INSTITUTE OF TERRESTRIAL ECOLOGY
(NATURAL ENVIRONMENT RESEARCH COUNCIL)

NCC/NERC CONTRACT HF3/03/333
ITE PROJECT T08010B1
Interim report to Nature Conservancy Council

BATS AND POLLUTION

I L BOYD

Monks Wood Experimental Station
Abbots Ripton
HUNTINGDON
Cambs PE17 2LS

September 1987

INSTITUTE OF TERRESTRIAL ECOLOGY
(NATURAL ENVIRONMENT RESEARCH COUNCIL)

NCC/NERC CONTRACT HF3/03/333
ITE PROJECT T08020B1
Interim report to Nature Conservancy Council

BATS AND POLLUTION

I L Boyd

Monks Wood Experimental Station
Abbots Ripton
HUNTINGDON
Cambs PE17 2LS

September 1987

C O N T E N T S

1 THE EFFECT OF PERMETHRIN ON THE BEHAVIOUR AND SURVIVAL OF PIPISTRELLE BATS

1.1 Summary

Adult female pipistrelle bats were kept in large outdoor enclosures. One group (Group 2, n = 16) was exposed to wood blocks, placed in the roosting boxes, which were dosed with permethrin while another group (Group 1, n = 15) was given blocks dosed with solvent alone. Although bats in both groups died during the experiment, there was no significant difference between the number of deaths in each group. Most deaths occurred at the start of the experiment and could be attributed to poor weather at that time. Most females gave birth during the experiment and there was no difference between the 2 groups in the birth rate, survival of young or growth of the young. Weight changes in females from the 2 groups were not significantly different at any stage and the pattern of food consumption was also similar. Food consumption was directly related to the expected energetic requirements of lactation. An index of feeding activity also showed this pattern. Overall, there was no effect of permethrin on the behaviour or survival of pipistrelle bats as measured by the methods used in this study and this confirms some previous observations.

1.2 Introduction

Permethrin is highly liposoluble with a solubility in water of 0.2 mg/l (Worthing, 1983). It is a contact insecticide with a low vapour pressure which is produced in at least 15 commercial formulations in Britain for agricultural, veterinary, domestic and public hygiene uses. It has low toxicity to mammals and birds with LD_{50} values normally exceeding 100 mg/kg (Worthing, 1987).

Evidence from a variety of sources suggests that chlorinated insecticides used for the treatment of structural timbers could be an important cause of decline in bat populations (Clark, 1981a, Racey & Swift, 1986; Boyd, 1987). In Britain, there has been a gradual move away from the use of more toxic insecticides, such as lindane, and an increased use of permethrin-based insecticides. Racey & Swift (1986) showed that pipistrelle bats kept in cages which had been treated with permethrin survived with the same frequency as controls. The purpose of this study was to extend the work of Racey and Swift to examine the effect of permethrin on the behaviour and survival of free-flying bats which were not continually exposed to the pesticide. The study was also designed to provide a comparison between the action of permethrin and that of lindane given in similar conditions (Boyd, 1987).

1.3 Materials and Methods

1.3.1 Study animals

Adult female pipistrelle bats (Pipistrellus pipistrellus) were collected from a roost site in Cambridgeshire during May 1987. All bats were trained to feed on mealworms (larvae of Tenebrio molitor) and were given food and water ad-lib throughout the study. A powdered mineral and vitamin supplement (Vionate, ER Squibb & Sons Ltd, Hounslow, Middlesex, UK) plus powdered vitamin C (The Boots Co, Nottingham, UK) was also given daily as a dressing on the food. Before the start of experiments, bats were kept in cages (250 mm wide x 100 mm deep x 140 mm high) consisting of two connected chambers, one of which was kept shaded, while the other had a course nylon mesh front and was exposed to natural daylengths. The inside of each cage was lined by finer nylon mesh to allow the bats to climb. Analysis of the mealworms and the plywood used to build the

cages showed they contained no pesticide.

1.3.2 Pesticide dosing procedure

Bats were exposed to wood blocks which had been dosed with a commerical preparation of permethrin normally intended for the treatment of wood ("Cuprinol", 1.6 g/l permethrin in a mixture of mineral oil (5%) and white spirit (95%)). Clean planed pine blocks

present before dosing. A brush was used to apply the pesticide, as recommended in the instructions. Three coats were applied, the solvent being allowed to dry between each coat. Wood blocks for use as controls were given identical coatings of white spirit containing 5% light white mineral oil (Sigma Chemical Company, Poole, Dorset). End grain formed less than 13% of the surface area of the wood blocks. The end grain was not sealed.

1.3.3 Experimental design

Two groups of adult pipistrelle bats were kept in separate enclosures (9 m wide x 10 m long x 2 m high) where they were allowed to fly freely. There was a feeding point at the centre of each enclosure on a platform 1.5 m above ground level, which was protected from the weather by a timber roof 0.5 m above the platform. Bats had direct access to the food from one side only because it was placed, together with water, on the floor of a timber box (150 mm wide x 100 mm deep x 140 mm high), the east-facing side of which had been removed. This box was lined with nylon mesh to allow the bats to climb inside. Adjacent to this was a second similarly lined roosting box (100 mm wide x 100 mm deep x 140 mm high) connected to the first by a hole (60 mm diameter) to allow access for the bats. There was an inspection door at the front of this box but otherwise it was closed to the outside. A 15 watt heater kept the temperature of this box between 30 and 43°C.

In order to measure the activity patterns of bats, arrivals at and departures from the central feeding platform in each enclosure were recorded. Both boxes were placed on a single 0-5000 g Sartorius electronic balance (accuracy 0.1 g) which was connected to an Epson HX20 microcomputer stored under the feeding platform. The computer was programmed to record plus and minus weight changes of the balance in excess of 3 g. This was achieved by taking readings from the balance at intervals of 2 secs throughout the experiment and comparing the balance reading at time t with that at time t-1. If a significant weight change (>3 g) had occurred, then the time of the event was recorded, together with the balance reading. Further balance readings and times were then taken at 2-sec intervals for a further minute. When there was no significant change in the balance reading, each reading was saved and averaged over a 30-min period. This average provided a base-line reading. All records were stored on magnetic cartridge tapes prior to further analysis. An identical apparatus was used in both enclosures.

Four other roosting boxes (120 mm wide x 40 mm deep x 120 mm high) were also provided in each enclosure. Bats gained entrance to these boxes through a slit on the underside, and a backboard, which extended below the box, was provided to allow the bats to alight before crawling into the box. Both the backboard and the insides were lined with nylon mesh. One of these boxes was located on each

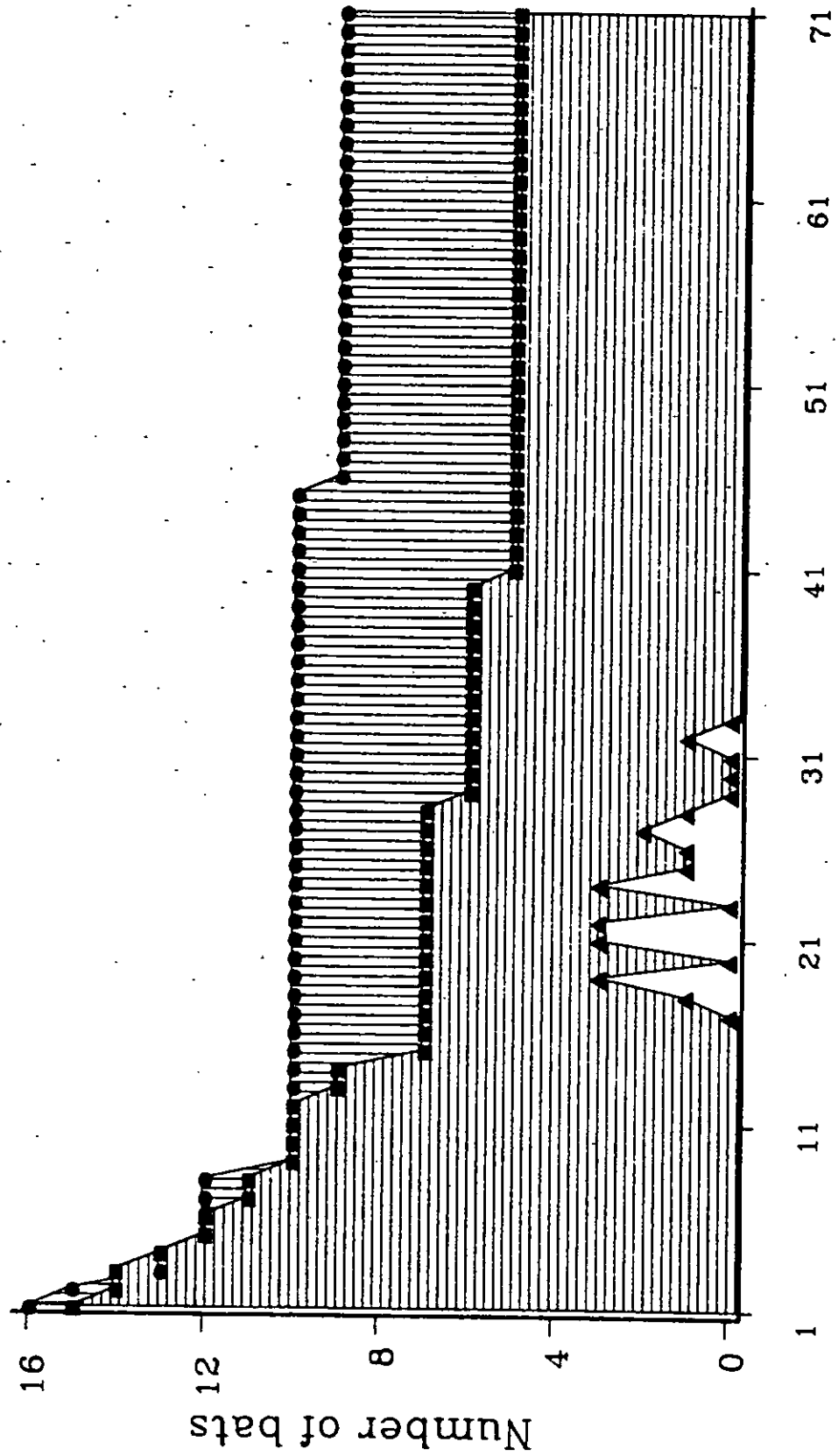


Figure 1.1. Record of number of bats present in Group 1 (squares) and Group 2 (dots) during the study. The triangles show the number of bats born on each day.

of the 4 sides of the enclosure with the entrance at 1.75 m above the ground. This arrangement was identical for both enclosures.

The roosting boxes and feeding platform (plus roof) were constructed of birch plywood which was shown to have no pesticide residues before the start of the experiment. The enclosures, which were located adjacent to one another, were constructed mainly of steel poles and wire, with nylon mesh (mesh size 6 mm) forming the sides and roof.

In order to simulate the type of exposure to pesticide that bats experience in the wild, a block of wood, which had been dosed with permethrin by the method described above, was placed in each roosting box. One group of bats acted as controls (Group 1, n = 15), where a wood block dosed with solvent alone was placed in each box, while the other group (Group 2, n = 16) was given blocks dosed with permethrin. One of the boxes used by Group 2 was given a block dosed with solvent alone and this box was chosen at random.

1.4 Results

1.4.1 Survival of adults

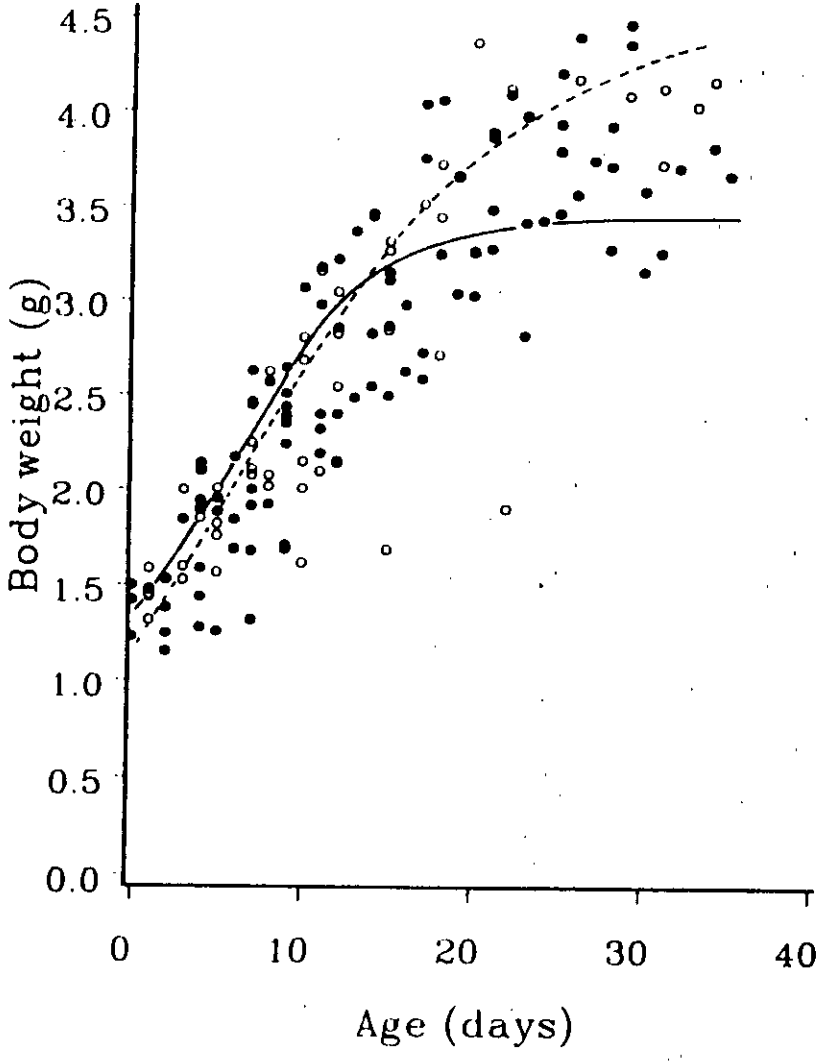
During the 76-day period of the study, 10 bats from Group 1 (67%) and 7 bats from Group 2 (44%) died. There was no significant difference between the number of bats which survived from each group (Fisher's Exact Test, P=0.285). Most mortality occurred in both groups in the first 15 days, which was immediately before the birth period (Figure 1.1). This mortality was closely correlated with a period of high rainfall which, combined with the unfamiliarity of the enclosure to the bats at this early stage of the experiment, was probably the main cause of this early mortality.

1.4.2 Survival and growth of young

Some adults had died before the period of parturition began, but of those females which survived, 7 (100%) from Group 1 and 8 (80%) from Group 2 gave birth. In total 8 (5 males: 3 females) and 11 (7 males and 4 females) young were born in Groups 1 and 2 respectively, there being 1 set of twins from Group 1 and 3 sets from Group 2. The mean dates of parturition were 23.0 ± 1.9 for Group 1 and 23.5 ± 1.0 for Group 2. These dates were not significantly different (P>0.05) from each other, but the overall mean date of parturition was significantly earlier (t=2.762, df=34, P<0.01) than had been found in a sample of females from the same colony in the previous year (Boyd & Myhill, in press). One adult from Group 1 died during parturition.

Growth curves were fitted to body weights and radius lengths of young using the Gompertz model (Zullinger et al, 1984) and the means of the parameters of this equation are given in Table 1.1 (Figure 1.2). Boyd & Myhill (in press) considered that the growth of males did not differ from that of females, so both sexes were combined in this study. The estimated asymptotic size (A), the age at the inflection point (I), and the growth rate constant (K), were not significantly different between the 2 groups except for the growth rate constant (K) for body weight which was greater for Group 1 than for Group 2, suggesting that young in Group 1 grew faster overall. However, the shape of the growth curve of body weight in Group 1 was influenced by outlying points (Figure 2.1) and may not be

Figure 1.2 The body weight and radius length of newborn bats related to age for young born in Group 1 (circles) and Group 2 (dots). Curves were fitted by least squares regression using a Gompertz growth model (Zullinger et al, 1984). Dashed lines show average growth in Group 1 while solid lines show average growth in Group 2.



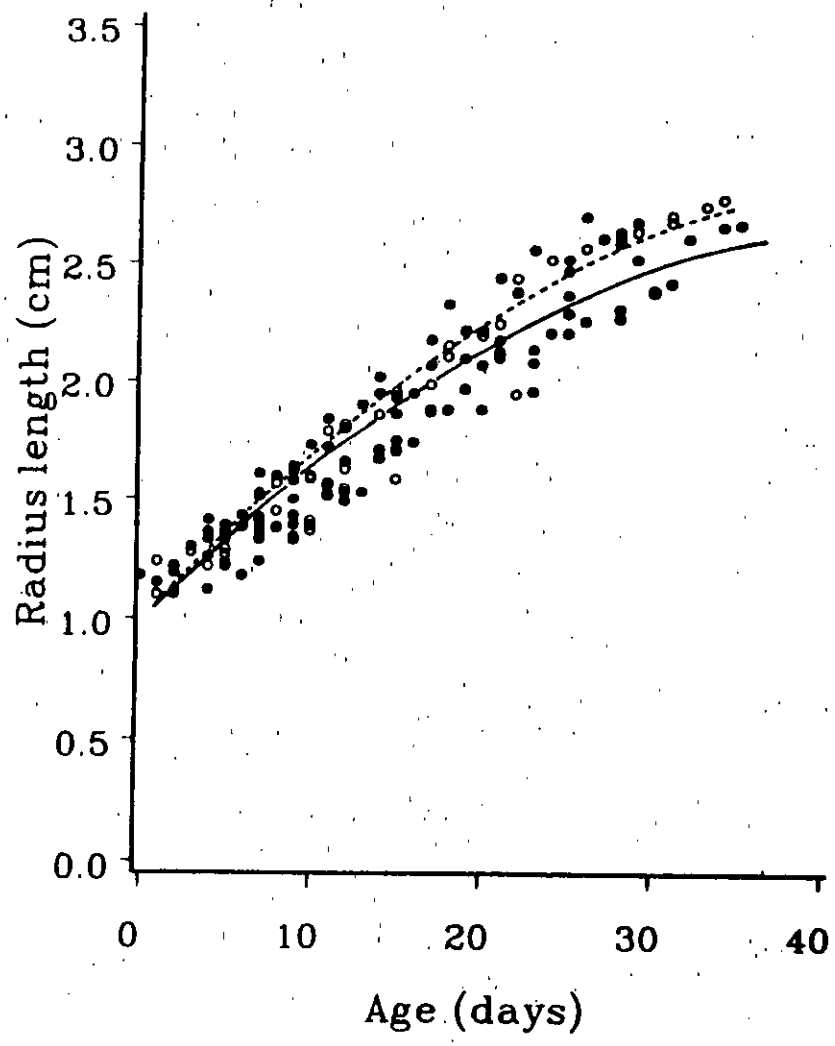


Table 1.1 Arithmetic mean for each of the parameters for Gompertz growth models fitted to the growth for each bat from the 2 groups by least squares regression.

		Group 1			Group 2					
	Parameter	Mean	SE	n	Mean	SE	n	t	df	P
radius len	A	2.944	0.228	5	3.367	0.189	11	1.316	14	>0.05
	K	0.055	0.005	5	0.050	0.004	11	0.729	14	>0.05
	I	0.728	2.609	5	3.564	1.147	11	1.174	14	>0.05
body mass	A	3.317	0.494	5	4.621	0.401	11	1.904	14	>0.05
	K	0.186	0.040	5	0.096	0.010	11	3.011	14	<0.01*
	I	0.458	1.009	5	4.377	1.587	11	1.576	14	<0.05

* denotes significance

Table 1.2 Arithmetic mean for each of the parameters for Gompertz growth models fitted to the growth data for each bat from the current study and from a previous study (Boyd & Myhill, in press).

		1986			1987					
	Parameter	Mean	SE	n	Mean	SE	n	t	df	P
radius len	A	3.214	0.106	21	3.235	0.152	16	0.117	35	>0.05
	K	0.116	0.007	21	0.052	0.003	16	7.566	35	<0.00*
	I	6.452	0.877	21	2.678	1.134	16	2.677	35	<0.025*
body mass	A	4.215	0.126	21	4.214	0.344	16	0.003	35	>0.05
	K	0.206	0.013	21	0.125	0.017	16	3.855	35	<0.001*
	I	5.425	0.638	21	3.153	1.208	16	1.774	35	>0.05

* denotes significance

Table 1.3 Number of bats surviving to weaning (30 days) or dying before weaning in a group exposed to permethrin (Group 2) and a control group (Group 1).

	Group 1	Group 2
No. Surviving	3	11
No. dying	5	0

representative. The average growth pattern was similar to that described for bats from the same colony in the previous year only in terms of the asymptotic size (A) (Table 1.2). Otherwise, there were significant differences in the pattern of growth between years.

Survival of young bats to weaning age (c.30 days) was significantly better in the group exposed to permethrin (Group 2) than in the controls (Group 1) (Table 1.3). This was partly a consequence of a higher mortality of mothers in Group 1 than Group 2 leading to a higher mortality of the young bats. After weaning, further mortality occurred because of difficulties in training young bats to feed on mealworms. This meant that only 3 (1 from Group 1 and 2 from Group 2) of the young bats survived to the end of the experiment.

Therefore, the pattern of survival was similar in the 2 groups and there was no evidence that exposure of young bats, or their mothers, to wood treated with permethrin caused premature death or a reduced growth rate.

1.4.3 Growth and food consumption of mothers

There was no significant difference between the body weights of females in the 2 groups throughout the study (Figure 1.3), although body weight did vary significantly with time in both groups (Group 1, $F(14,74)=4.34$ $P<0.001$; Groups 2, $F(14,131)=8.97$, $P<0.001$). The initial increase in weight during the first 15 days was partly caused by death of some lighter bats during this time (Figure 1.1) but there was also a general weight increase in those which survived. There was rapid weight loss through the period of parturition (Figures 1.1 & 1.2). Most newborn pipistrelles weigh about 1 g at birth and, together with loss of fluids and the placenta, this will largely account for the reduction in maternal weight from about 8 to 6 g through the period of parturition. During lactation, maternal weights stabilised at about 6.5 g, although there was a slight significant ($F(8,77)=3.84$, $P<0.001$) increase in the weight of bats in Groups 2 through lactation.

The variation in food consumption through the study was similar in the 2 groups and was not significantly different at any stage of the study (Figure 1.4). Food consumption began to increase after day 15 from 2 to 4 g/day/bat between Days 35 and 45. After Day 45, food consumption began to decline. This shows that food consumption increased at a roughly constant rate in both groups during the first 20 days of lactation and then stabilised during the last 10 days. This may reflect the energetic stress on mothers during lactation.

1.4.4 Activity patterns

The method used to measure activity was probably most indicative of feeding activity because the balance recorded arrivals at, and departures from the feeding site. This suggested that feeding activity, as would be expected, occurred between dusk and dawn, although there was a slight increase in activity during the early afternoon (Figure 1.5). This could have been caused by observer distrubance, though the analysis ignored the hour each day in which observations were made.

For the purposes of analysis, the activity record was divided into 3 parts for each group. The first of these (Days 1-18) showed

Figure 1.3 The mean (± SEM) body weight of bats from Group 1 (circles) and Group 2 (dots) during the study.

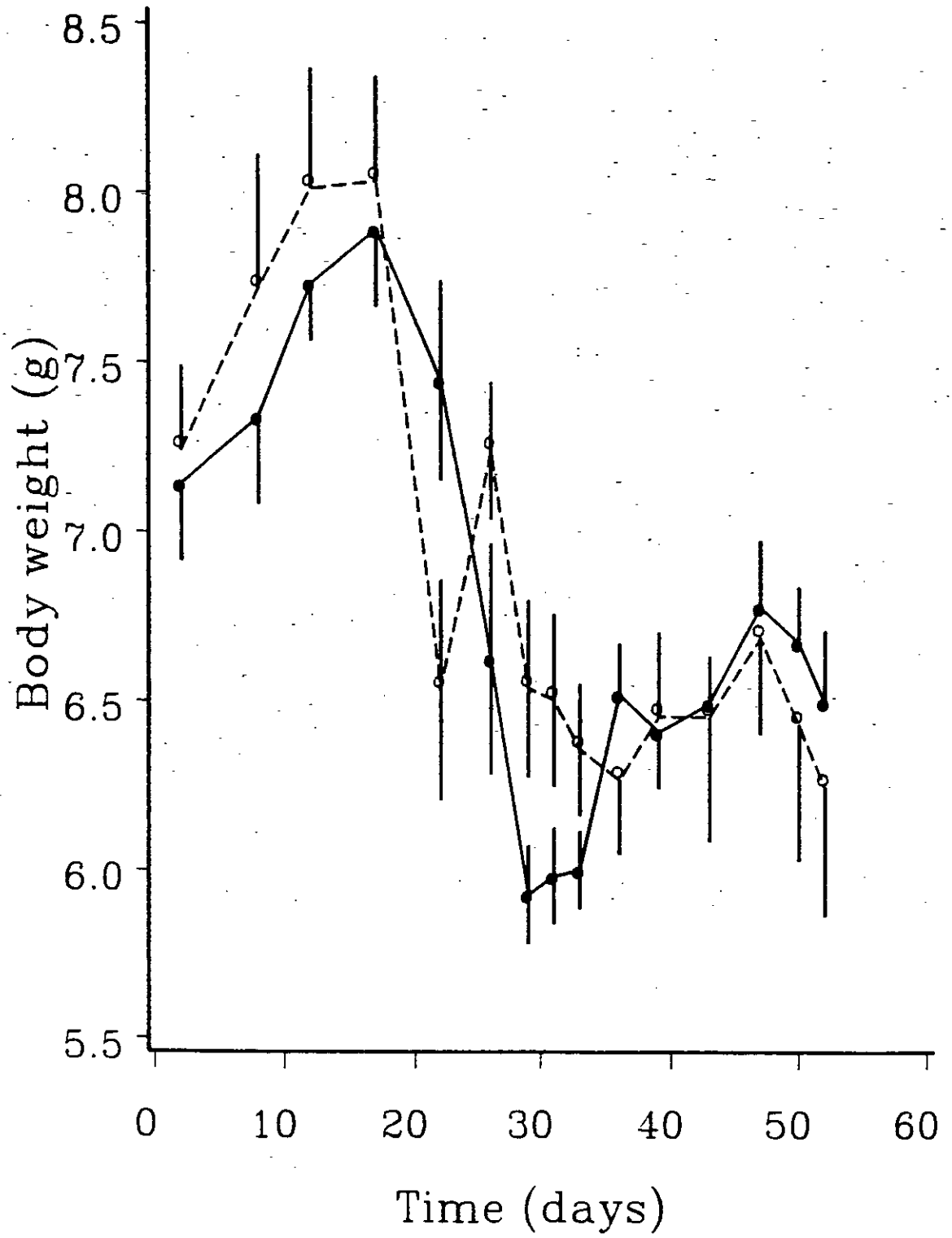
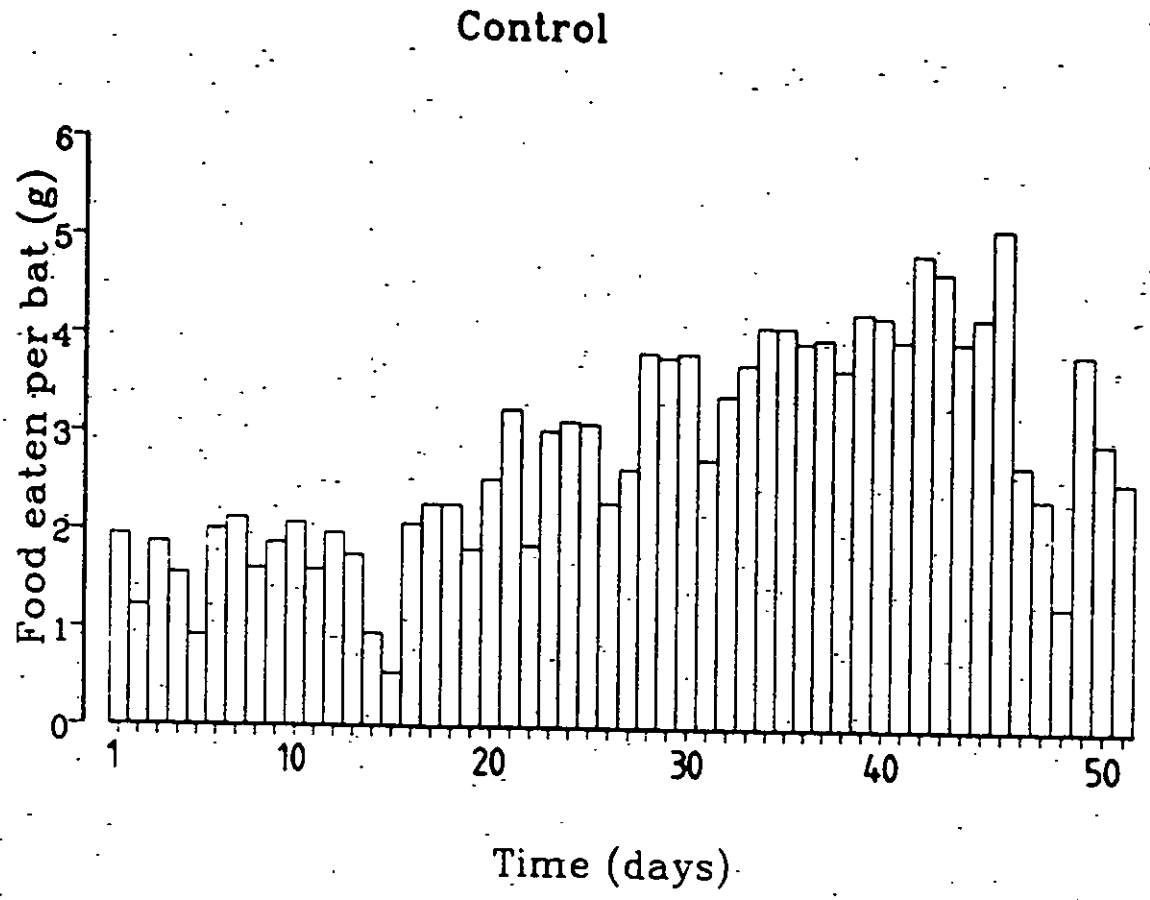


Figure 1.4 **Variation in the food consumption of bats from Group 1 (controls) and Group 2 (Permethrin) during the study.**



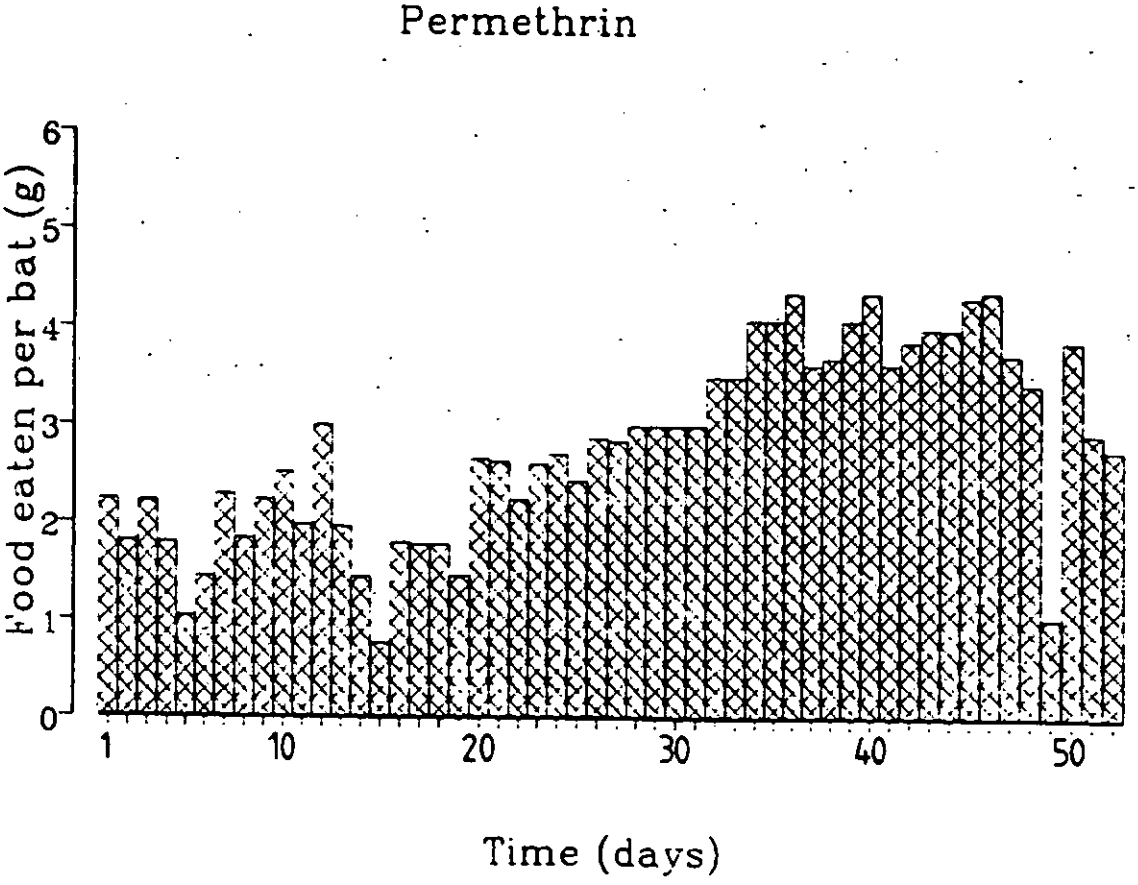
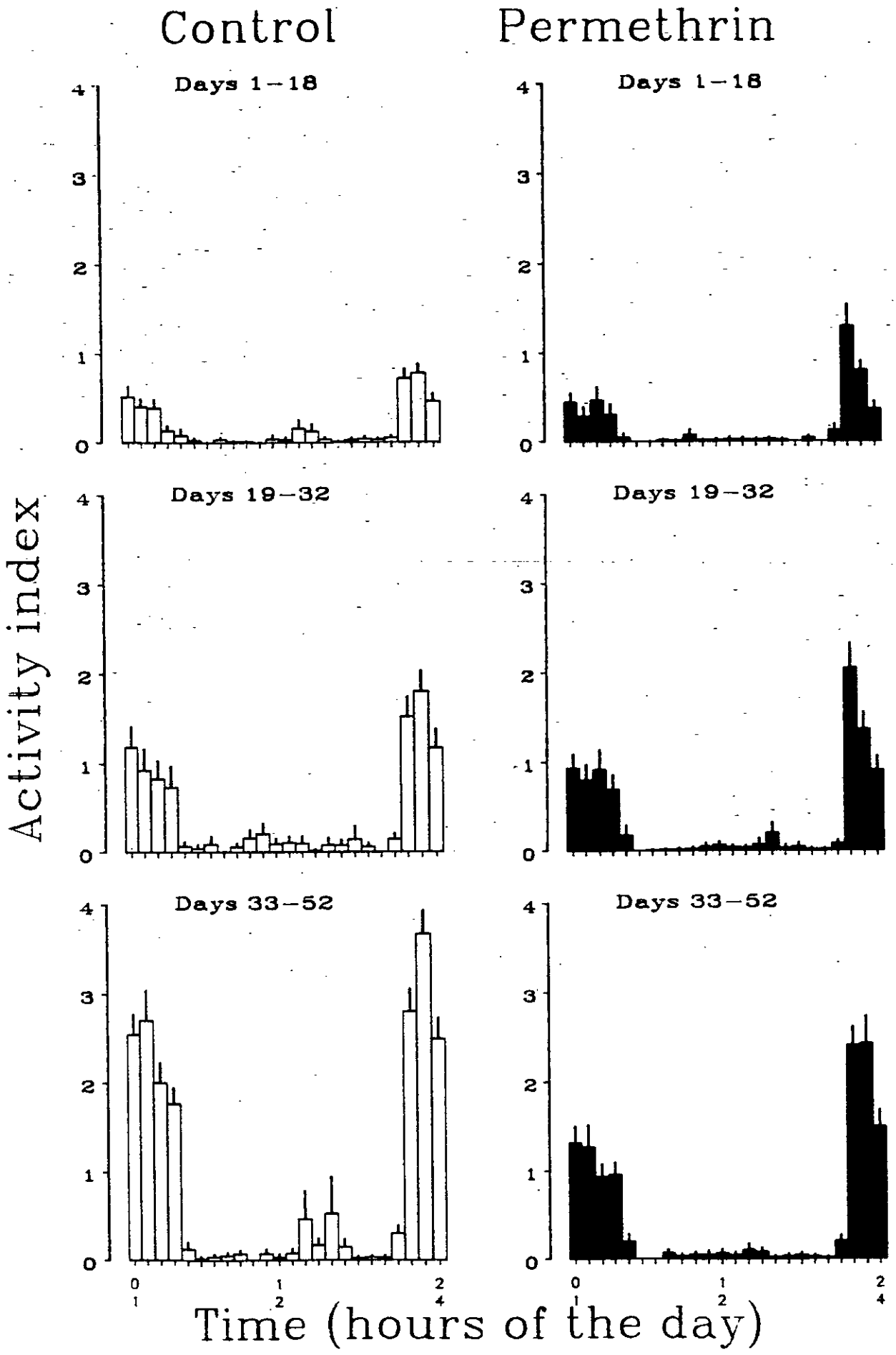


Figure 1.5 Acitivity index (see text for explanation) for bats from Group 1 (control) and Group 2 (Permethrin) during 3 phases of the study representing the period before birth (Day 1-18), while births were taking place (Days 19-32) and for the remainder of lactation (Days 33-52).



activity during the late stage of gestation while the second (Days 19-32) showed activity during the birth period, which includes early lactation for most bats (Figure 1.1), and the third (Days 33-52) shows activity during the later stages of lactation. During the first and second periods (Days 1-18 and 19-32) there was no difference during any hour of the day between the activity of the 2 groups, but during the third period (Days 33-52) there was significantly more activity in the controls than in the dosed group (Figure 1.5) between hours 23 and 03 ($P<0.05$). Otherwise the pattern of activity in the 2 groups was similar.

The total activity was lowest in late pregnancy, higher during the birth period and early lactation and highest later in lactation (Figure 1.5). There was a bimodal pattern of activity during Days 33-52 in the controls (Group 1) and some signs of bimodality occurred in other periods for both groups. This is shown by the extended tail-off in activity between midnight and dawn (c.04.00). This would occur if the large first peak in activity after dusk was followed by a similar peak before dawn.

1.5 Discussion

This study showed no effect of permethrin on the behaviour and survival of pipistrelle bats. Using a similar experimental design, Boyd (1987) demonstrated that lindane caused reduced survival in pipistrelles. However, it was unfortunate that, in this study, bats in the control group died during the experiment, suggesting that the animals were stressed by their captivity. Racey and Swift (1986) also had this problem, which stems partly from the difficulties associated with keeping bats in captivity (Racey, 1970). There were several possible reasons for the deaths of bats in the first 2 weeks. Previous experience has shown that, even after being trained to feed and adapt to captivity in cages, some bats take several weeks fully to adapt to living in outdoor enclosures. In the 2 weeks following the start of the experiment, there was heavy rain which resulted in some bats becoming wet. In general, it was these individuals which died during the early part of the Experiment. There was no evidence that these individuals were diseased, but many were pregnant.

This study has confirmed the results of Racey and Swift (1986) that permethrin is significantly less toxic to bats than most other organochlorine insecticides. It also agrees with the LD_{50} tests on other mammals (Worthing, 1987), which show a relatively low toxicity of permethrin, and it suggests that these tests give a good indication of the relative toxicity of these chemicals in bats.

As with lindane (Boyd, 1987) there was little indication that permethrin affected behaviour. However, bats in the control group showed a higher level of activity during the main phase of lactation, but this may have been an artefact caused by the different number of bats in the 2 groups at this stage of the Experiment. Fewer bats were present in the control group and, if there was competition for space at the feeding point, then the activity per bat may have been reduced in the group with the highest density (Group 2). Alternatively, the method of recording could not distinguish between bats which arrived at, or departed from, the feeding point simulaneously. This could have artifically reduced the activity recorded per bat for Group 2.

Swift (1980) also observed a bimodal distribution of activity in wild pipistrelles during lactation. She concluded that the bats were adapting their activity to the dusk and dawn peaks in insect availability.

Between the peaks, mothers returned to suckle their young. In this study, food was always available and there was still a bimodal distribution of activity, albeit much reduced in the second peak. This suggests that, during lactation in these bats, the rate of milk production and the rate of digestion of food may be linked to the availability of food at dusk and dawn.

Clark and Krynitsky (1978) suggested that organochlorines could affect the growth and survival of young little brown bats (Myotis lucifugus). Boyd and Myhill (in press) have described the comparative neonatal growth of pipistrelles and showed variation between colonies in the growth of young which may reflect different feeding conditions for mothers before birth. Some parameters of the model fitted to the data for growth of young born in this study were not significantly different from those defined by Boyd and Myhill (in press). There was no effect of permethrin treatment on the growth or survival of young. Boyd and Myhill observed that bats which gave birth late had young with high growth rates compared with bats which gave birth early. This trend was confirmed in this study where bats gave birth earlier than in the previous year and there was a concomitant low growth rate of the young.

Evidence suggests that permethrin is a safe insecticide for use in bat roosts. Providing its insecticidal properties are as potent as those of lindane, it should be preferred to lindane as an insectide for use on structural timber, not only because of its lower toxicity to mammals and birds, but because it has a low vapour pressure, making it likely to be effective for longer, and reducing the required frequency of wood treatment.

2. SENSITIVITY OF BATS TO PESTICIDES

A considerable number of field studies have related the presence of pesticides in the tissues of bats to the use of these chemicals in the environment. Clarke (1981a) reviewed much of this literature, but no author has yet provided a synthesis by which the effect of pesticides on bat populations may be modelled. Sensitivity of bats to pesticides may be determined in several ways; the ability of bats to detoxify a pesticide, thereby affecting its rate of accumulation in the tissues; the direct toxicity of a pesticide, or, at the level of the population, how rapidly a population will return to its equilibrium size after mortality of some bats due to pesticide poisoning. The ultimate questions asked by those involved in conserving bat populations relate more to the survival of populations rather than individuals. Whilst studies of pesticide toxicity to individuals can often lead to sensible inferences about the effects on populations, there is a need to place studies of individuals in the context of population dynamics.

This section of the report examines the sensitivity of individuals to pesticides by describing the annual cycle of fat deposition in bats and by examining the detoxication of pesticides in a pilot study using an in vitro technique.

2.1 Sensitivity of individuals to pesticides - annual cycle of fat deposition in pipistrelle bats

2.1.1 Summary

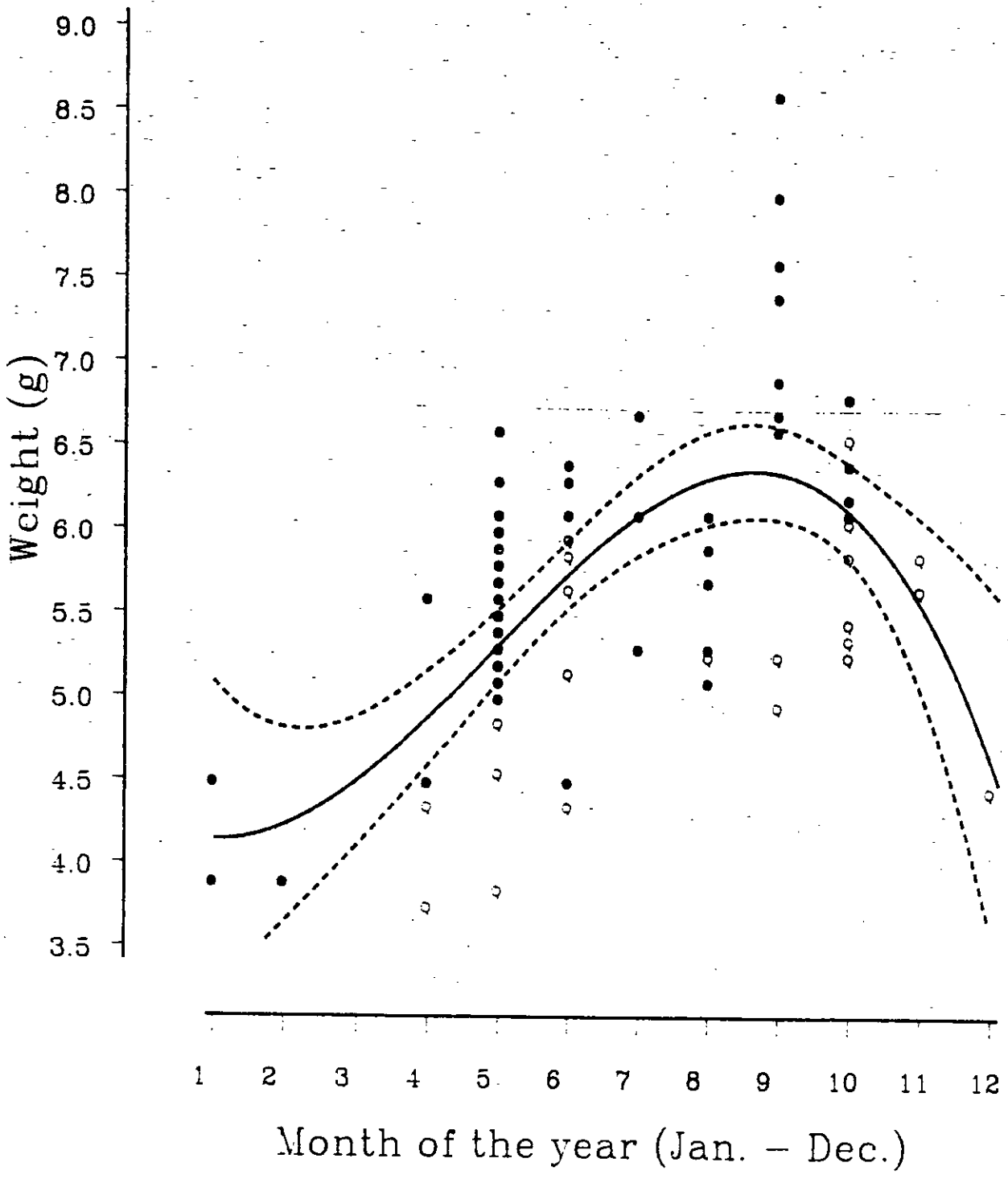
Weights of pipistrelles caught at different times through the year or kept free-flying in captivity, showed an annual cycle of body weight from a minimum of about 4.5 g at the end of hibernation to over 6.0 g immediately before hibernation. Carcass analysis of bats showed a relationship between body weight and weight of fat, so that, above 4.8 to 5.5 g, there was a 1:1 relationship between body weight and fat weight. This implies that the annual cycle of weight is indicative of levels of fat in the body which has important implications for the ability of bats to resist poisoning by fat-soluble pesticides and for the temporal relation between exposure to pesticides and toxic effects.

2.1.2 Introduction

Many pesticides are highly lipophilic molecules which mainly partition into the fatty tissues. It is generally recognised that large depots of storage fat can buffer animals against the toxic effects of these pesticides by absorbing most of the pesticide in the body, thus reducing the pesticide concentration in target tissues, particularly the brain (Clark & Kroll, 1977; Clark, 1981b). Therefore, individuals in good condition, as measured by body fat content, will be most resistant to pesticide poisoning. However, high levels of body fat will allow pesticides to accumulate and there will be released when the fat is metabolised.

In seasonal environments, episodic reproduction and hibernation can place widely varying metabolic stresses on individuals. Many mammals show seasonal cycles of fat deposition (Boyd & Myhill, 1987) which can be indicative of these metabolic stresses and which will cause seasonal variation in the ability of individuals to resist exposure to pesticides. The aim of this study was to describe changes in the fat content of pipistrelle bats through the year and to assess the consequences of these changes for pesticide toxicity.

Figure 2.1.1 The weight of adult pipistrelle bats caught in the wild at different times of year (some data supplied by R E Stebbings). Females are represented by dots and males by open circles. Pregnant females caught in June were excluded. The curve is a cubic polynomial fitted to the data by least-squares regression. Dashed lines show the 95% confidence limits of this curve. The regression equation was : weight = 4.300 - 0.294 month + 0.152 month^2 - 0.010 month^3.



2.1.3 Materials and Methods

All bats used in the study were captured under NCC licence at sites in eastern England. Bats were captured in the wild over a number of years, but bats used for carcass analysis were collected during 1986 and 1987. Some of these were kept free-flying in captivity for up to 6 months as part of other studies before analysis.

Bats captured in the wild were weighed (accuracy 0.1 g) before being released and these weights were used to construct a model of the annual cycle of body weight of bats, using a least squares cubic polymonial regression.

Bats which were used to study the weight changes in individuals over an extended period in captivity were eventually sacrificed for carcass analysis to provide an indication of fat content in relation to body weight. Some of these bats died in captivity, but they were also included in the analysis because the causes of death were unlikely to affect any relation between body weight and fat content. Bats were weighed immediately after death and dissected to remove all subcutaneous adipose tissue located mainly in the abdominal and lumbar regions. The interscapular brown adipose tissue was also easily dissected as a discrete mass. The skin was removed and these tissues, together with the liver and the remainder of the cracass, plus the skin, were weighed separately (accuracy 0.001 g). The whole carcass was then ground with sand (3-10 g) in a clean beaker with a glass rod until completely macerated. Anhydrous sodium sulphate (2-7 g) powder (conditioned at 700°C for 5 h) was added and mixed in to absorb water. This mixture was extracted with 5 ml aliquots of distilled hexane over a period of 30-60 minutes with 50 ml hexane. Extracts were allowed to settle for at least 2 h, after which 25 ml of each was pipetted into a pre-weighed (accuracy 0.0001 g) glass vial. The hexane was evaporated with a continuous stream of air over the top of each vial for 3 days before the vials were reweighed. The weight difference was taken to be the weight of half the extractable lipid in the tissue.

2.1.4 Results

Weights of bats caught at different times of year showed a significant variation ($F_{10,86}=34.66$, $P<0.001$) with a minimum of 4.5 g in February-March and a maximum of over 6.0 g in August - October (Figure 2.1.1). Few measurements were available for the hibernation period (October to March) when pipistrelles are not often caught. A cubic polynomial model gave a significant fit to the data ($r^2=0.355$, $P<0.01$).

There was a significant positive correlation between the weight of extractable fat and the weight of subcutaneous adipose tissue ($r=0.775$, $P<0.001$) and brown adipose tissue ($r=0.676$, $P<0.001$), but there was no correlation between liver weight and weight of extractable fat ($r=0.259$, $P<0.06$). Changes in body weight may be attributed to changes in the size of the fat and non-fat portions of the carcass. Given that the increase in fat weight cannot be greater than the increase in body weight, the relationship between body weight and fat weight will approach linearity, with slope of 1, if changes in body weight are caused by changes in fat weight alone. However, the relationship between fat weight and body weight was not simple (Figure 2.1.2). At weights above 4.8 to 5.5 g, fat

Figure 2.1.2 The total weight of dissectable fat (subcutaneous fat weight plus brown fat weight) related to body weight. The linear model fitted to the data when body weight was less than 5.3 g was : fat weight = 0.051 body weight - 0.127 (r^2=0.086). The model when body weight was greater than 5.3 g was fat weight = 0.540 body weight - 2.688 (r^2=0.600).

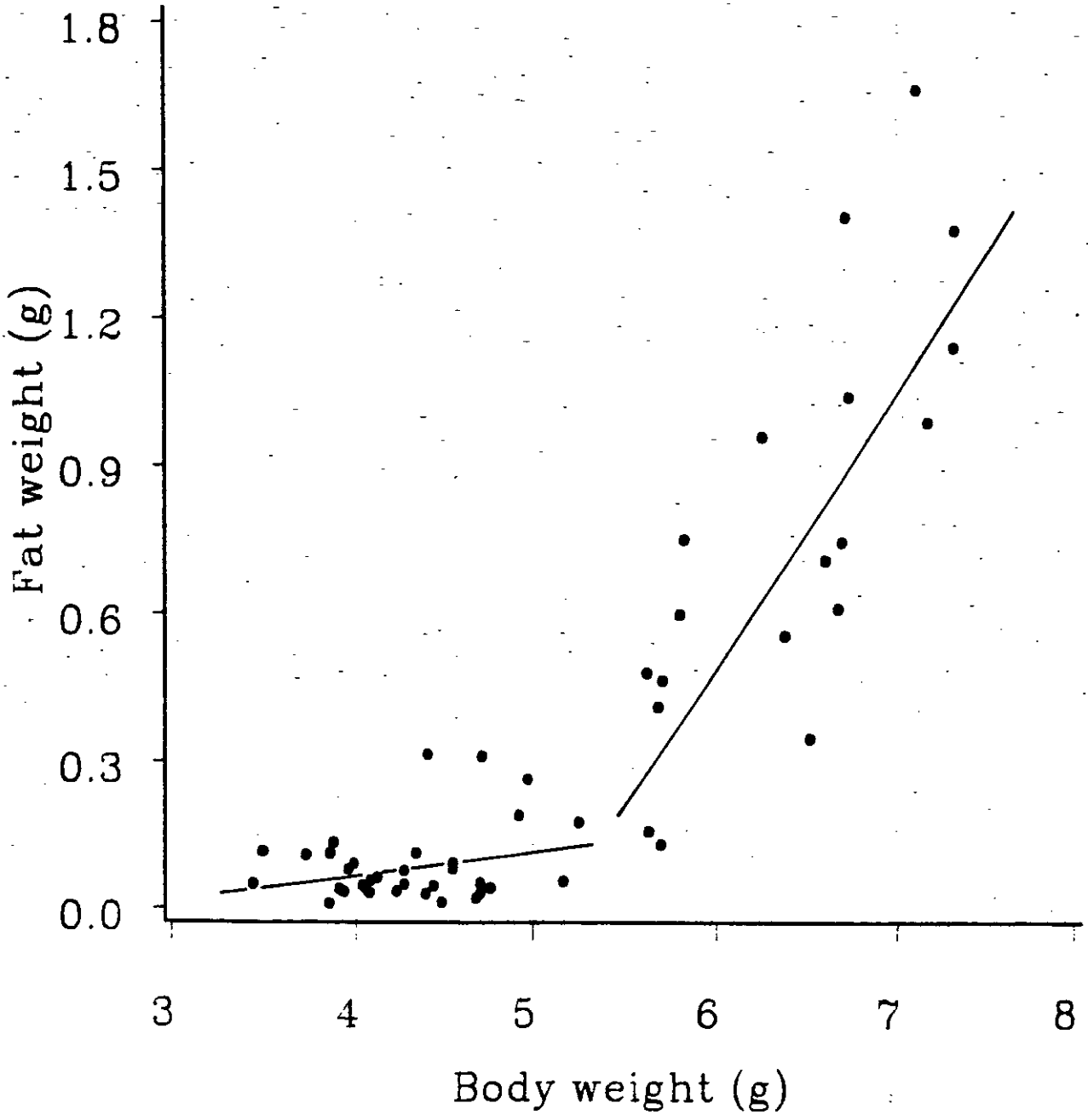


Figure 2.1.3 The weight of dissectable subcutaneous fat related to body weight.

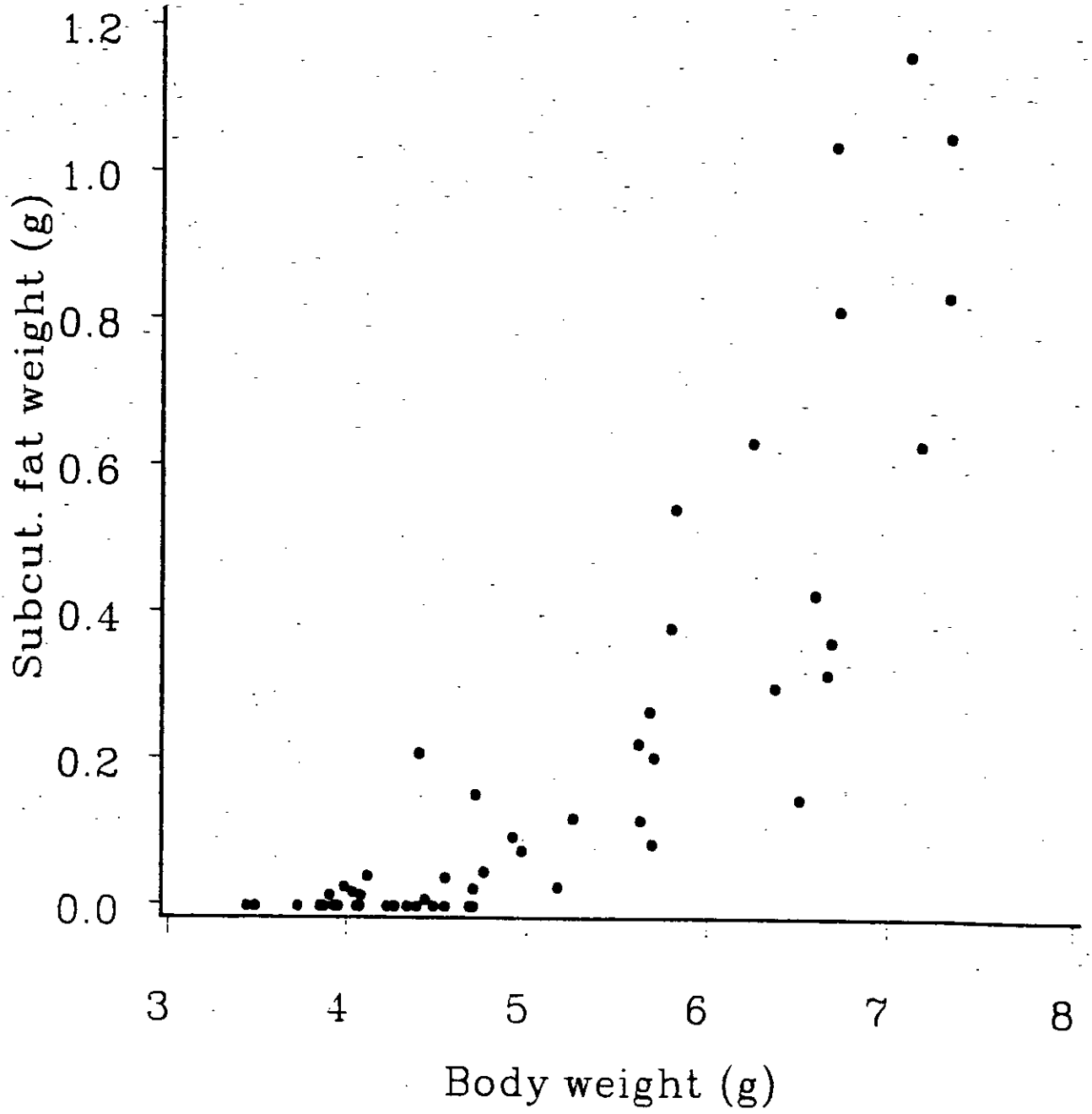


Figure 2.1.4 The lean body weight (body weight - subcutaneous fat weight - brown fat weight) related to body weight. (Lean body weight = 0.682 body weight + 1.273, r^2=0.931).

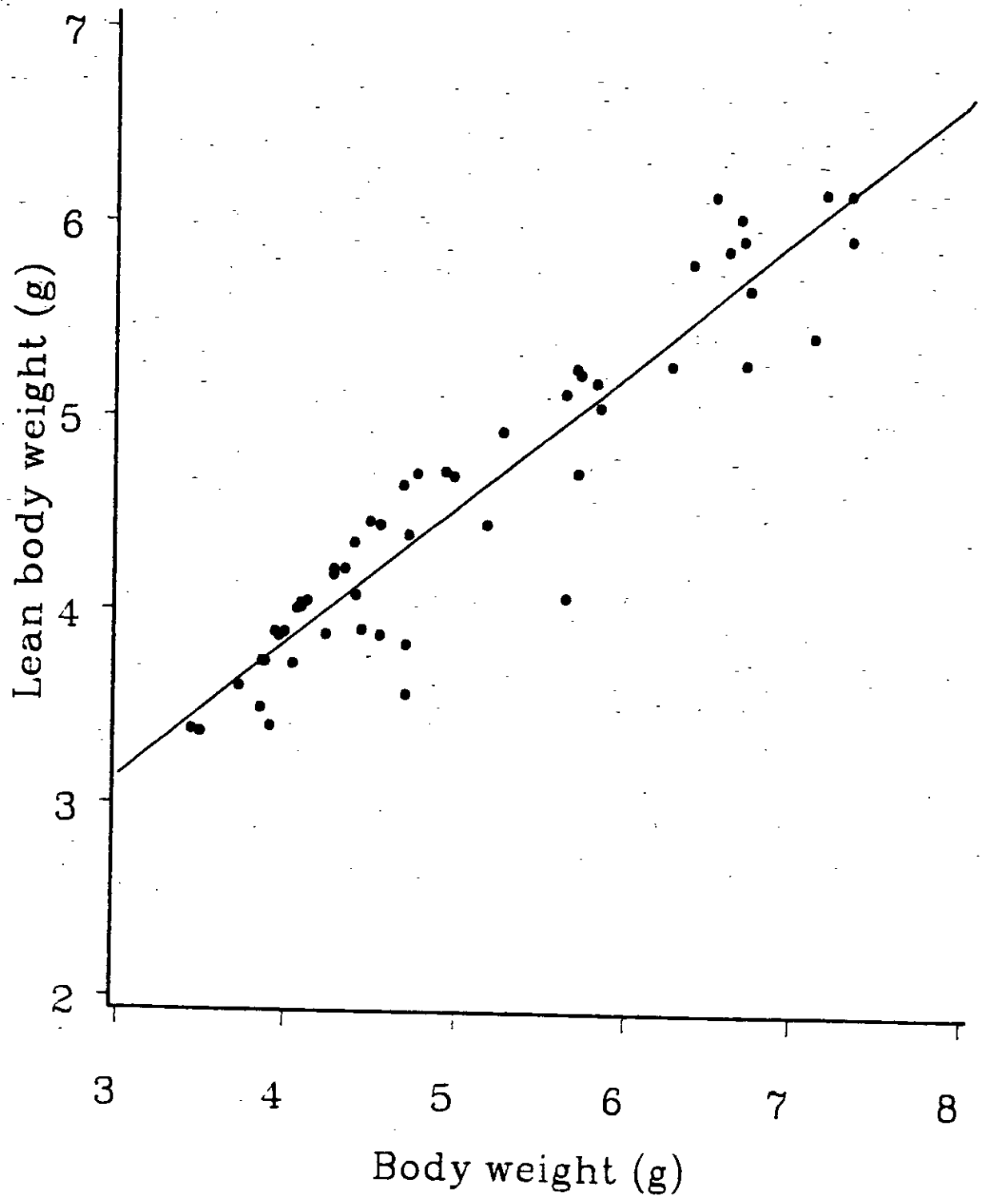


Figure 2.1.5 The liver weight related to body weight. (Liver weight = 0.053 body weight −0.025, r^2=0.553).

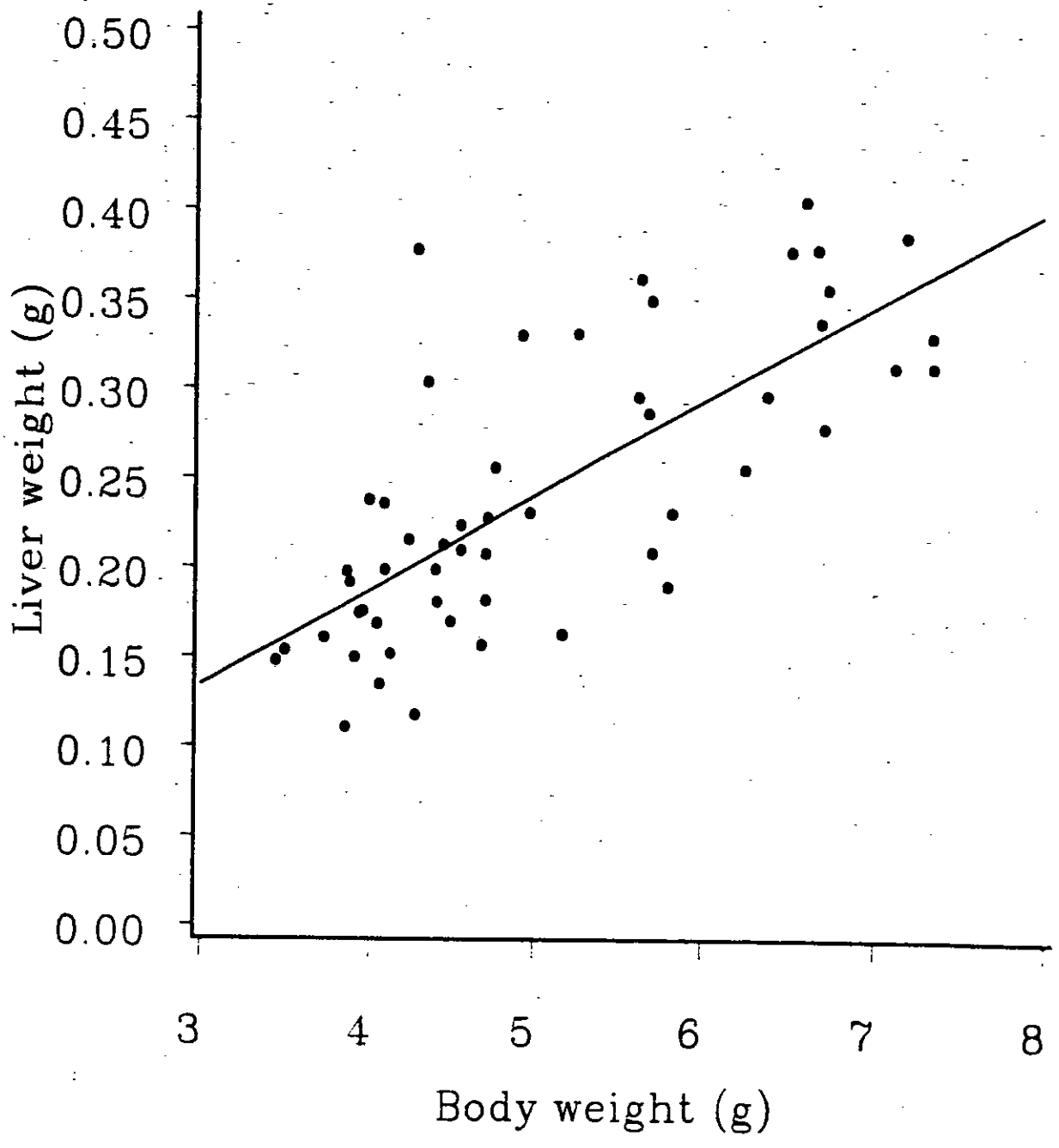
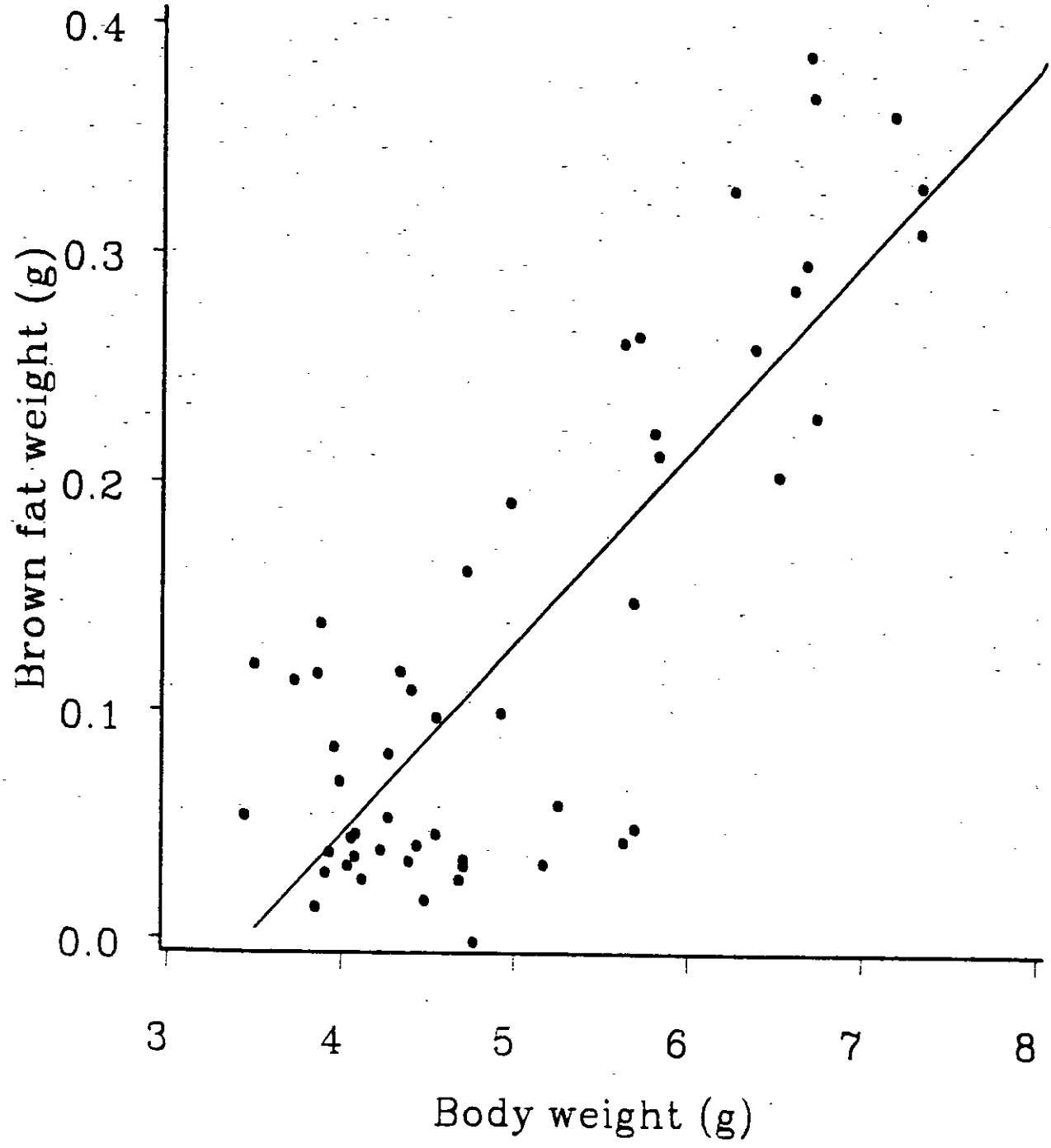


Figure 2.1.6 The brown fat weight related to body weight. (Brown fat weight = 0.090 body weight -0.315, r^2=0.685).



weight increased roughly in proportion to body weight but below this, fat weight was roughly constant and approached zero. There was a transition between growth of the non-fatty and the fatty portions of the carcass. A linear regression was used to model the relationship between body weight and fat content for carcasses weighing greater than 5.3 g. While this weight was chosen arbitrarily, it was found to give the best fit linear model compared to other models using higher and lower weights as the cut off.

This pattern of fat weight in relation to body weight was largely a consequence of changes in the weight of subcutaneous fat (Figure 2.1.3). The lean body weight (body weight - subcutaneous fat weight - brown fat weight), liver weight and brown fat weight increased linearly with body weight (Figures 2.1.4, 2.1.5., 2.1.6).

2.1.5 Discussion

These data show that there is a significant degree of variation in the quantity of fat in pipistrelle bats through the year which will mean that the ability of individuals to resist poisoning by pesticides will also vary through the year. Even during the summer, there was considerable variation in the fat weights, showing that some bats would be more resistant to pesticide poisoning than others. Different pesticides are metabolised at different rates (Walker, 1975) and the dynamics of fat deposition will form a complex interaction with the dynamics of pesticide detoxication.

In the case of pesticides, such as dieldrin and the DDT metabolite, DDE, which have a low rate of detoxication in mammals (Walker, 1975), bats may be able to accumulate amounts of these pesticides during the summer, when many different roost sites may be used, without any apparent effect on behaviour or survival, but with the reduction of fat reserves through hibernation (October to March), the concentration in target tissues will increase rapidly and could reach lethal levels. This is significant because, deaths due to pesticide poisoning will probably occur in places remote from where pesticides have been used. Clark and Krymitsky (1983) found higher levels of DDE in brown fat than white fat. Reduction of white fat through hibernation will then lead to accumulation of pesticides in brown fat. Since the function of brown fat is to produce heat by the rapid metabolism of triglycerides at arousal, bats may be particularly vulnerable to poisoning by organochlorines at the end of hibernation when brown fat is metabolised.

While fatty tissue cannot be viewed as metabolically inert, it may act to increase the accumulation of pesticides, such as gamma-HCH, which can be metabolised fairly rapidly (Walker, 1975), by increasing the half-life of the pesticide. Hence, Boyd et al (in prep) found higher total weights of gamma-HCH in bats which survived dosing with gamma-HCH than in those which died, mainly because the survivors had large amounts of fat giving them low concentrations of gamma-HCH in tissues.

2.2 Metabolism of Pesticides

2.2.1 Summary

This pilot study was to investigate a method of assessing the capacity of bats to metabolise pesticides (using an in vitro technique). The method involved a modified aldrin epoxidase assay

which was developed on vole liver microsomes; it has to be tested in bats.

2.2.2 Introduction

The detoxication of pesticides usually takes place in the liver. Often, the dose of pesticide which an animal can endure is determined by the rate at which it is metabolised by the liver so that, in the case of chemicals such as dieldrin or PCB's where metabolism is slow, accumulation will occur and a lethal concentration can soon be reached, even with moderately low levels of exposure. The usual method of detoxication is to change the liposoluble pesticide into a derivative which is water-soluble by an oxidation step, involving the hepatic monoxygenase enzymes (Walker & Zorgani, 1974; Portig & Koransky, 1977; Chipman & Walker, 1979). Dechlorination and conjugation with glutathione, a co-factor to the dehydrochlorinase enzyme, is also involved in the metabolism of some pesticides, such as DDT, DDE and gamma-HCH (Walker, 1975). The water-soluable metabolite can then be excreted via the kidneys.

While it is always desirable to know how a toxicant will affect all aspects of the biology of an animal, such as a bat, it is sometimes more productive in the short-term to consider fundamental aspects of the biochemistry of pesticide metabolism. To this end, several authors (Walker & Zorgani, 1973; Portig & Koransky, 19771 Chipman & Walker, 1979; Knight et al 1981) have described methods for extracting enzymes from liver by isolating them in-situ on the microsomal fraction. These microsomes will contain most of the enzymes responsible for pesticide detoxication and provide an opportunity to study the ability of animals to metabolise pesticides in vitro. This has some advantages over whole-animal studies. For example, it is possible to carry out several assays on the same individuals; from knowledge of the total microsomal weight, it is possible to calculate the total capacity of individuals to metabolise a pesticide; it is also possible to compare the ability of different species to metabolise different pesticides under standard conditions and it provides a rapid, cheap method to screen for pesticide sensitivity in many species of wildlife.

Bats are unusual mammals because they are heterothermic. This allows them to reduce their metabolic rate while inactive, which has clear energetic advantages, but it also has possible implications for their ability to metabolise pesticides. The activity of the hepatic enzymes responsible for detoxication may be maximal for the few hours each day when bats are feeding, digesting or suckling. Conversely, bat liver enzymes may be effective over a wide temperature range. It has also been suggested that bats are inherently less able to metabolise pesticides than other mammals (Litterst et al, 1974).

The purpose of this study was to test the hypothesis that the capacity of bats to metabolise pesticides is determined by their diurnal cycle of body temperature, and also to compare their metabolic capacity with that of another small mammal, the bank vole (Clethrionomys glareolus) at normal active body temperatures. This made use of an in vitro aldrin epoxidase assay modified to be performed on small amounts of liver microsomal tissue.

2.2.3 Material and methods

Preparation of liver microsomes

Voles were killed by cervical dislocation and then weighed. The liver was removed immediately after death and cooled to 4°C. All procedures were carried out in a cold room at 4°C, or in apparatus cooled to 4°C. Following weighing (accuracy 0.001 g), the liver was chopped finely with scissors and homogenised in 1.15% KCl. This was then centrifuged at 11,000 g for 30 minutes to deposit cell debris and organelles. The post-mitochondrial supernatant was removed and centrifuged at 104,000 g for 1 hour. The resulting pellet of microsomes was re-suspended in KCl using a glass homogeniser, and microsomes were washed by centrifugation at 104,000 g for a further 30-40 minutes. The pellet was then resuspended as before to a concentration of 2 g liver/ml.

Aldrin epoxidase assay

Monoxygenase activities of microsomal preparations can be determined using aldrin as a substrate. This highly liposoluble compound partitions almost completely into the microsomal membrane, and it is transformed to its stable metabolite dieldrin. Substrate concentrations were calculated in terms of nmol aldrin/mg microsomal protein as an estimate of the substrate concentrating on contract with the enzyme.

Incubations took place at 20 and 39°C with a combination of microsomes at different dilutions, a reaction mixture and a generating mixture. The reaction mixture consisted of 0.1725 g nicotinamide, 0.26 g KCl and 2.145 g NaH PO_4 (all obtained from Sigma Cemical Co., Fancy Road, Poole, Dorset, UK) made up in 250 ml of distilled water and adjusted to pH 7.4. The generating mixture consisted of 19 mg NADP, 33 mg glucose-6-phosphate and 20 µl (c.32 units) glucose-6-phosphate dehdrogenase (all obtained from BCL, Bell Lane, Lewes, East Sussex, UK) in 1 ml distilled water. Fifty microlitres of each microsomal dilution (5 serial dilutions from 50 to 3.125 µl made up to volume with reaction mixture) set up in replicate with a no substrate control of 25 µl microsomes and 25 µl reaction mixture, was placed in cooled test tubes and 0.43 ml of cold reaction mixture was added. Twenty microlitres of generating mixture was then added and all tubes were preincubated in a shaker bath, set to the required incubation temperature, for 90 seconds. Twenty microlitres 0.2 mg/ml aldrin substrate (dissolved in absolute alcohol) was then added to initiate the reaction. At this point, test tubes were also vortex mixed. After 3 minutes the reaction was terminated by adding 1 ml distilled hexane and vortex mixing. The supernatant was removed to a storage vial and the extraction was repeated twice. The concentration of dieldrin in the extracts was measured using gas chromatography.

2.4.3 Protein assay

The total amount of microsomal protein extracted from each liver was measured by the Lowry method. This involved a mixture of 3 reagents: (A) 150 ml distilled water containing 3 g sodium carbonate plus 0.6 g sodium hydroxide (0.4%); (B) 10 ml distilled water containing 0.1 g potassium-sodium tartarate plus 0.5 g copper sulphate (pentahydrate) or 0.032 g copper sulphate (anhydrous); (C) Folin-Ciocalteu reagent diluted 1:1 with distilled water. Reagents

were made up fresh for each assay and glassware was washed in a potassium hydroxide soak. Reagent B (3 ml) was added to 150 ml reagent A and stirred. Five millilitres of this mixture was added to 1 ml protein samples (replicate standard curves of 20-200 µg/ml bovine serum albumin plus unknown samples diluted to 1:100 and assayed in replicate). After mixing and leaving for 10 minutes at room temperature, 0.5 ml reagent C was added and mixed in. After a further 20 minutes, the absorbance was measured at 750 nm against a distilled water blank in a spectrophotometer. The unknown protein samples were then calibrated against the standard curve.

2.2.4 Results and discussion

Eleven assays of vole liver monoxygenase activity have been partially completed. However, the results, in terms of the quantity of dieldrin in the final extracts are not yet available because of the temporary absence of gas chromatographic equipment at Monks Wood. All other parts of these assay are complete. Each assay was carried out twice, one at 39°C and again at 20°C. The intention is to repeat these assays using liver microsomes from pipistrelle bats, thus giving a comparison between the activity shown by bats and voles at different incubation temperatures. From this we may be able to conclude whether bats have similar detoxication enzyme activity to voles and whether this activity is maintained at temperatures equivalent to ambient.

Future Developments

It is clearly early days for this work, but if the techniques described here prove to be satisfactory, they hold promise for future studies in vertebrate ecotoxicology. The method is a quick and cheap alternative to using large numbers of live animals in experiments and the results may be of more use in the long-term. It also has many benefits when rare species are concerned, because wide-ranging conclusions can be reached from studies of a small number of specimens.

One approach for the future would be to continue and expand the study as described above to include more species of British wildlife, particularly other bats, and to investigate the phenomenon of enzyme induction and its importance in ecotoxicology. Induction is caused by low chronic levels of exposure to a pesticide, such as DDE, which causes an increase in the monoxygenase enzymes in the liver. This in turn may allow a certain degree of resistance to subsequent acute exposure to other pesticides which are metabolised by the same enzyme system.

Another development would be to culture hepatocytes in vitro so that we would not require to sacrifice animals for these studies, and so that we would have an unchanging standard tissue on which to carry out tests. For example, it may be possible to culture hepatocytes from a rare species of bat and then have the capacity to test it for resistance to new pesticides which come on to the market.

2.3 Sensitivity of Bat Populations to Pesticides - Population Dynamics of Brown Long-eared Bats (Plecotus Auritus) in Bat-Boxes at Thetford Forest

2.3.1 Summary

A population of brown long-eared bats (Plecotus auritus), which occupied bat boxes at Thetford Forest, was studied for 10 years. Individuals were ringed and recaught at frequent intervals throughout their lives.

Immigration was probably a small proportion of the total recruitment, the remainder of the recruitment coming from reproduction within the population. A redistribution of the boxes over a wider area after 8 years had no effect on male recruitment and only a slight transient effect on female recruitment.

The total population increased during the study from 73 to 140 bats, giving a doubling time of approximately 10 years. The female population showed exponential growth with an intrinsic rate of increase of 0.072, while growth of the male population was asymptotic. The juvenile sex ratio was biased towards males during the first 5 years ($P<0.05$), but did not vary from unity thereafter. The overall sex ratio was biased significantly towards females in the first 4 years and the last 3 years of the study.

Estimates of survival excluded bats which may have emigrated. The annual survival rate, estimated from bats of known-age, showed female survival to be 0.86 after correction for the rate of increase and the uncorrected male suvival rate was 0.60. Annual survival rates for each year of the study were also obtained from a maximum likelihood estimate and these showed that the mean female survival rate for the period of study was 0.780 ± 0.035 (SEM) while the mean male survival rate was 0.623 ± 0.076 (SEM).

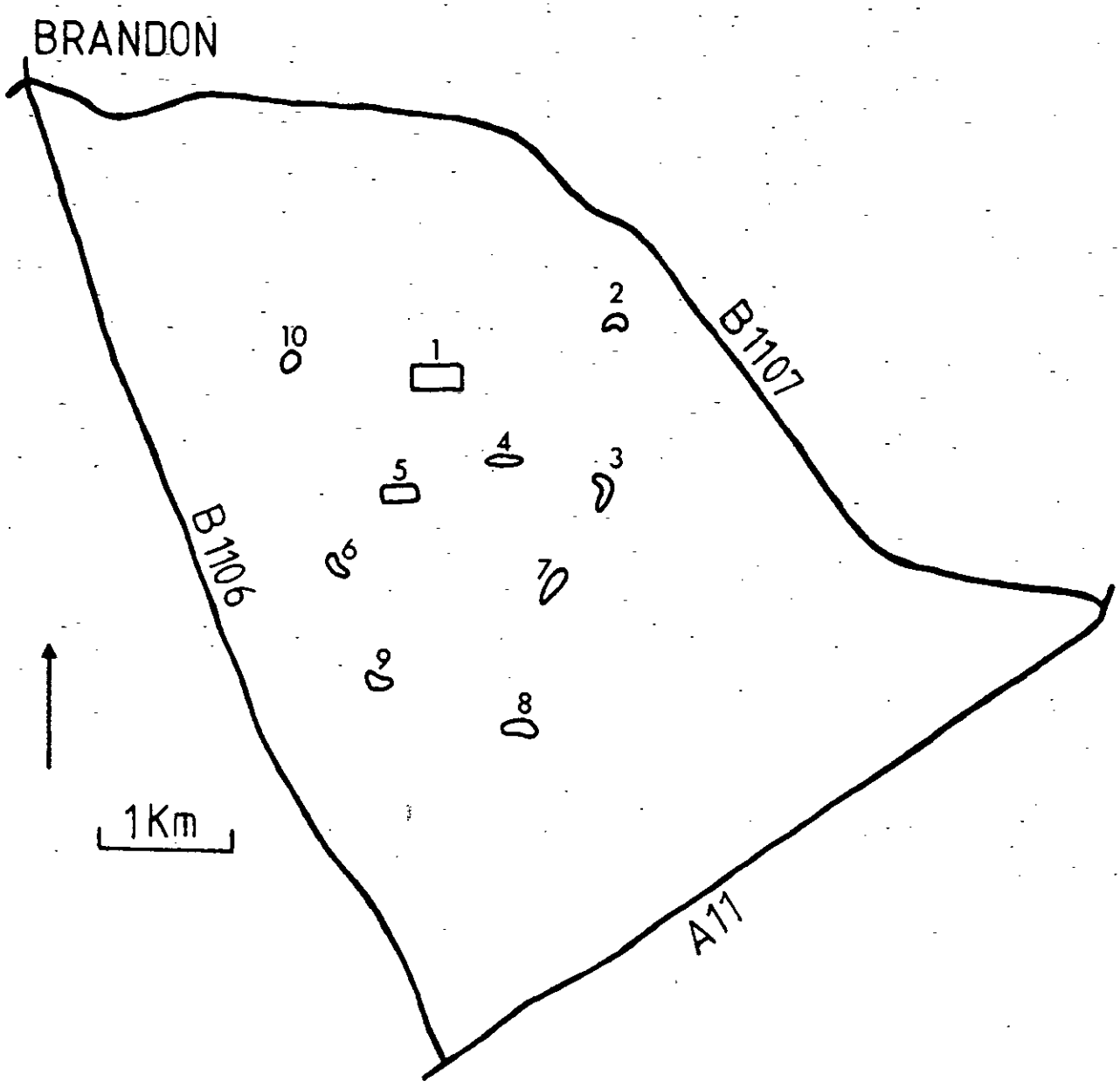
The estimate of the mean number of young born per female each year was 0.548 ±0.094 (SEM).

2.3.2 Introduction

The brown long-eared bat (Plecotus auritus) is a typical woodland species and is the second most abundant of the British bats (Stebbings, 1977). It hibernates through the winter, but spends the summer at roosts adjacent to the feeding areas. The young are born in early July, and reach morphometric maturity within a few weeks of birth. However, females may not always become sexually mature in time to give birth the following year (Stebbings, 1966). Body condition affects sexual maturity in males (Speakman & Racey, 1986). Like most other temperate zone bat species, brown long-eared bats have low annual mortality and reproductive rates (Stebbings, 1966; Stebbings, 1970; Tuttle & Stevenson, 1982) compared with other small mammals. Therefore, the trends of brown long-eared bat population may be sensitive to small changes in average survival and reproduction.

The placement of suitable boxes in a section of Thetford Forest in 1975 provided an opportunity to investigate their use by brown long-eared bats. Before the study began, a population of these bats was known to roost in buildings on the edge of the forest and it was postulated that, given extra roost sites in the forest, some of these bats would colonise the forest in summer. Regular inspections of these boxes over a period of 10 years gave information about the bats using the boxes and allowed population size, reproduction and mortality rates to be estimated. This paper describes these population parameters and

Figure 2.3.1 Map of the study area showing the main roads and the relative positions of the 10 sites where bat boxes were placed.



relates them to the management and conservation of bat populations.

2.3.3 Materials and methods

Study area and bat boxes

Boxes were erected in the Thetford Warren and Downham Highlodge Warren sections of Thetford Forest, Suffolk, which consists of approximately 10,000 ha, mainly planted with mixed Corsican (Pinus nigra) and Scots pine (Pinus sylvestris). Planting began in 1922 and much of the forest is now in an advanced state of maturity. Felling and replanting of some areas has occurred. Boxes were erected in 2 phases; the first was in September 1975 when 480 boxes were placed on 60 trees evenly spaced on the perimeter of a rectangular 7 ha forest block, which was located in the main body of the forest (Figure 2.2.1, Site 1). On each tree, boxes were arranged in two groups of 4, one at 3 and the other at 5 metres above the ground. Each group had a box facing north, south, east and west. During the second phase, a further 162 boxes were erected at 10 other sites during 1984 and 1985 (Figure 2.3.1, Sites 2-10). In this case, 18 boxes were placed on 6 trees at each site with 3 boxes per tree at a height of 5 metres above the ground. These boxes faced north, south-east and south-west. At the same time, the number of boxes at Site 1 was reduced to 160. The arrangement on each tree remained the same at Site 1, but the number of trees with boxes was reduced to 20 which were evenly spaced on the perimeter of the forest block. The selection of sites for boxes was made in consultation with the forest managers and with the future management of the forest in mind.
Boxes were constructed of untreated rough-cut wood and measured approximately 10 x 15 x 15 cm internally (Stebbings & Walsh, 1985). Each had a removeable top to allow inspection and removal of bats. The back-board extended below the box to allow the bats to alight and climb up into the box through a slit on the underside.

Measurements of bats

Boxes were checked 2 to 4 times each year between June 1977 and June 1987. Visits were made before and after the birth period each year but not during the birth period (late June and July) to avoid disturbance during lactation. Bats were removed from boxes for identification. Several species of bats used the boxes regularly, but brown-long eared bats were the most abundant. Each newly caught bat was ringed with a numbered alloy bat ring (Mammal Society, Burlington House, Picadilly, London) sexed and classed as juvenile (young of the year) immature or adult. Juvenile bats up to 5 months old were recognised by their grey colour, soft membranes and large finger joints. Females were classed as immature if they had brown fur and were judged to be nulliporous, or at least had not been suckled, as shown by the presence or absence of nipple distension. Males were considered as adults when dark brown pigmentation at the distal end of the eipididymis was dispersed.

Data analysis

Estimates of annual survival were based on the assumptions that the number of bats which lose rings is insignificant in proportion to the total marked population and that marked bats which were not recorded for more than 3 years were dead or were otherwise uncatchable. The assumption about ring loss was not tested, but no ring loss in 45 free-flying bats kept in captivity over a period

of several months, which suggests that ring loss is extremely rare. The assumption that missing individuals were no longer present in the local population was examined by comparing the probability of recovery after years of absence. Of 191 cases where bats went missing for 1 year in the first 7 years after the start of recording, 51 (27%) were recaught within 3 years. Of these recaptures, 39 (76%) were recaught within 1 year of absence, 47 (92%) were recaught within 2 years and the remainder were recaught within 3 years. No bats were recaught after more than 3 years absence.

Each year of the study was defined by the reproductive season, and ran from the beginning of July until the end of June in the following calendar year. This meant that cohorts could be equated directly to measurements based on real time. the number of bats alive in year n was estimated as the sum of the number caught in that year plus the number which were caught in subsequent years and, by interpolation, must have been alive in the year in question. It was not possible to assign all newly marked bats to a specific year class because some bats were first caught as adults.

Annual survival was estimated by 2 methods. One of these used aged cohorts where individuals which were first ringed as juveniles were used to give an empirical estimate of annual survival using least-squares regression. This gives a single estimate averaged over year classes and calendar years. Data from young born in different years were pooled. The second method was the maximum likelihood estimate of annual survival first described by Cormack (1964) for use on sightings of marked animals. This involved analysis of unaged cohorts and provided an estimate of survival (P) which was the probability that a bat which was alive in the year i will also be alive in the year i+1. The method enables annual variation to be assessed. At least 4 assumptions are involved in this approach which are common to all mark-recapture techniques. The first is that ringed bats are recaptured randomly, and all individuals have the same chance of recapture. This would mean that, after ringing, some bats would not be recaught even though they were present in the study area. This assumption was tested by comparing the number of times a bat was captured with its expectation of recapture in the following year (Table 2.3.1). If significant non-randomness of this type existed, we might expect the probability of recapture to decline sharply after the first capture. Comparing the number of years in which a bat had been captured with its expectation of recapture showed no such tendency.

A second assumption is that ringing and subsequent handling do not affect survival. This is implicit in ringing studies and cannot be tested directly. However, there were few indications that rings injured bats and no deaths were observed as a direct result of handling. Thirdly, it is assumed that survival is independent of age. This assumption was tested using the aged cohort analysis of survival and was found to be upheld. The final assumption is that catching effort is not related to catching success. The method used for catching bats precluded this possibility.

The observations used in the maximum likelihood estimates of annual survival are:

a_i = number of previously ringed bats seen in year i.
b_i = number of new bats ringed in year i.
c_i = number of bats caught in year i and not after year i.

Table 2.3.1 Probability of recapturing bats in relation to the number of times caught.

Capture no.	Probability of recapture	
	Males	Females
1	0.4725	0.6347
2	0.5116	0.6763
3	0.7045	0.7447
4	0.5806	0.7286
5	0.3889	0.5769
6	0.4286	0.8000

These observations were used to calculate 2 parameters, p_i, the probability that a bat alive in year i survived to the next year (survival rate), and E_i, the probability of catching a bat if it was alive in year i (catching effort). These parameters are given by:

$$P_i = \frac{(a_i + b_i - c_i)\, v_i\, (a_{i+1} + b_{i+1}) - (a_i + b_i - c_i)(a_{i+1})(c_{i+1})}{(a_i + b_i)\, v_i\, (a_{i+1} + b_{i+1} - c_{i+1})}$$

$$E_i = \frac{a_i\, (a_{i-1} + b_{i-1} - C_{i-2})}{(a_{i-1} + b_{i-1})\, (v_{i-1})\, (P_{i-1})}$$

where

$V_i = \Sigma\ (b_i - c_i)$

The annual reproductive rate was estimated from the total number of juveniles caught in their year of birth expressed as a proportion of the total number of adult females estimated to be alive in that year.

2.3.4 Results

Recruitment of marked bats

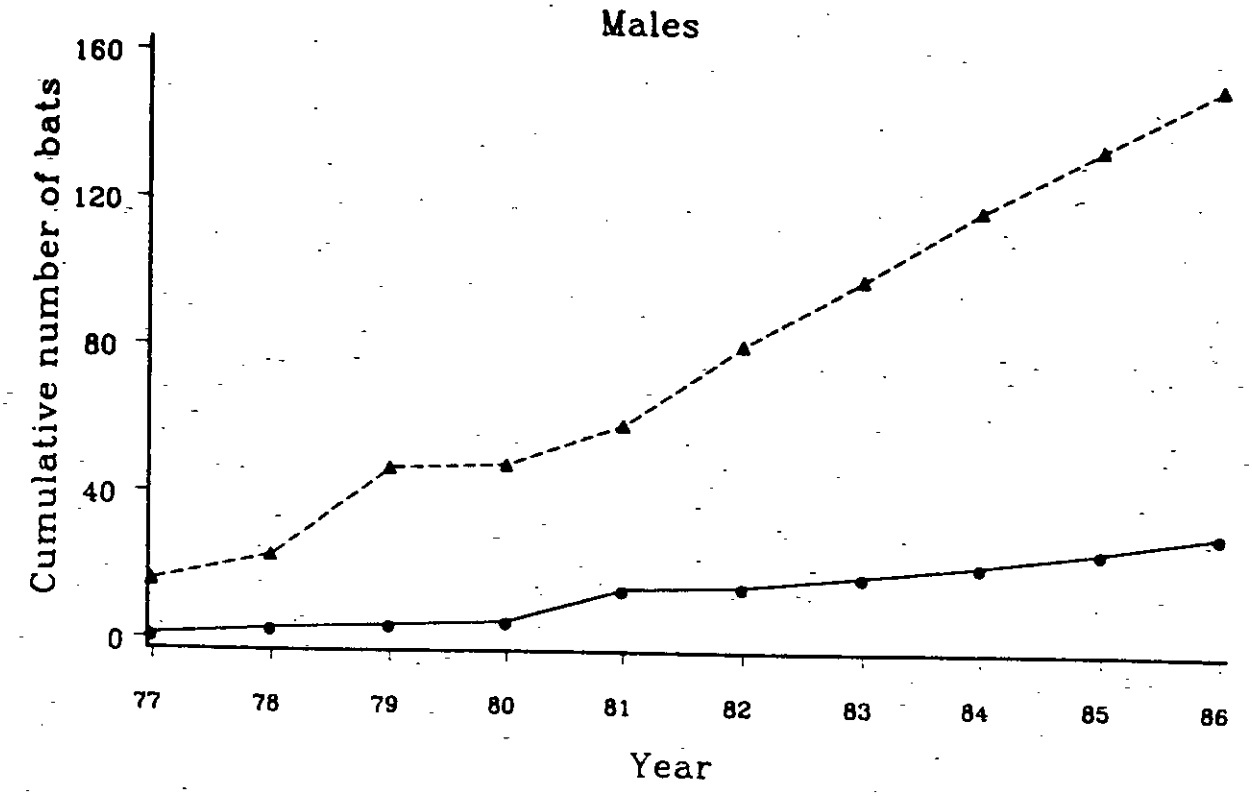
Major unknown variables in this study were the rates of immigration and emigration. It was not possible to measure emigration, and immigration included the capture of bats which could have been resident in the study area but were box-shy up to the time of first capture. The importance of immigration as a source of recruitment to the marked
population could be measured from the cumulative recruitment of juvenile and adult bats of the two sexes (Figure 2.3.2). Amongst females, there was an initial high rate of recruitment of unknown adults during 1977-78 when bats were first marked but, thereafter, the annual rate of recruitment was 5.22 ± 1.32 (SEM) adult bats with a maximum occurring in 1985-86 after the redistribution of the boxes. Amongst males, there was no initial high rate of recruitment as in females suggesting either that few males were present or that they were less easily captured than females. The annual rate of recruitment of previously unmarked adult males was not significantly different from that of females with a mean of 3.22 ± 0.86 (SEM) bats ($t = 1.269$, $df = 16$, $P>0.2$). The change in box distribution had no effect on the number of new adult males captured. However, as the population increased (Figure 2.3.3) and juvenile production increased (Figure 2.3.2), recruitment of adults formed a progressively smaller proportion of the total recruitment through time.

The annual rate of recruitment of juvenile bats to the marked population (13.4 ± 2.57 (SEM) for females and 15.2 ± 2.21 (SEM) for males) was significantly greater than for adults in both sexes ($P<0.025$ in both cases).

Dispersion

Bats made use of most of the boxes placed at Site 1 during the study and there was no tendency for the bats to occupy particular boxes or for individuals to be found only in certain boxes. Bats were often caught in clusters of 20 or more individuals in a single box. Forty of the 335 bats first marked at Site 1 (Figure 2.3.1) were subsequently recaptured at other sites (4, 5 and 10) but 79 bats

Figure 2.5.2 The cumulative number of male and female bats ringed as juveniles (triangles, dashed lines) or as adults (dots, solid lines) with time through the study.



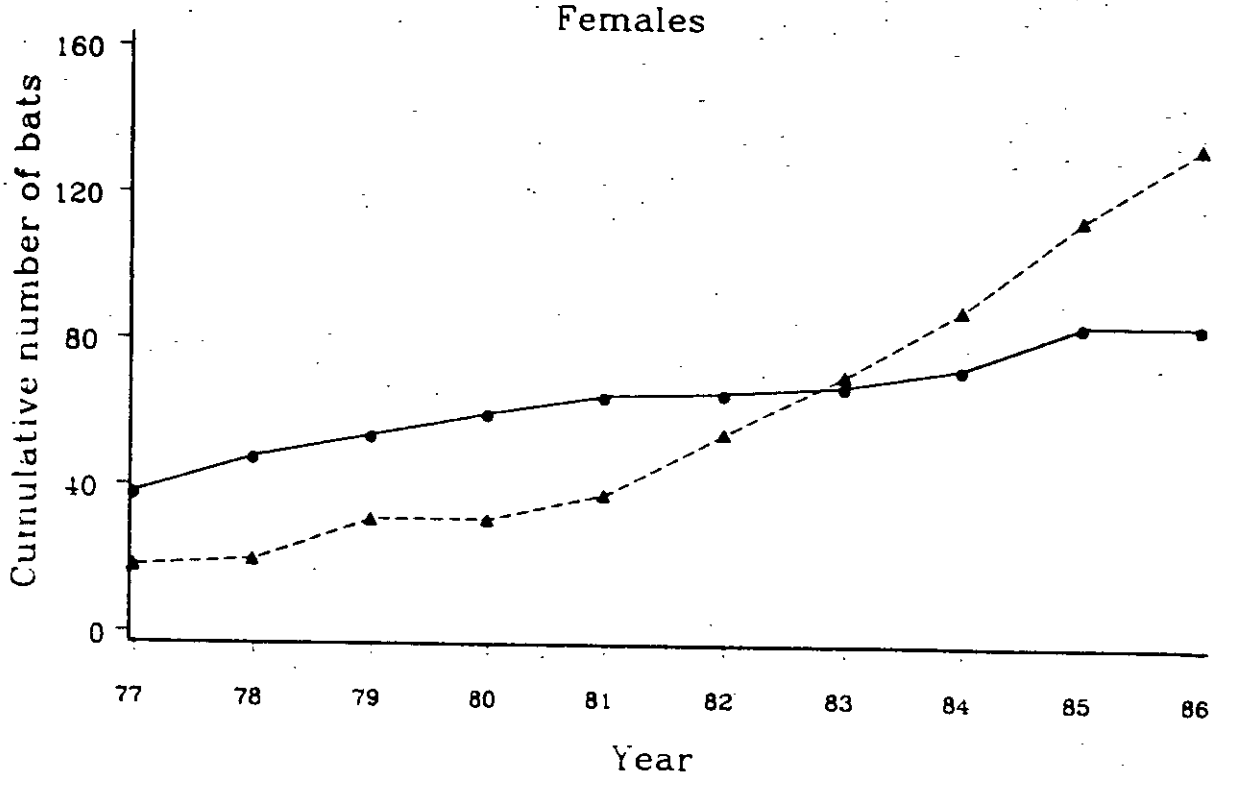
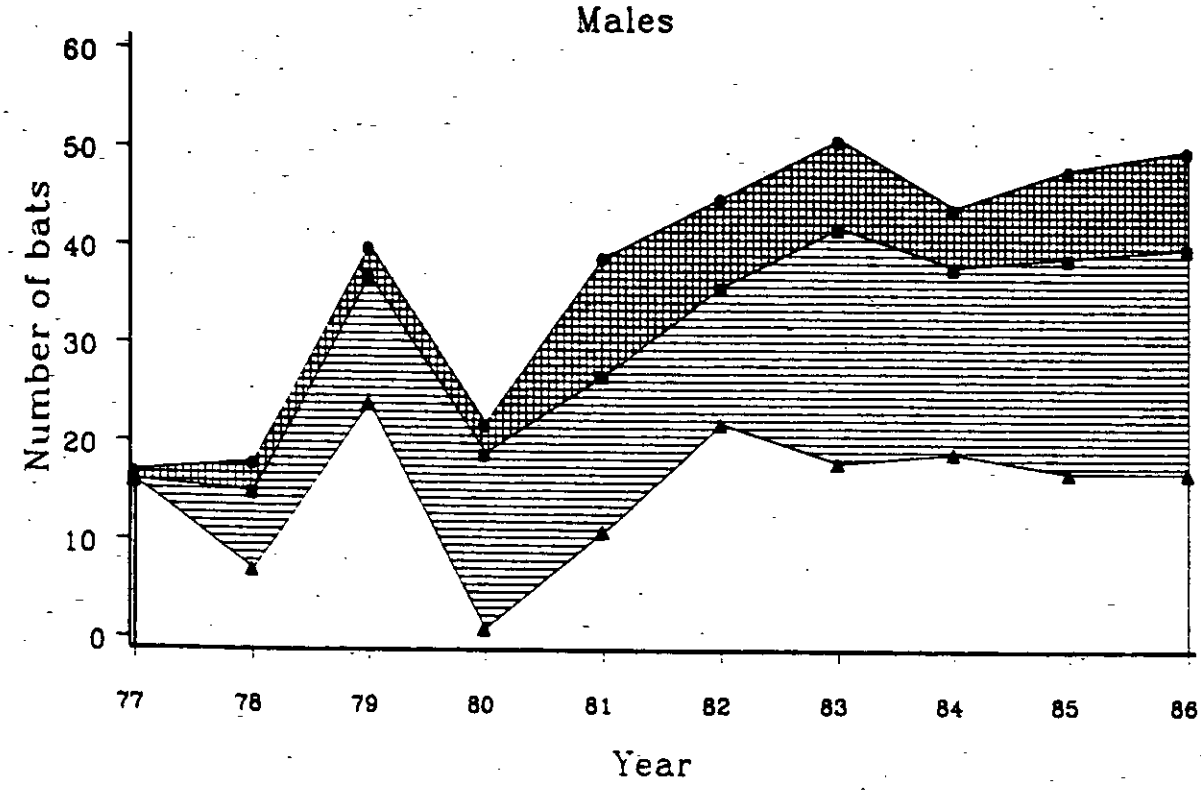
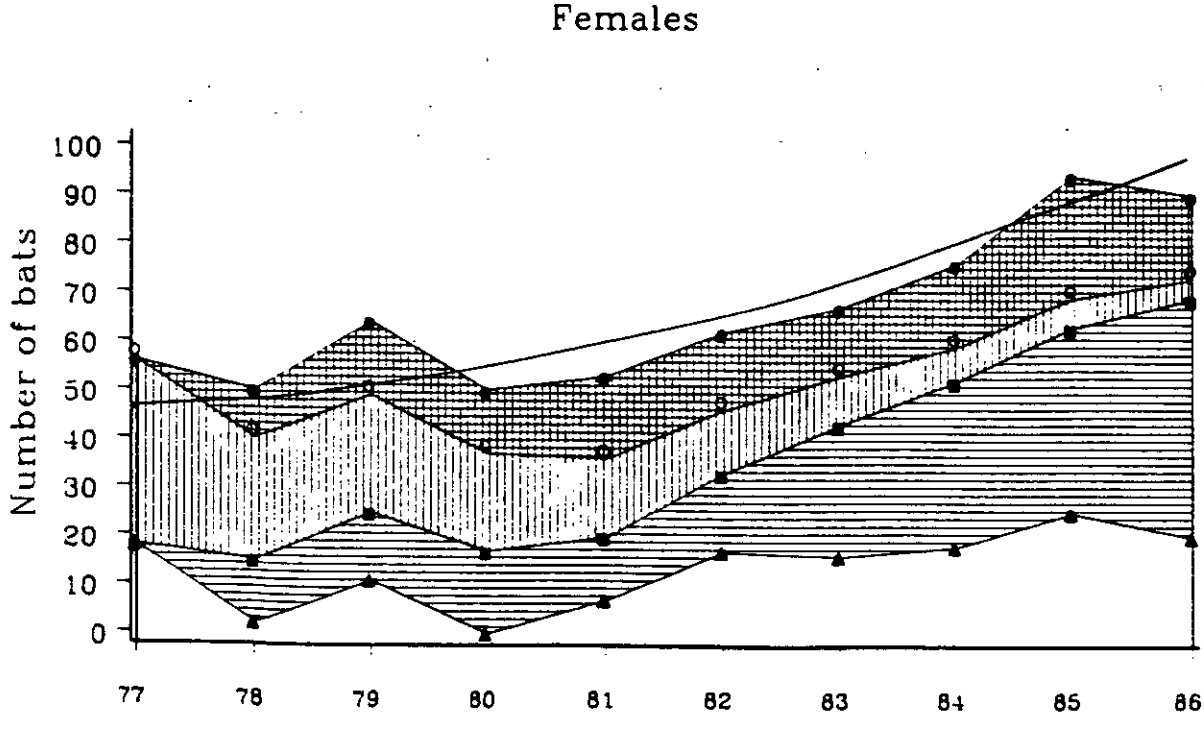


Figure 2.5.3 The number of bats of each sex known to be alive in each year of the study. Unhatched sections shown the number of juveniles and horizontal hatching shows the number of adults which were ringed as juveniles. In the case of females, vertical hatching shows the number of adult females ringed in the first year of the study while cross-hatched sections show the number ringed in subsequent years. Only 1 adult male was ringed in the first year so cross-hatching shows the total number of males ringed as adults. An exponential curve (R^2 = 0.978) was fitted to the data for number of females by least squares regression (number of females = 43.477 x exp (0.072 x time)).





were also caught exclusively at sites other than Site 1 and most of these were first caught as juveniles. Site surveys were too infrequent to allow a complete analysis of dispersion but it seems that bats were able to find newly sited boxed which were often occupied preferentially by young bats.

Population size

The number of bats estimated to be alive or still living in the study area increased with time (Figure 2.3.3). The increase in females gave a good approximation to an exponential model (Figure 2.3.3) while in males the increase was asymptotic. The number of bats present was underestimated by the methods used in this study and such error would be greatest at the beginning and end of the study. At the beginning of the study, it may have taken several years before all bats were marked, but evidence in Figure 2.3.2 suggests this was not the case for either sex, because recruitment of previously unmarked adults remained low and fairly constant throughout. The high probability of capture in any year (Tables 2.3.3 and 2.3.4), would also have reduced errors towards the end of the study.

As the study progressed, a larger proportion of the population was composed of bats which had been ringed as juveniles (Figure 2.3.3). As expected from Figure 2.3.2, a proportion of the adult population was always composed of individuals which were first ringed as adults and which could have been immigrants. However, the increase in the size of the population as a whole was largely due to recruitment of juveniles born within the study population.

Sex ratio

There was a marked difference in the composition of the male and female sections of the population throughout the study. The sex ratio of juvenile bats (152 males : 134 females) during the study also did not vary significantly from 1:1 ($X^2 = 1.13$, $P>0.25$), but significantly more juvenile males than females were present during the first 5 years (59 males : 39 females, $X^2 = 4.55$, $P<0.05$) while there was no difference during the second 5 year period (93 males : 96 females, $X^2 = 0.05$, $P<0.75$). The sex ratio of the total marked population also varied through the study (Table 2.3.2) with the proportion of males increasing during the first 5 years and then showing some decline thereafter.

Survival

If the annual survival rate was constant, then the number of animals in successive age classes should have followed a negative exponential curve. The exponent of the expression describing this curve for a stationary population represents the instantaneous mortality rate and the natural logarithm of this value is the annual survival rate (Chapman & Robson, 1960). In a changing population, the intrinsic rate of increase can be added to the instantaneous mortality rate and the natural logarithm of this will give the true annual survival. This will compensate for the skewed age distribution in a changing population. Using only bats of known age, a frequency distribution of the number of bats alive in different age classes was calculated (Figure 2.3.4). The distributions for both males and females gave good fits to an exponential curve suggesting a constant annual survival rate. A

Table 2.3.2 Number of adult females, number of juveniles, estimated fecundity rate and the sex ratio of the total marked population in each year of the study. The Chi-square probabilities for each sex ratio being not significantly different from unity are shown.

Year	No adult females	No Juveniles	Fecundity rate	Sex ratio (males/female)	P
1977-78	38	34	0.895	0.304	<0.005
1978-79	48	9	0.188	0.360	<0.005
1979-80	53	35	0.660	0.625	<0.025
1980-81	50	1	0.020	0.440	<0.005
1981-82	46	18	0.391	0.736	>0.05
1982-83	45	39	0.867	0.726	>0.05
1983-84	51	34	0.667	0.761	>0.05
1984-85	58	37	0.638	0.579	<0.005
1985-86	69	42	0.609	0.511	<0.005
1986-87	70	37	0.529	0.556	<0.005

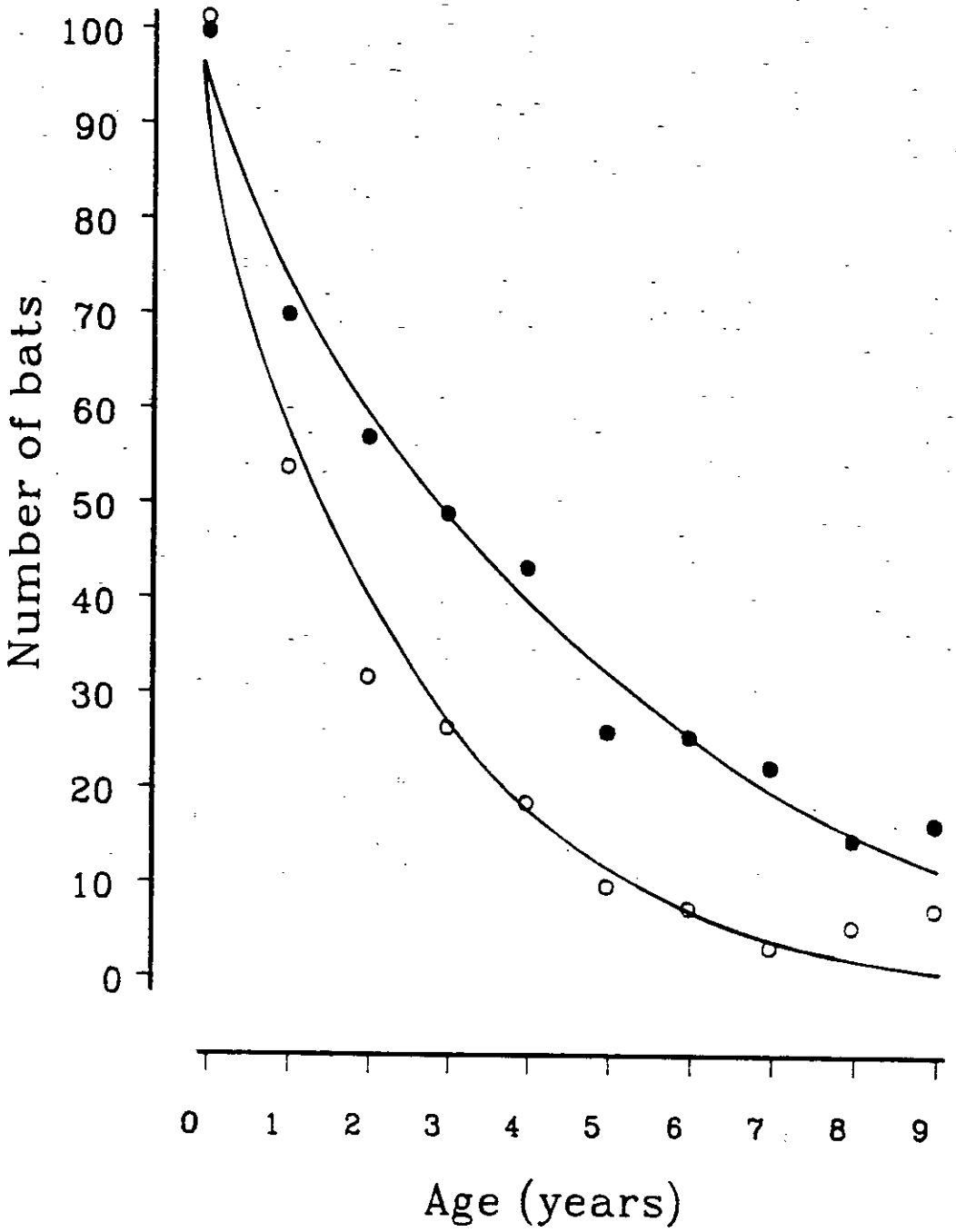
Table 2.3.3 Estimates of annual survival rates (P) of female brown long-eared bats and estimates of proportion alive that were caught (E). a is the number of previously ringed bats caught, b is the number of new bats ringed, and c is the number of bats caught in the year concerned but which were not caught again.

Year	a	b	c	P	E
1977-78	0	56	18	0.6797	
1978-79	37	12	3	0.9787	0.9720
1979-80	43	17	20	0.7107	0.8776
1980-81	34	6	9	0.7874	0.7249
1981-82	38	12	9	0.8250	0.9122
1982-83	43	18	13	0.7957	0.9713
1983-84	47	18	14	0.7948	0.9486
1984-85	51	23	19	0.7581	0.9500
1985-86	54	37	25	0.7253	0.9289
1986-87	70	20	90		

Table 2.3.4 Estimates of annual survival rates (P) of male brown long-eared bats and estimates of proportion alive that were caught (E). Other parameters are as described for Table 2.3.3.

Year	a	b	c	P	E
1977-78	0	17	8	0.5420	
1978-79	8	9	3	0.9455	0.8682
1979-80	13	25	20	0.5254	0.7548
1980-81	12	2	3	0.8560	0.5410
1981-82	17	20	17	0.5532	0.8213
1982-83	21	23	15	0.6591	0.9326
1983-84	30	21	29	0.4893	1.0000
1984-85	18	22	17	0.6261	0.7213
1985-86	24	21	20	0.5556	0.8163
1986-87	28	22	50		

Figure 2.5.4 The number of bats alive in successive age classes given an initial population size of 100 bats for each sex. Numbers of males are shown by circles and numbers of females are shown by dots. In total, 134 female and 152 male bats of known age were used in the analysis. Exponential curves were fitted by least squares regression (number of females = 94.8 x exp (-0.222 x age), r^2 =0.989; number of males = 96.7 x exp (-0.508 x age), r^2 = 0.981)).



correction was only made for the rate of increased in the case of females where the intrinsic rate of increase was obtained from the exponential model fitted to the estimates of population size (Figure 2.3.3). The uncorrected annual survival rate for males was 0.602 and for females it was 0.800. When corrected for the rate of increase and skewness of the age distribution, female annual survival was 0.861.

Cormack's method for measuring annual survial gave 9 independent estimates of the annual survival rate through the period of the study (Table 2.3.3 and 2.3.4). The overall annual survival was estimated from the geometric mean of the annual survival estimates and gave 0.623 ± 0.076 (SEM) for males and 0.780 ± 0.035 (SEM) for females. Variation in survival between years was greater for males than females. The estimates of survival from the frequency distributions of male and female numbers in each age class (Figure 2.3.4) were within the expected range of estimates produced by the above method (two-tailed test, $P>0.05$ for both sexes). The mean proportion alive which were caught each year was estimated to be 0.807 ± 0.050 (SEM) for males and 0.911 ± 0.029 for females (Tables 2.2.3 and 2.2.4).

A small number of adults was recruited to the marked population each year (Figure 2.3.2) and, in order to determine if these bats were transient, immigrants or resident, their survival in the marked population was compared with the survival of known-age bats using Cormack's unaged cohort method. Females ringed as adults had a mean annual survival rate of 0.773 ± 0.055 (SEM) and this was compared with 0.780 ± 0.024 (SEM) for females ringed as juveniles. Males ringed as adults had a mean annual survival rate of 0.674 ± 0.068 (SEM) and this compared with 0.611 ± 0.051 (SEM). Neither of these pairs of means were significantly different ($P>0.5$ in both cases). Therefore bats ringed as adults had survival rates similar to those of bats ringed as juveniles suggesting they were resident in the study area.

Fecundity

Population fecundity, which is the average number of offspring produced in the population in a year, could not be measured directly at the time of birth because the disturbance caused by handling lactating females and their young would probably have affected juvenile survival. Young bats which died during birth, lactation or the period of weaning would not have been included in the total number of juvenile bats found each year. Also, while most young bats were ringed in the period before hibernation, some were missed and were first ringed after emergence from hibernation. This was most marked in 1984-85 when only 8 of 37 juvenile bats were ringed before hibernation. This means that any estimate of fecundity, or of survival (see Figure 2.3.4 and above), will not include bats which died between birth and first capture and will be a minimum estimate of fecundity.

The average annual fecundity rate for 1977 to 1985 inclusive was 0.548 ± 0.094 (SEM) but there was considerable variation between years especially during the first 5 years (Table 2.3.2).

2.3.5 Discussion

It was not possible to estimate directly how much immigration and emigration affected the dynamics of this population of brown long-eared bats. The term survival in the context of this study is used to mean the suvival of individuals in the marked and catchable population and will exclude bats which emigrated. Little is known about dispersal in bats although some species occupy traditional sites where individuals can be caught in successive years throughout life (Stebbings & Arnold, in press; Thompson, 1987). Brown long-eared bats are also known to occupy traditional roost sites (Stebbings, 1966; Stebbings, 1970) and it seems probable that the bats in this study were normally resident in Thetford Forest from Spring to Autumn. However, their feeding ranges are unknown.

Immigration may have occurred during the study, the best indication of this being the fairly constant annual recruitment of adults to the marked population, but some of these individuals could have been bats which had gone unmarked as local juveniles in the previous year. Taking account of this, plus the lack of evidence that apparent immigrants were transient individuals, suggests that immigration of adults was a minor source of recruitment. It is not possible to come to the same conclusion about juveniles, some of which could have been immigrants, but in order to support juvenile recruitment at the observed rate, some highly productive populations would be required in the vicinity of Thetford Forest, or bats could have moved into the area from some distance.

A change in the dispersion of boxes in 1984-85 appeared to have little influence on the size of the marked population. However, more adult females than usual were ringed at this time, so the wider pattern of distribution may have caused the feeding ranges of other females to be included, thereby drawing them into the marked population. Conversely, this increase was sufficiently small that it could have arisen by chance.

The frequency of capture of individual bats was high, which suggests that these bats used the boxes readily and had alternative roost sites. The pattern of recruitment of adults does not suggest that a significant residual population of unmarked bats was present. Moreover, if some bats were particularly difficult to catch, those which were recruited as adults would have given a reduced survival rate when compared with bats recruited as juveniles.

One advantage of studying populations in forestry plantations is that the habitat is quite uniform over large areas. While some felling and replanting occurred in the vicinity of the boxes during the course of the study, there is no reason to suspect that the changes in the population occurred because of habitat changes. Rather the increase in the population was probably due to the increased number of roost sites provided by the boxes.

Two independent estimates of the survival rate of males and females were obtained and both gave similar results. The estimate obtained using the technique of unaged cohort analysis provided a better overall picture of survival than the known-age cohort analysis, which loses accuracy due to small sample sizes in the older age classes. Unaged cohort analysis has probably been the most widely used method for measuring survival rates in bats (Davis, 1966; Goehring, 1972; Humphrey & Cope, 1977; Foster, Humphrey & Humphrey,

1978; Keen & Hitchcock, 1980; Stevenson & Tuttle, 1981; Thompson, 1987). The estimates of annual survival in the Thetford population were similar to those found in another population of brown long-eared bats in Britain (Stebbings, 1970) and were within the range of survival values found for bats in general (Humphrey & Cope, 1977; Tuttle & Stevenson, 1982). However, not all studies of bat survival rates using unaged cohorts satisfy the assumptions inherent in mark-recapture data and therefore inter-study comparisons of absolute survival rates could be misleading (Humphrey & Cope, 1977). In particular, the assumption that there is no age-specific component in survival is often not tested. As in this study, Stevenson & Tuttle (1981) found no significant age-specific effect on survival in the gray bat (Myotis grisescens). Many bat studies also do not distinguish between winter and summer populations, the composition of which could be quite different. This study refers specifically to a summer population.

Patterns of survival can vary significantly between colonies of the same species (Stevenson & Tuttle, 1981). This may be related to environmental factors such as human disturbance although food abundance and climate probably have a more general role in determining survival rates (Keen & Hitchcock, 1980). Most significantly in this study, male survival rates were significantly less than those for females. Stevenson & Tuttle (1981) found no overall difference in survival rates of the two sexes in the gray bat. Keen & Hitchcock (1980) observed greater male than female survival in the little brown bat (Myotis lucifugus) although Humphrey & Cope (1976) found greater female than male survival in the same species. The difference between survival rates in the two sexes in this study were greater than those found in most other studies and agree with the difference observed by Stebbings (1977). This suggests that inter-sexual differences in the ecology and behaviour of brown long-eared bats cause a higher death rate amongst males than females.

The measure of fecundity used in this study assumed that most females gave birth for the first time at the end of their first year. Females of some vespertilionid bats may become sexually mature in their first autumn (Racey, 1974) and, for Plecotus males, sexual development in their first autumn is dependent on body condition (Speakman & Racey, 1986). Stebbings (1966) suggested that males became sexually mature in their first year while some females do not reproduce until their second year. The age of sexual maturity in vespertilionides is from 3 to 16 months in both sexes (Tuttle & Stevenson, 1982), but this variation may simply reflect the normal probability of seasonally-breeding individuals of any age having developed gonads in any breeding season.

The annual fecundity rate was subject to several unqualified errors. Probably the most significant of these was that some juveniles were not caught until the spring after weaning and since much of the first year mortality, immigration or emigration probably occurred in the intervening time between birth and first capture in spring, the number captured may have given a poor indication of the number born.

The marked population increase during the study from 73 to 140 individuals gave a doubling time of about 10 years. This increase was mainly the result of recruitment of bats produced within the population, showing that the population was more than

self-sustaining. This rate of increase was consistent with the usual survival and reproductive rates expected in temperate zone vespertilionid bats (Tuttle & Stevenson, 1982). The dynamics of bat populations are more similar to those of large rather than small mammals (Caughley, 1966). This has implications for the stability of bat populations exposed to certain types of disruption, such as pesticide poisoning or roost destruction. Relatively small reductions in fecundity and survival, especially in females, could cause numbers to decline. Bat populations will then be slow to return to stabilise after disruption.

2.4 Stability of bat populations

2.4.1 Introduction

In Section 3.5, it was shown that a population of brown long-eared bats had an average annual survival rate of 0.86 for females and 0.60 for males, while there was an annual fecundity rate of 0.55. These parameters are similar to those of other bat populations (Tuttle & Stevenson, 1981) and they are sufficient to allow some inference to be made about the stability of bat populations subjected to abnormally high mortality or reduced reproduction caused by, for example, pesticide poisoning.

The dynamics of most bat populations are simplified by the absence of long periods of sexual immaturity and an apparent constant survival rate throughout life. However, no data exist to measure age-specific reproductive rates in females and it has to be assumed that all individuals make an equal contribution to reproductive output, irrespective of age. In fact this is highly improbable in long-lived mammals, but, providing the age distributions are not highly skewed towards either the old or young age classes, due to a rapid increase or decline in the population, any effect of age on reproductive value will be averaged out through time. It also has to be assumed that males are always in excess and that their numbers do not normally limit the rate of increase of the population, and so, for most purposes, they can be ignored. This is probably a true assumption for many bats which are usually polygynous (Bradbury, 1977; Bradbury, 1979).

The purpose of this section of the report is to illustrate and quantify the stability of bat populations by using the data collected from brown long-eared bats at Thetford Forest.

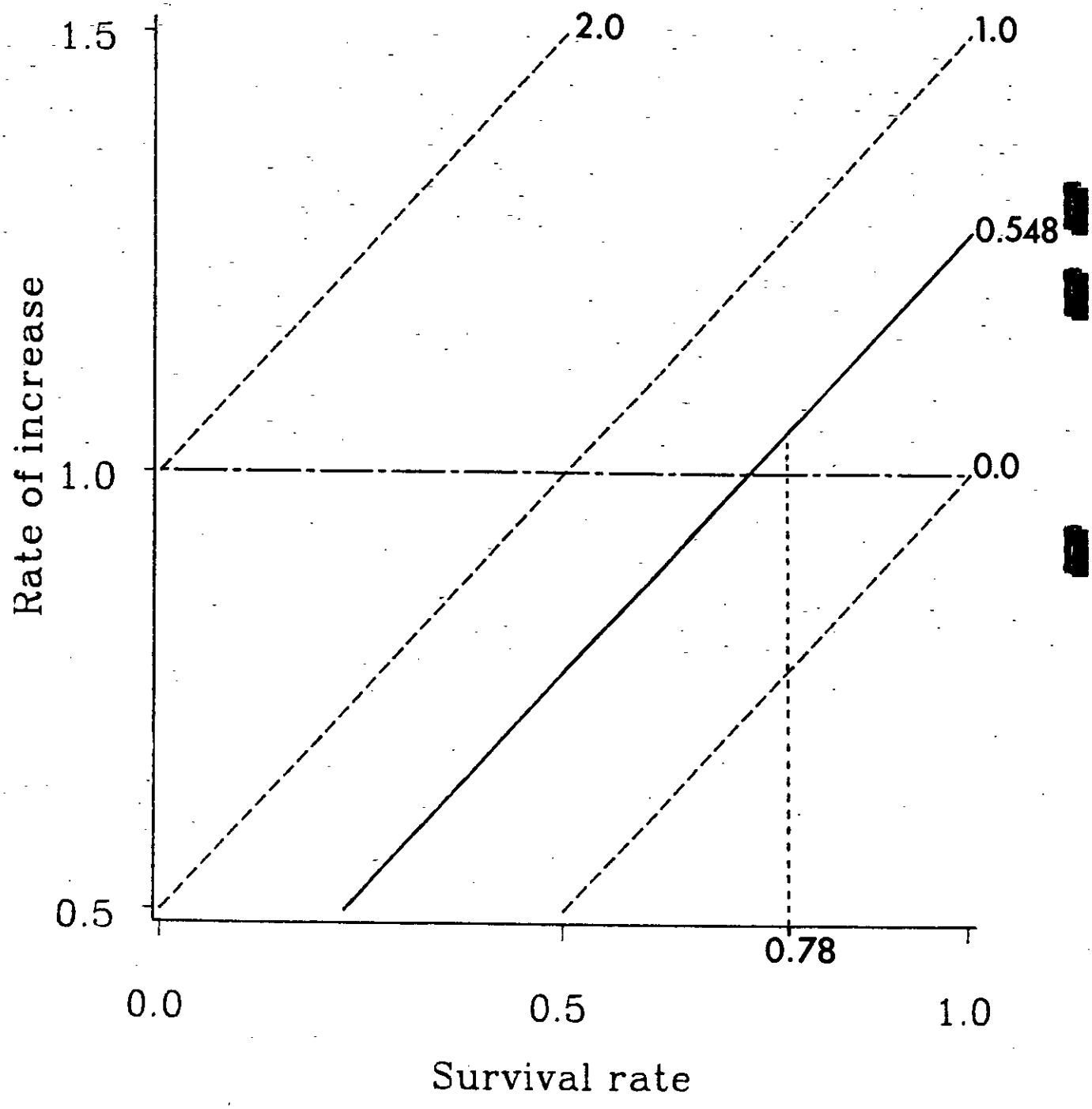
2.4.2 Methods, results & discussion

If all survival and fecundity rates are constant in time then:

$$\sum_{x=0}^{x=n} l_x \, m_x \, e^{-rx=1}$$

where l is the probability of a female surviving from birth to age x, m is the mean number of female offspring produced by a female of age x (in this case half the average fecundity rate), r is the intrinsic rate of increase in the population and n is the age of the oldest female. From this we know that the size of the population, where population growth is independent of density, at time t is given by

Figure 2.4.1 Rate of increase in a hypothetical bat population as a function of female survival rate at different reproductive rates. The solid line shows the relationship when the reproductive rate defined in section 2.3.4 was used and a 1:1 sex ratio at birth was assumed.



$$N_t = N_o.e^{-kt}$$

where k is the instantaneous mortality rate. The natural logarithm of this value gives the rate of increase of the population. When the rate of increase is greater than 1, then the population is increasing and when the rate of increase is less than 1 it is in decline. The rate of increase of the female section of the brown long-eared bat population at Thetford Forest (Section 2.1) was 1.075. Given the other parameters of the female population, it is possible to show how the rate of increase changes with variations in survival at a constant reproductive rate (Figure 2.4.1). This shows that a decline in female survival of greater than 5% would cause this population to go into decline. The simplified nature of the population dynamics means that a reduced birth rate is equivalent to reduced survival, so small changes in the birth rate may also have a major influence on the rate of increase.

However, it would be unusual for some factor, such as contamination of a winter roost with a pesticide, to affect only survival or birth rate. Most probably, both would be affected because the death of reproductively mature females will have a consequent effect on the birth rate. Therefore, in a population consisting of about 90 females (the size of the Thetford brown long-eared population in 1986) an additional average mortality of 2 or 3 adult females each year could cause the population to decline.

While temperate zone bats are subjected to many of the metabolic and energetic stresses commensurate with being small (McNab, 1982), their population dynamics are similar to those of large mammals (Caughley, 1966). It is easy to understand why large mammals, which are rare relative to most small mammals, must be managed with some care, because they have slow intrinsic rates of increase. Where bats are concerned, this is not intuitively obvious and, in many cases, the long-term stability of bat populations may be threatened by any small, and probably unseen, increase in mortality from any cause.

3. ACKNOWLEDGEMENTS

Data for the analysis of the population dynamics of brown long-eared bats at Thetford Forest were provided by Dr R E Stebbings.

4. REFERENCES

BOYD, I.L. 1987. The effect of gamma-HCH (Lindane) on the behaviour and survival of pipistrelle bats. ITE Contract Report to Nature Conservancy Council.

BOYD, I.L. & MYHILL, D.G. (1987). Seasonal changes in condition, reproduction and fecundity in the wild European rabbit (Oryctolagus cuniculus). J. Zool. Lond., 212, 223-233.

BOYD, I.L. & MYHILL, D.G. in press. Variations in the post-natal growth of Pipistrelle bats (Pipistrellus pipistrellus). J. Zool., Lond.

BOYD, I.L., MYHILL, D.G. & MITCHELL-JONES, A.J. in prep. Uptake of gamma-HCH by Pipistrelle bats and its effect on survival.

BRADBURY, J.W. 1977. Social organization and communication. In Biology of bats, Vol 3, Ed W A Winsatt, pp 1-72. New York : Academic Press.

BRADBURY, J.W. 1979. Behavioural aspects of reproduction in Chiroptera. J. Reprod. Fertil., 56, 431-438.

CHAPMAN, D.G. & ROBSON, D.S. (1960). The analysis of a catch curve. Biometrics, 16, 354-68.

CHIPMAN, J.K. & WALKER, C.H. 1979. The metabolism of dieldrin and two of its analogues : the relationship between rates of microsomal metabolism and rates of excretion of metabolites in the male rat. Biochem. Pharmacol., 28, 1337-1345.

CLARK, D.R., Jr. 1981a. Bats and environmental contaminants : a review. United States Department of the Interior, Fish and Wildlife Service, Special Scientific Report - Wildlife, , 235.

CLARK, D.R., Jr 1981b. Death in bats from DDE, DDT or dieldrin : diagnosis via residues in carcass fat. Bull. Environment. Contam. Toxicol., 26, 367-374.

CLARK, D.R. Jr & KROLL, J.C. 1977. Effects of DDE on experimentally poisoned free-tailed bats (Tadarida brasiliensis) : lethal brain concentrations. J. Toxicol. Environ. Health, 3, 893-901.

CLARK, D.R., Jr & KRYNITSKY, A. 1978. Organochlorine residues and reproduction in the little brown bat, Laurel, Maryland - June 1976. Pest. Mont. J., 12, 113-116.

CLARK, D.R., Jr & KRYNITSKY, A. 1983. DDE in brown and white fat of hibernating bats. Environ. Poll., 31, 287-299.

CORMACK, R.M. 1964. Estimates of survival from the sighting of marked animals. Biometrika, 51, 429-438.

DAVIS, W.H. (1966). Population dynamics of the bat Pipistrellus subflavus. J. Mammal, 47, 383-396.

FOSTER, G.W., HUMPHREY, S.R. & HUMPHREY, P.P. (1978). Survival rate of young southeastern brown bats, Myotis austroriparius, in Florida. J. Mammal., 59, 299-304.

GOEHRING, H.H. 1972. Twenty-year study of Eptesicus fuscus in Minnesota. J. Mammal., 53, 201-207.

HUMPHREY, S.R. & COPE, J.B. 1976. Population ecology of the little brown bat (Myotis lucifugus) in Indiana and north-central Kentucky. Special Publication of the Americal Society of Mammalogists, 4, 1-81.

HUMPHREY, S.R. & COPE, J.B. 1977. Survival rates of the endangered Indiana bat, Myotis sodalis. J. Mammal., 58, 32-36.

KEEN, R. & HITCHCOCK, H.B. 1980. Survival and longevity of the little brown bat (Myotis lucifugus) in southeastern Ontario. J. Mammal., 61, 1-7.

KNIGHT, G.C.; WALKER, C.H.; CABOT, D.C. & HARRIS, M.P. 1981. The activity of two hepatic microsomal enzymes in sea birds. Comp. Biochem. Physiol., 68c, 127-132.

LITTERST, C.L; REAGAN, R.L. & GRAM, T.E. 1974. Microsomal drug metabolizing enzyme activity in livers of bats. Comp. Biochem. Physiol., 47B, 163-167.

McNALS, B.K. 1982. Evolutionary alternatives in the physiological ecology of bats. In Ecology of bats, Ed T H Kunz, pp151-200. New York : Plenum.

PORTIG, K.S.J. & KORANSKY, W. 1977. Oxidative transformation of hexachlorocyclohexane in rats and with rat liver microsomes. Naungn - Schmeideberg's Arch. Pharmacol, 298, 115-128.

RACEY, P.A. 1974. Ageing and assessment of reproductive status of Pipistrelle bats, Pipistrellus pipistrellus. J. Zool., Lond., 19, 175-189.

RACEY, P.A. 1982. Ecology of bat reproduction. In: Ecology of bats, Ed. T.H. Kunz, pp 57-104, Plenum Press, London.

SPEAKMAN, J.R. & RACEY, P.A. 1986. The influence of body condition on sexual development of male brown long-eared bats (Plecotus auritus) in the wild. J. Zool., Lond., 210, 515-525.

STEBBINGS, R.E. 1966. A population study of bats of the Genus Plecotus. J. Zool., Lond., 150, 53-75.

STEBBINGS, R.E. 1970. A comparative study of Plecotus auritus and P. austriacus (Chiroptera, Vespertillionidae) inhabiting one roost. Bijdr. Dierk., 40, 91-94.

STEBBINGS, R.E. 1977. Order Chiroptera, Bats. In: Handbook of British Mammals, Eds. G.B. Corbet & H.N. Southern, pp 68-128, Blackwell, London.

STEBBINGS, R.E. & GRIFFITHS, F. 1986. Distribution and status of bats in Europe. Institute of Terrestrial Ecology, Huntingdon, pp 142.

STEVENSON, D.E. & TUTTLE, M.D. 1981. Survivorship in the endangered gray bat (Myotis grisescens). J. Mammal., 62, 244-257.

THOMPSON, M.J.A. 1987. Longevity and survival of female pipistrelle bats (Pipistrellus pipistrellus) on the Vale of York, England. J. Zool., 211, 209-14.

TUTTLE, M.D. & STEVENSON, D. 1982. Growth and survival of bats. In: Ecology of bats, Ed. T.H. Kunz, pp 105-150, Plenum Press, London.

WALKER, C.H. & EL ZORGANI, G.A. 1973. Metabolism of a dieldrin analogue - secondary oxidation by liver microsomes. Life Sciences, 12, 585-593.

WORTHING, C.R. 1987. The pesticide manual, a world compendium. The British Crop Protection Council.

ZULLINGER, E.M. RICKLEFS, R.E., REDFORD, K.H. & MACE, G.M. 1984. Fitting sigmoidal equations to mammalian growth curves. J. Mammal., 65, 607-636.