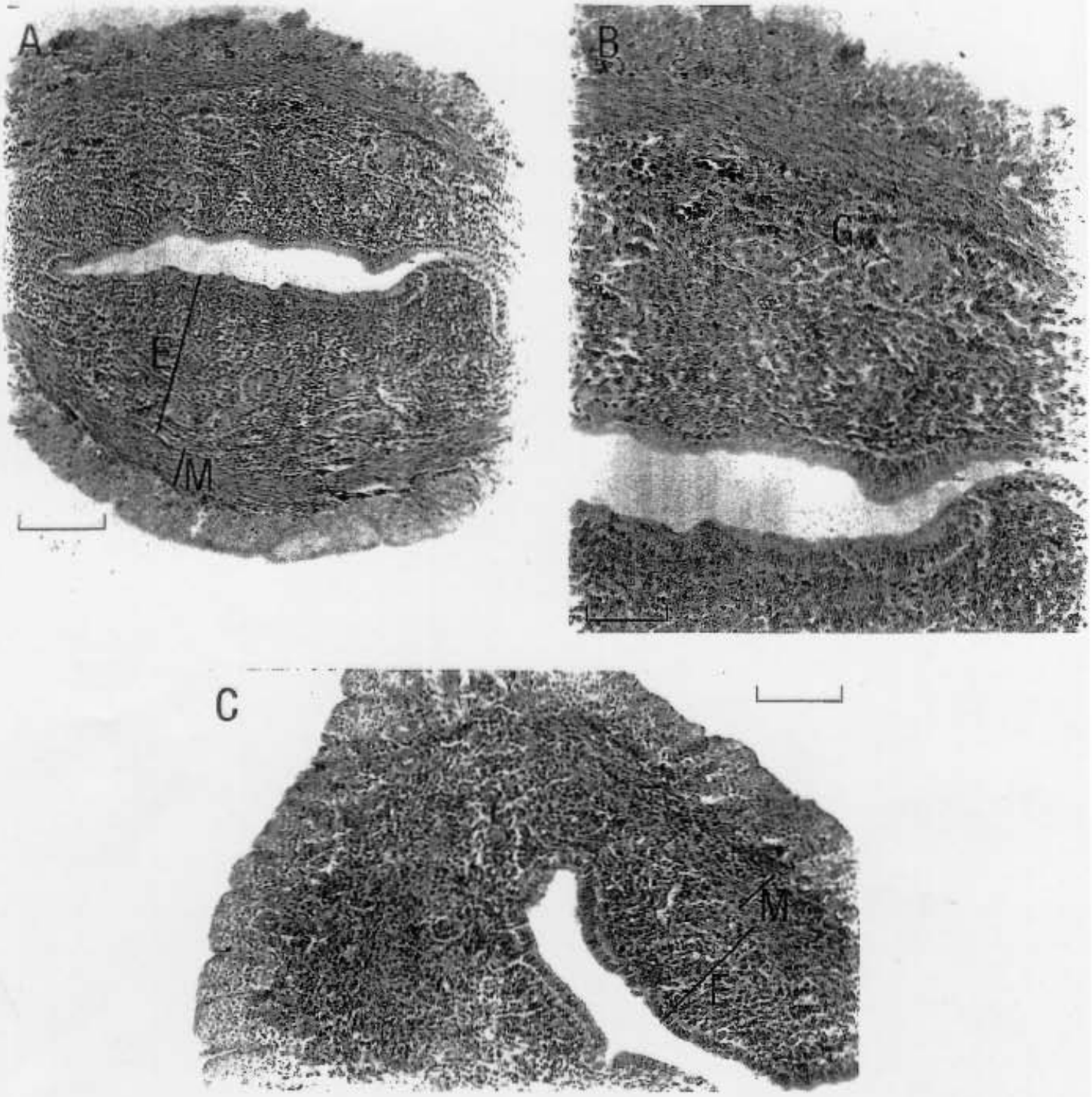

Plate 4.6: C/S through the uterine horns of female *Rhabdomys pumilio* that had been exposed to 15°C and either to an *ad lib.* diet (A and B) or to a food-restricted diet (C), showing a reduction in the thickness of the endometrium (E) and myometrium (M) and the number of glands (G) in C compared to A and B. Scale bars represent 0.1mm (A) and 0.05mm (B & C).

Table 4.5. Statistical analysis (MANOVA) of the effects of temperature and a reduced diet on the mean measurements (mm) of the different layers of the uterine horn for female *Rhabdomys pumilio*.

Dependent variable	Temperature		Food		Food and temperature	
	P value	F value	P value	F value	P value	F value
Uterine wall	0.3431	0.906	<0.0001*****	25.376	0.7185	0.13
Myo-metrium	0.3703	0.808	0.0005*****	12.653	0.7492	0.103
Endo-metrium	0.1936	1.71	<0.0001*****	20.17	0.1868	1.76

***** significant difference at P<0.05.

Table 4.6. Statistical analysis (MANOVA) of the effects of temperature and reduced diet on the vascularisation of the uterine horn and the number of glands present in the uterine wall of female *Rhabdomys pumilio*.

Dependent variable	Temperature		Food		Food and temperature	
	P value	F value	P value	F value	P value	F value
Vascular-isation	0.0889	2.985	0.8059	0.0609	0.2889	1.1439
Gland number	0.0383*****	4.48	0.2097	1.61	0.0906	2.95

***** significant difference at P<0.05.

4.2.3 *Energetics:*

The energy intake (both g/animal/day and kJ/animal/day) for the mice on the food-restricted diet was significantly lower than that of the mice on the control diet (P<0.0001) during the last week of the experiment. There was also a significant interaction between food intake and the

temperature, where mice at 26^0C on the control diet ate significantly more food than the other mice, while the food-restricted mice at the same temperature ate significantly less food compared to the other mice (P=0.01). Temperature acting alone had no significant effect on the amount of food eaten (g/animal/day and kJ/animal/day) by the mice (Fig.4.10 & Table 4.7).

The mice in the cold room produced more faeces which contained significantly more energy than the mice in the warm room (P<0.05), while the mice exposed to the food restricted diet produced significantly less faecal energy than did the mice on the control diets (P<0.01) (Fig. 4.10 & Table 4.7). The interaction between food availability and temperature, however, did not have a significant influence on the amount of faecal energy produced by the mice (P>0.05).

Table 4.7: Results of the statistical analysis (MANOVA) of the effects of food and temperature on the energy intake, energy output, energy assimilated and the digestive efficiency of female *Rhabdomys pumilio*. (energy measured in kJ/animal/day).

Dependent variable	Temperature (P value)	Food (P value)	Interaction (P value)
Energy input	0.9306	<0.0001*****	0.01*****
Food eaten (g/animal/day)	0.9306	<0.0001*****	0.01*****
Energy output	0.0431*****	0.0014*****	0.2138
Faeces produced (g/animal/day)	0.0166*****	0.0026*****	0.2638
Energy assimilated	0.024*****	0.0106*****	0.0471*****
Digestive efficiency (%)	0.0064*****	0.4968	0.4403

*****significant difference at P<0.05.

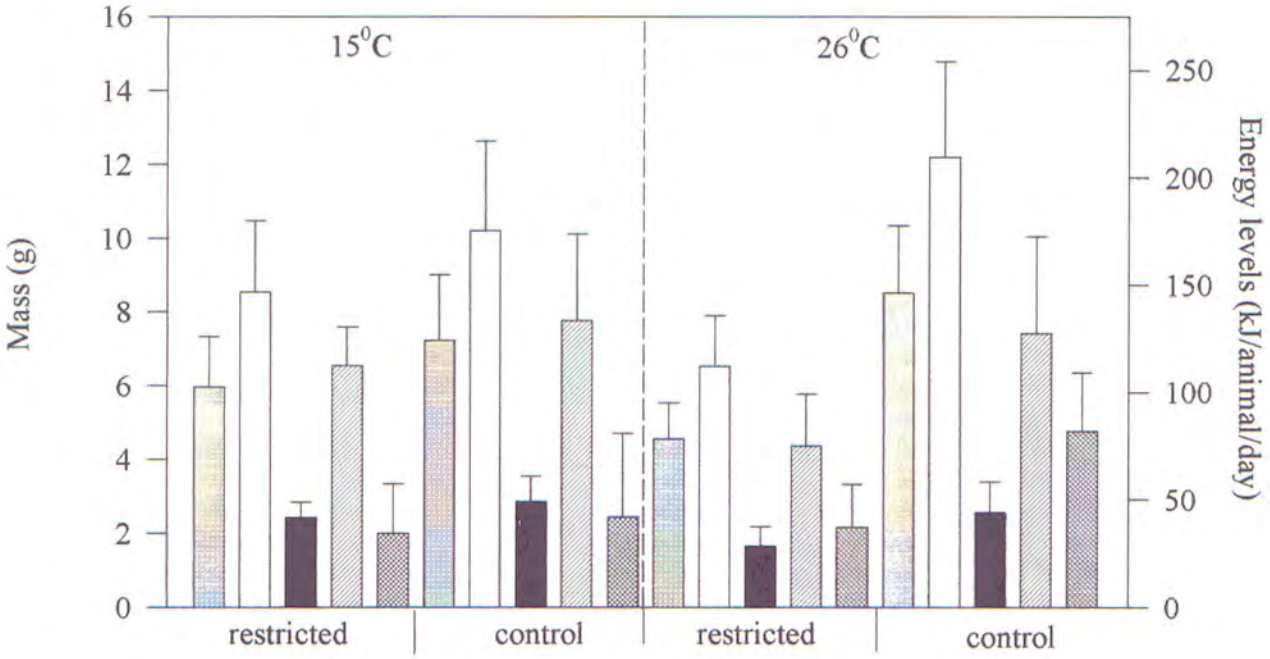

Figure 4.10: Mean dailyenergetics of female *Rhabdomys pumilio,* exposed to different temperatures and to different diets, for the final week of the experiment: food intake (g)
faeces produced (g)
energy input (kJ/animal/day)
energy output (kJ/animal/day)
energy assimilated (kJ/animal/day)

Temperature, food and an interaction between these two factors all had a significant effect on the amount of energy that was assimilated by the mice. Mice exposed to the low temperatures, to a restriction in food availability and to low temperatures with a restriction in food availability all assimilated significantly less energy than the mice from the other groups ($P<0.05$ for all) (Fig. 4.10 & Table 4.7).

The digestive efficiency of the mice in the cold room was significantly lower than that of the warm room mice ($P<0.01$), however, food and an interaction between food and temperature had no significant influence on the digestive efficiencies of the mice ($P>0.05$) (Fig. 4.11. & Table 4.7).

Table 4.8: Changes in energy flux for the different experimental groups from the start of the experiment (week 0) to the end of the experiment (week 8) for female *Rhabdomys pumilio* exposed to one of two temperatures and one of two diets.

Group	Energy input		Energy output		Faecal production (g)		Energy assimilation		Digestive efficiency	
	0	**8**	**0**	**8**	**0**	**8**	**0**	**8**	**0**	**8**
15FR	176.6± 33.5	146.2± 34.9	94.4± 35.5	112.1± 19.1	2.03± 0.78	2.41± 0.44	82.2± 23.0	34.1± 24.4*	47.6± 12.6	21.5± 13.8*
15AL	155.6± 40.0	177.2± 44.2	89.1± 40.9	136.8± 41.9*	2.13± 0.85	2.86± 0.70	66.5± 30.5	40.5± 41.5	43.3± 17.9	21.3± 20.1*
26FR	142.8± 60.2	112.3± 23.7	66.9± 35.7	75± 24.4	1.4± 0.74	1.66± 0.53	75.9± 32.8	37.3± 19.9*	54.2± 13.4	32.9± 15.7*
26AL	177.1± 33.6	209.6± 44.5	91.6± 32.8	127.5± 45.5*	1.93± 0.62	2.57± 0.83*	85.4± 34.1	82.1± 27.3	48.2± 15.6	40.6± 14.6

***** significant difference at $P<0.05$.

The energy intake (food intake) during the last week of the experiment (week 8) was not significantly different from that in week 0 for any of the groups of mice. In terms of faecal production (g), mice from the 26AL group produced significantly more faeces during week 8 ($P<0.05$) while the other groups showed no significant difference. The energy output during week

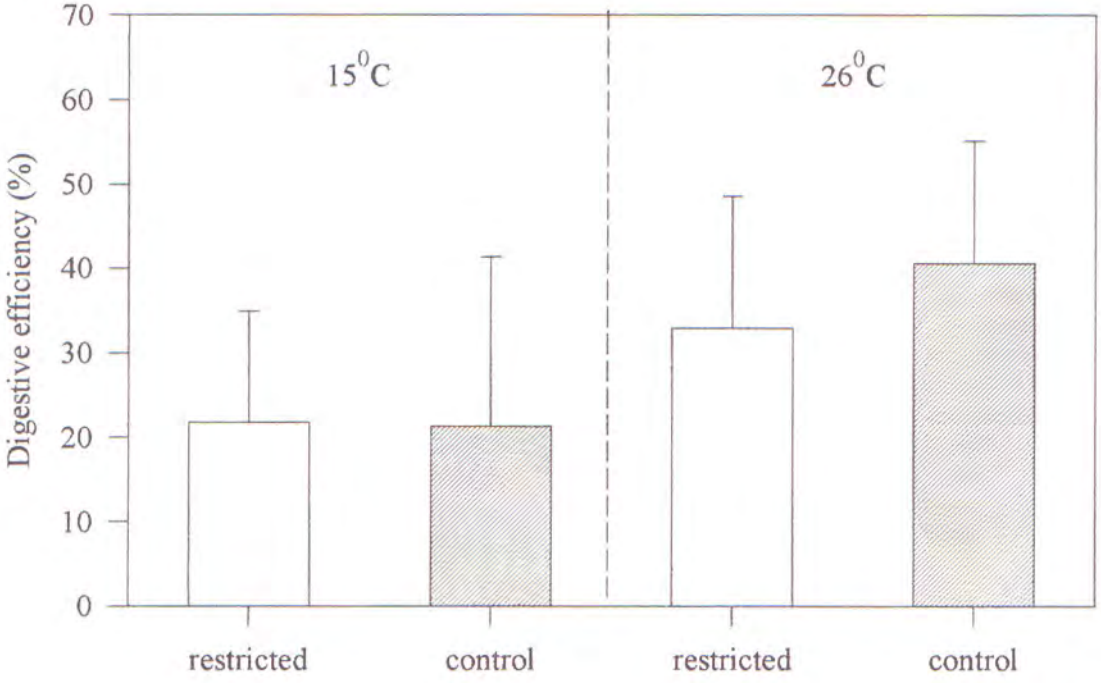

Figure 4.11: The digestive efficiency of female *Rhabdomys pumilio* exposed to one of two temperatures and one of two diets, ☐ food-restricted and ▨ control diet.

8 was also significantly higher for mice from both control groups ($P<0.05$), while the energy assimilation for the food-restricted mice was significantly lower compared to those levels experienced in the first week of the experiment ($P<0.003$). While digestive efficiency had not significantly changed for the 26AL mice, it was significantly lower for the other three mouse groups. (Table 4.8).

To summarise, it is evident that food had the greatest effect on the reproduction of female *Rhabdomys pumilio*. Not only did a reduction in food significantly affect the mass of the females and their reproductive organs, but it also significantly reduced follicular activity and the thickness of the uterine horns. In the ovaries the numbers of secondary follicles, Graafian follicles and corpora lutea were significantly reduced in the food-restricted mice while the vascularisation of the corpora lutea was significantly increased. While the endometrium and myometrium were significantly reduced in thickness with reduced food availability, the number of glands in the endometrium was greater in the mice exposed to high temperatures. Neither temperature nor food had an effect on the vascularisation of the uterus.

Throughout this experiment temperature and an interaction between food and temperature had little effect on the reproductive activity of the females.

Although fat had no significant effect on the size of the reproductive organs of the females, the time spent in physical activity did have an effect, where animals that spent large amounts of time physically active had the smallest reproductive organs.

CHAPTER FIVE
DISCUSSION: THE EFFECTS OF REDUCED FOOD AVAILABILITY AND LOW AMBIENT TEMPERATURE ON REPRODUCTIVE ACTIVITY.

Of the many environmental factors that can influence the timing of mammalian reproduction, food availability is thought to be the most important cause of seasonal reproduction (Bronson, 1989; Bronson & Heideman, 1994). Indeed a number of experiments have been conducted on rodents and other mammals to test their response to varying food availability, either by providing populations with long-term food supplementation (eg: Alibhai, 1985; Cittadino *et al.*, 1994; Duquette & Millar, 1995; Galindo-Leal & Krebs, 1998; Millar *et al.*, 1998), short-term food supplementation (eg. Pinter & Negus, 1965), short-term food deprivation (eg. Bazhan *et al.*, 1996) or long-term food restriction (eg: Hamilton & Bronson, 1985). Although these experiments have studied the effects of food availability in different manners, the fundamental results are similar. Animals that are provided with a food supplement of either green plant matter (Pinter & Negus, 1965; Alibhai, 1985) or oats and sunflower seeds (Galindo-Leal & Krebs, 1998) all show an increase in their reproductive activity, while those mice that are exposed to either short-term or long-term food deprivation (Hamilton & Bronson, 1985; Bazhan *et al.*, 1996) all experience some form of depression in their reproductive activity. Interestingly, in the studies involving both males and females of a species, the results indicate that the females respond to a greater extent than do the males (Hamilton & Bronson, 1985 -wild house mouse; Cittadino *et al.*, 1994- Pampean grassland mouse; Galindo-Leal & Krebs, 1998- rock mouse), supporting suggestions that males are generally less susceptible to reproductive controlling factors than are the females (Bronson, 1989; Bronson & Heideman, 1994).

The results of the current study on *Rhabdomys pumilio* support the findings of these other experiments. Firstly, food deprivation was an important factor in controlling reproduction. A 10% reduction in food availability in the experiment resulted in a reduction in spermatogenic activity and follicular development and a reduction in the masses of the reproductive organs of males and females. Secondly, in female *Rhabdomys pumilio*, follicular development was significantly

inhibited by a reduction in food availability irrespective of the temperature, and a similar, significant trend was seen in the mass of the ovaries and uterus. However, in the males, maximum inhibition of their reproductive activity was achieved when the animals were exposed to food reduction and low ambient temperature. These results, therefore, support the idea that male reproductive activity is less easily inhibited than that of the females.

This differential sensitivity of the two sexes to environmental cues may be related to the higher energy costs incurred by female mammals during their reproductive activity compared to those experienced by the males. Although the energetic costs of gametogenesis are probably not that different for males and females, and the demands for energy by female mammals during early pregnancy are only slightly increased, the main costs of reproductive activity for the females are during late lactation when she is nursing a litter of neonates that weigh more than she does (Bronson & Perrigo, 1987; Bronson, 1989). It therefore seems that the different susceptibilities to environmental inhibition of reproduction in male and female *Rhabdomys pumilio* may be explained in terms of their differing energetic contribution after fertilization, where the males are not required to expend any further energy towards reproduction while the females have to carry all the extra costs of looking after the neonates.

It is clear from these results that energy availability is one of the important factors playing a role in controlling the reproduction of *Rhabdomys pumilio*. The assimilation of energy is probably the most important basis to life. Energy is required firstly for three primary demands; namely cellular maintenance, thermoregulation and for locomotor costs of obtaining food. If there is excess energy available once these primary demands have been met, this remaining energy can be used for growth or reproductive activity, or it can be stored as fat for later use (Bronson, 1989). However, if the availability of energy is decreased through, for example, a restriction in food resources, the requirements of the primary demands are provided for, while reproductive activity, growth and fat storage may be halted or reduced depending on other climatic conditions.

The amount of energy required by an animal to survive depends primarily on ambient temperature. If the availability of food remains high when ambient temperatures are low, an animal may still be able to maintain reproductive activity (eg. the house mouse - Bronson, 1989). However, if, in combination with reduced ambient temperature, the food supply is reduced, the energetic costs of thermoregulation will increase, the available energy will decrease and reproduction may be inhibited (Bronson, 1989).

Besides the direct energy costs of reproduction and the reliance on food availability and ambient temperature, the amount of body fat in storage had an effect on the reproductive activity of the four striped field mice. For whatever reason, the males and females used in this study had variable abilities to resist fat loss and a few animals that had been deprived of food had retained a certain amount of subcutaneous fat. Although it has been reported that fat reserves are important for successful reproduction in female mammals (see Duquette & Millar, 1995), there has been little other evidence to support this (Bronson & Manning, 1991; see Duquette & Millar, 1995). In the current study there was no statistically significant effect of fat reserves on the size of the females' reproductive organs, however the male experiment presented quite different results, where males with the larger fat reserves had the heaviest reproductive organs and the highest levels of spermatogenic activity. It is thus possible that while fat reserves have a significant influence on the reproductive activity of males, these reserves play little role in the reproductive status of the females of the same species. The results suggest that female reproductive activity may be inhibited before their fat reserves are expended.

Under natural conditions, rodents will spend much of their time in thermally buffered nests, however they must emerge from their nests to forage for food irrespective of the climatic conditions. If food is scarce, then they will spend more time searching for food and thus the costs required in finding the food will increase. What is also important is the amount of time a rodent must spend foraging in relation to the ambient temperature to which the rodent is exposed while foraging (Bronson, 1989). If food is scarce and the ambient temperatures are low, then the caloric costs of the prolonged foraging could outweigh the caloric gain (Bronson, 1989). Simply, this means that the animal will expend more energy while foraging than it will gain from the food it

has found. This will result in a loss of body fat and a reduction in reproductive activity. Although I have found no previous studies that relate reproductive activity of rodents with physical activity, it was found in this study that physical activity had a significant effect on the size of the female reproductive organs of the mice. Females that spent more time out of their nest, exposed to the external climatic conditions, generally had the smallest reproductive organs, while those mice spending the majority of their time in their buffered nests had the larger reproductive organs. The different levels of activity shown by the mice might explain why some females were able to resist fat loss while others did not. However, no conclusions can be drawn at this stage for the species as a whole, as the physical activity of the males was not observed during their experiment.

Few studies have been conducted on the effects of ambient temperature alone on reproductive activity. It has been suggested, however, that an animal's reaction to temperature changes or challenges will depend on both its genes and its previous experience (Bronson, 1989). Bronson (1989) goes on to say that low ambient temperature seems to have little effect on the reproductive capability of male mammals however, for females of small size, low temperatures can cause them to depress their productivity. Although the effects of low ambient temperature are tested more often, there is evidence that high temperatures can also affect reproduction. Sharaishi (1962- cited by Harris, 1979) found that the Japanese Harvest mouse, *Micromys japonicus*, did not breed during the hottest part of the year, indicating that extreme high temperatures (and low temperatures) can cause reproduction in rodents to cease.

In the current study, low ambient temperature, by itself, had a much smaller effect on the reproduction of the female four striped field mice than did food deprivation acting alone, although temperature did have a significant effect on ovarian mass and the number of glands in the endometrium, however the significant effect on the ovarian mass was only present when the effects of physical activity were removed. For the male four striped field mice temperature, alone, had a greater effect on their reproduction, although this was still less than the effect of food, where body mass, testes mass, spermatogenic activity, epididymal sperm storage and the size of

the Leydig cell nucleus were all significantly smaller or lower in the mice from the cold conditions. The accessory gland mass was also significantly reduced in the cold room mice but only when the effects of the fat reserves had been removed.

These results indicate that, despite Bronson (1989) saying that male mammals seem to be less effected by low temperature, male *Rhabdomys pumilio* were more effected by temperature changes than were the females. The difference in response to temperature by the males and females may be explained by Bronson and Perrigo (1987) who emphasized that low temperature is not of overwhelming importance for breeding of wild house mice (as may be the case for other rodents) and that temperature needs to work in conjunction with some other factor like food availability.

Testing the interaction between temperature and food availability showed that there was a significant influence on the size of the testes (when fat effects were removed) and on spermatogenic activity for the males where low food availability combined with low temperature resulted in the smallest and least active testes. For the females, low temperature and low food availability significantly reduced the uterine horn mass (once the effects of physical activity had been removed) while high temperature combined with low food availability resulted in a significantly reduced number of corpora lutea.

A similar experiment (warm room temperature = 21^0C, cold room temperature = 5^0C, *ad libitum* diet and food restricted diet = 90% of mean daily food) conducted on female house mice by Marsteller and Lynch (1987) indicated that the significant effect of food restriction was real at both temperatures, where the food restriction reduced both mean body weight and growth rates of females and that temperature had negligible effects upon reproduction when food was freely available. While these results are the same as those of the present study, Marsteller and Lynch found that there was a significant interaction between low temperature and low food availability, where low temperature magnified the effects of food restriction.

It has been suggested that animals exposed to prolonged periods of stress could have delayed or suppressed oestrous behaviour, reduced ovulation rate or a reduction in their fertility (Daley *et al.*, 1999). Such stresses include periods of being exposed to temperatures below the animal's level of thermoneutrality (Selye, 1980- cited by Bronson, 1989). This stress concept is based on the fact that when animals are exposed to some physiological stress, high levels of ACTH are common, the adrenal glands enlarge and cortisol levels increase (Hamilton, 1998). The increase in cortisol levels will cause an increase in the negative feedback potency of oestradiol and the combination of cortisol and oestradiol will decrease the LH pulse frequency (Daley, *et al.*, 1999). There will also be a reduction in the production of FSH, and this will inhibit reproduction (Bronson, 1989). However, during the present study on *Rhabdomys pumilio*, a concurrent study using the same specimens indicated that the adrenal glands of the mice exposed to the food-restricted diet and to the low ambient temperatures were significantly smaller ($P < 0.05$) than those of the control mice and those of the mice exposed to the high ambient temperature (K. Wilkins - Rhodes University, *pers comm.*). This suggests that the mice within this study were not stressed by the experimental conditions and that the changes in their reproductive activity were not controlled by the adrenal glands and cortisol.

The results for the present study have shown that male and female *Rhabdomys pumilio* do respond differently to environmental factors in terms of their reproductive activity and do so to varying degrees. The males remain reproductively active irrespective of the temperature if there is sufficient food available, and reproduction is inhibited when food abundance and ambient temperature decrease together. If only food abundance decreases and the ambient temperature remains high, males respond in various ways depending on their body fat reserves, by continuing reproductive activity (high fat reserves) or by reducing reproductive activity but not halting it (medium fat reserves) or by halting reproductive activity completely (no fat reserves).

For female *Rhabdomys pumilio*, a temperature of 15^0C has no substantial effect on reproduction and food availability is the key cue. Irrespective of the temperature, when the females were exposed to reduced food availability their reproductive activity was inhibited. It is, however, unclear from the histological analysis of the ovaries whether follicular development was halted,

as the corpora lutea in the food-restricted females were more vascularised compared to those of the control mice. This suggests that the corpora lutea were fairly new, however there was little sign of primary and secondary follicles, indicating that follicular development had halted but that perhaps the degeneration of the corpora lutea had been suspended.

This variation in response to controlling factors of reproduction by the two sexes of a species is typical of an opportunistic breeding strategy (Bronson, 1989; Bronson & Heideman, 1994). In its purest form, opportunism is independent of photoperiod influences and dictates that males remain sexually ready throughout the year and that females either remain sexually active throughout the year or breed seasonally depending on the availability of energy and nutrient resources (Bronson & Heideman, 1994). In a previous study on *Rhabdomys pumilio*, it was shown that short daylength had no inhibitory effect on the reproductive status of males (Jackson & Bernard, 1999) and, as discussed in Chapter two, the breeding strategies of these mice varies from the more equatorial countries where it is found, to the more temperate areas of its distribution. Since, in addition to the above mentioned points, it has been shown in the present study that the reproductive activity of these mice is inhibited by a reduction in food availability and, or a reduction in ambient temperature, it is possible to conclude that *Rhabdomys pumilio*, in the Eastern Cape, is truly opportunistic and will reproduce whenever the availability of energy allows.

Being opportunistic can be advantageous to populations of these mice in the Eastern Cape of South Africa. As mentioned earlier (Chapter 2), this region is found on a climatic transition zone between summer and winter rainfall areas, and experiences considerable variation in the rainfall from year to year. The temperature variation of the region is also affected by this transition zone and, although the mean temperature changes from summer to winter remain fairly stable through the years, variation can occur. This variation of temperature and rainfall causes the Eastern Cape to have seasonal but highly unpredictable climatic conditions. If these mice were strictly seasonal, as previously reported, there would be periods during the normal breeding season (summer) when reproductive activity would be unsuccessful. However, for an opportunistic species this reduction in reproductive success would not occur to as great an extent and during periods of increased

energy levels, reproductive success would be high, irrespective of the time in year. Such opportunistic tendencies have been shown by David and Jarvis (1985) and by Jackson (*pers. obs.*) where reproductively active four striped field mice have been trapped during some winters, in both Cape Town (Western Cape) and in Grahamstown (Eastern Cape).

The endocrine control of reproduction is well known and a model for the way in which food restriction might affect reproduction has been proposed by Bronson (1989). Previous studies have shown that food restriction reduces the production of LH significantly, but has less of an effect on the production of FSH (Sisk & Bronson, 1986; Meredith *et al.*, 1986). This change in LH production is caused by a change in the pulsatile release of GnRH and will initially affect the Leydig cells, testosterone production and the accessory glands in the males and will affect ovulation and the production of corpora lutea in the females (Niswender & Nett, 1988; Meredith *et al.*, 1986). If food restriction is prolonged then both steroidogenesis and gametogenesis will be affected (Bronson & Heideman, 1994). In the current study, the males were subjected to four weeks of food-restriction and, while in some of them gametogenesis had been inhibited, it was evident from the size of the Leydig cells, that a larger number of the males had experienced a reduction in steroidogenesis.

For the females, more complex results were obtained. Firstly, the uterine horns of the females that were exposed to the food-restricted diet were significantly smaller than those of the control females. In addition they were less glandular and less vascularised which indicate reduced activity (see Borg, *et al.*, 1978). Since active uterine horns require oestrogen priming followed by prolonged exposure to progesterone before the ovum can be implanted (Bronson, 1989), it is most likely that the females that had been deprived of food had reduced levels of estrogen and possibly progesterone. If starvation causes a reduction in LH production, this will inhibit or reduce the rate of ovulation and the number of corpora lutea will be reduced. Since the corpora lutea are the main source of progesterone, the progesterone levels will decline as a result of food deprivation. Under normal circumstances, in some mammals, if pregnancy does not occur then the presence of progesterone in the uterus causes the production of Prostaglandin $F_{2\%}$ ($PGF_{2\%}$) which then causes the corpora lutea to degenerate (Niswender & Nett, 1988). However, if there

is a reduction in progesterone levels, this may result in a reduction in $PGF_{2\%}$ causing a cessation of the degeneration of the corpora lutea.

Although the hormonal aspect of the female's reproductive system was not examined in the present study, we can speculate that the presence of highly vascularised corpora lutea in the ovaries of the females that were exposed to the food-restricted diet was due to the reduction in the production of $PGF_{2\%}$, which caused a cessation of the degeneration of the corpora lutea of these mice. In the ovaries of the females that were maintained on an *ad libitum* diet the number of new corpora lutea remained high due to continuous ovulation and the degeneration of old corpora lutea. This normal cycle would then result in stages where the corpora lutea of these mice would be less vascularised compared to the food-restricted females depending on how long after ovulation the mice were killed.

Finally, it has been suggested that prolonged exposure to food restriction will inhibit FSH production and secretion, and this will then inhibit or retard follicular development and oestrogen production (Bronson, 1989). The present study on the females was of a prolonged nature (8 weeks), and in the animals that had been exposed to food restriction, the condition of the ovary suggests that follicular development had been inhibited and oestrogen levels were probably low. The very thin endometrium and myometrium of these mice support this suggestion.

To summarise, the reproductive activities of male and female *Rhabdomys pumilio* responded differently to environmental factors. In both the males and the females, food deprivation alone had a greater inhibitory effect than reduced ambient temperature acting alone. In males, a combination of reduced food availability and reduced ambient temperature was required for maximum inhibition of reproduction, while in the females a reduction in food availability, irrespective of the temperature, had the full inhibitory effect. This can be interpreted as indicating that the females are more susceptible to environmental inhibition than are the males.

Both sexes had varying abilities to resist fat loss when food was limited and in the males, the fat reserves had a significant effect on their reproductive activity. Levels of physical activity varied amongst the experimental females and in cases of high physical activity, the mass of the mice and their reproductive organs was significantly reduced.

In total, these results suggest that *Rhabdomys pumilio* is truly opportunistic in the Eastern Cape of South Africa, showing seasonal breeding when climatic conditions are seasonal, and is opportunistic in the best possible way, where males remain as active as possible (temperature dependent) and the females respond to the greatest degree ceasing reproductive activity under reduced food availability.

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