Grooming Behavior and Parasite Load in the Greater Horseshoe Bat (*Rhinolophus ferrumequinum*)

Diploma Thesis
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1 Introduction

1.1 Grooming Behaviour

1.1.1 Grooming in General

Grooming is a common behaviour amongst animals and is widely described in birds and mammals (e.g Clayton & Cotgreave 1994; Mooring & Hart 1995). We can distinguish between self-grooming and two forms of social-grooming, (1) allo-grooming that occurs between adults and their offspring and (2) allo-grooming that occurs between adults.

Grooming behaviour serves several functions, in addition to removing ectoparasites. For example, personal or self-grooming serves to maintain the condition of the fur, while social-grooming also plays a role in strengthening social bonds (e.g. Seyfarth & Cheney 1984; Wilkinson 1986; Muroyama 1994; reviewed by Dunbar 1988). The importance of grooming for removing ectoparasites has been studied in the impala *Aepyceros melampus* by Mooring *et al*.(1996a), who showed that when seeded with larval ticks, individuals prevented from grooming by fitted restraints ended up with a parasite load twenty times higher than unrestrained animals. In the same species, studied in the breeding season, territorial males, in comparison to females and bachelor males, spend more time scanning for intruders and less time grooming themselves. Consequently territorial males accumulate a higher load of ectoparasites (Mooring & Hart 1995; Mooring *et al.* 1996b). Bird species known to harbour more parasitic louse species spend more time on maintenance and grooming in particular than do host species with few lice (Cotgreave & Clayton 1994).

1.1.2 Grooming in Bats

Marshall (1982) and Wilkinson (1986) suggest that self-grooming in bats may be important in controlling ectoparasite load. The host's grooming behaviour may be a major factor for mortality of the permanent ectoparasites of bats (Marshall 1982). Auto-grooming also anoints the body with the animals own scent produced by skin glands (Begum & Alexander 1993).

Only self-grooming but no allo-grooming was observed in the long-nosed bat *Leptonycteris curasoae* (Fleming *et al.* 1998) and in *Carollia perspicillata* (Williams 1986). Allo-grooming is widely described in *Vampyrum spectrum* and *Desmodus rotundus* (Wilkinson 1985, 1986), *Phyllostomus hastatus* (McCracken & Bradbury 1981) and *Phyllostomus discolor* (Vehrencamp *et al.* 1977; Wilkinson 1987). Both self-grooming and allo-grooming have been documented in the common vampire bat *Desmodus rotundus* (Wilkinson 1986). The extent of grooming in bats may vary with age, temperature and health (Kunz 1976; Marshall 1982; McLean & Speakman 1997).

Wilkinson (1986) suggests that self-grooming serves a different function from allo-grooming and most probably acts to control ectoparasites. In *Desmodus rotundus* self-grooming occurs ten times more often than allo-grooming. He found that the proportion of time spent grooming and the amount of ectoparasite infestation correlated positively among tree roosts. However parasite counts were not taken in this study and Wilkinson did not distinguish between parasites that were living on the trees and that from indigenous ectoparasite load.

1.1.3 Grooming in *Rhinolophus ferrumequinum*

Self-grooming in captive Greater horseshoe bats was observed and described by Kolb (1981). Wings were almost always licked with the tongue and scratching was rare. Wings were cleaned on the inner and the outer side. Time spent grooming wings exceeded the time grooming the belly, back, sides, legs and mouth. Grooming took place after bats woke up and after feeding. Self-grooming also takes place before leaving the roost (Schober 1998). Allo-grooming has not been observed so far amongst in captivity living Greater horseshoe bats (Kulzer personal observation).

1.2 Parasitism, Ectoparasites and the Mite *Eyndhovenia euryalis*

1.2.1 Parasites in General

A parasite can be described in general as “an organism that lives in or on a host from which it derives food and other biological necessities” (Clayton & Moore 1997). For example ectoparasites can be described as parasites which live on the surface of a host and are dependent on at least one gene or its product from that host to complete their own life-cycle (Bennett 1998).

Parasites in general can reduce fitness as they can cause mortality, morbidity or reduce fecundity (Clayton & Moore 1997). Investigating host-parasite communities and within interactions are most interesting from different points of view. Host and parasite can co-evolve e.g. host towards resitance and parasites towards virulence (Clayton & Moore 1997). Hence, both host and parasite can have major impact on each others evolving. Parasites can thus exert selective pressures on their hosts. Parasites can, for example influence hosts lives by regulating host population size and demographic structure; or they may drive host population cycles (Anderson & May 1979; Freeland 1976; Dobson & Hudson, 1992; all reviewed in Goater & Holmes 1997). Parasites can even have an impact on host sexual selection (Møller 1990) and can select against inbreeding by hosts so that parasite resistance is increased (Coltman *et al.* 1999).

Transmission, or the passing of a parasite from one host to another, can be classed as horizontal or vertical. Horizontal transmission takes place between unrelated hosts and vertical between parent hosts and their offspring. One can also distinguish between endoparasites, which live inside their host’s body and ectoparasites which live externally (Walter & Procter 1999).

1.2.2 Ectoparasites

Ectoparasites can be divided in groups according to their life strategies as permanent, nest-dwelling and free living parasites (Brinck-Lindroth *et al.* 1975; In: Nilsson & Lundqvist 1979). Ectoparasites often are bloodsucking organisms, which live and feed on the external surfaces of their host. By removing blood, they reduce host energy and nutritional resources, thereby decreasing host’s fitness (Hart 1990; In: Mooring *et al.* 1996b). Studies of birds (Brown & Brown 1986) and mammals (Norval *et al.*1989) have shown that ectoparasite activity significantly reduces growth.

Or, to put it the other way round, a reduction of ectoparasite load results in an improvement of quality and quantity of offspring produced as, for example in the cliff swallow *Hirundo pyrrhonota* (Loye and Carroll 1991, in Møller *et al*. 1993). Blanco *et al.* (1997) for example suggest a commensal relationship between the Red billed chough *Pyrrhocorax pyrrhocorax* and its feather mite *Gabucinia delibata* instead, as it does not harm its host.

Whitaker (1988) describes two factors which may limit the detrimental effects on the host. First there has been shown to be an evolution in parasites towards reducing harm and injury to their hosts, and second, most of the time individuals harbour relatively small numbers of parasites.

1.2.3 Mites

Mites belong to the Phylum Arthropoda and in here to the Subphylum Chelicerata. Chelicerates do not have a separate head and anterior body region; the prosoma combines the functions of sensing, feeding and locomotion. Mites can have parasitic, commensal or mutualistic ecological interactions with animals. Mites have very variable generation times. They are cold-blooded and dependent on a critical temperature for reproduction. Generation time decreases and development rate increases as temperature increases. Some mites have a matchless population increase with very rapid lifecycles. Other mites with life cycles closely related to their host might only pass through a single generation per year (Walter & Procter 1999).

1.2.4 Bats and Parasites

Bat parasites have a high degree of host specificity. Many parasites are only found on a single bat species. There are the advantages of being well adapted to the host, but highly specified parasites also have the disadvantage that they cannot expand their population beyond their host's limits (Whitaker 1988). Bat-associated ectoparasites prefer different places on their host's body. Bugs, fleas, ticks, bat-flies and mites can be found in the fur. Some mite species hosted by bats are situated on ears and eyes. Wing-mites and mange mites of the genus *Nycteridocoptes* can be found on the wings (Walter 1996). Age of the colony, colony size and climate might have an influence on levels of infestation of bats in a colony (Marshall 1982; Walter 1996). Grooming, rotation of visited roosts or immune response are suggested as mechanisms to reduce parasite load (Walter 1996).

Greater horseshoe bats, for example harbour the tick *Ixodes vespertilionis*, the mite *Paraperiglischrus rhinolophus* or the flea *Rhinolophopsylla unipectinata* as ectoparasites (Walter 1996). The ectoparasite investigated in this study is exclusively the mite *Eyndhovenia euryalis* (see Figure 1) which belongs to the acari family Spinturnicidae. The family Spinturnicidae is exclusively associated with the mammalian order Chiroptera (Rudnik 1960; Uchikawa *et al.* 1994; Whitaker 1988). They feed on their host's blood and blood is commonly found in their digestive tract (Rudnik 1960). Infestation with Spinturnicidae is usually low. So the direct impact of being infested with Spinturnicidae is suggested to be low (Walter 1996). The mites spend their entire life on their host's body (Rudnik 1960). The lifecycle of all Spinturnicidae consists of five stages, egg, larva, protonymph, deutonymph, and adult (Rudnik 1960). The first two stages occur within the female body and birth is given directly to the protonymph. The protonymph moults once and develops into a male or female deutonymph, which moults into an adult. Spinturnicidae in Europe live up to 2 years and have probable a generation time of 4-5 weeks during summer. One female produce 6-8 eggs per cycle (Deunff, personal observation). Species studies of the genera *Spinturnix* and *Eyndhovenia* show reproduction and maximum population during summer. In winter some males and females survive in resistant stages (Deunff & Beaucournu 1981). *E. euryalis* is located on the wing-membrane and not very often on the tail membrane (e.g. see below) in summer and in the coat bordering the wings during winter (Deunff & Beaucournu 1981).

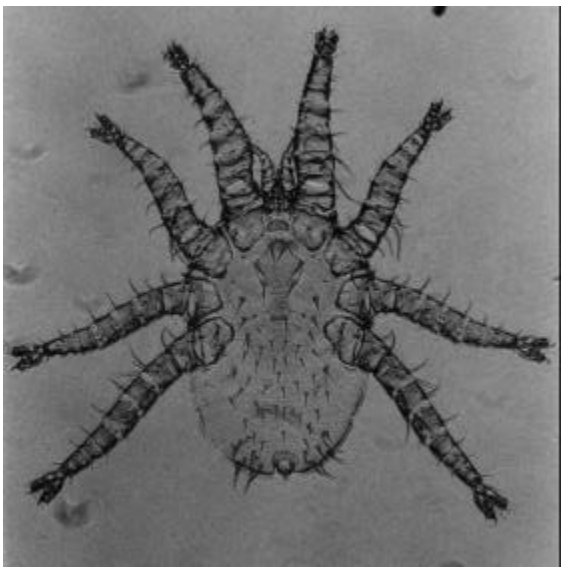

Figure 1 Eyndhovenia euryalis; female,
ventral view, collected from a female Greater horseshoe bat in Little Dean Hall (Forest of Dean)

(6 time obj lens/1cm=158.7 µm; picture size 80%)

1.2.5 Systematics of *Eyndhovenia euryalis* (Rudnik 1960)

Phylum:	Arthropoda
Subphylum:	Chelicerata
Class:	Arachnida
	Pseudoscorpionida
	Araneida
	Oplionida
Order:	Acarina, mites
	A Parasitiformes
	B Acariformes
Suborder:	Mesostigmata
	Metastigmata (=Ixodides)
Supercohort	Monogynaspida
Cohort:	Gamasina
Superfamily:	Parasitoidea
Family	Spinturnicidae
Genus	*Eyndhovenia*
Species	*Eyndhovenia euryalis*

1.3 Greater Horseshoe Bats

1.3.1 General

Bats are the only mammals capable of flying and they are classified in their own order, the Chiroptera. This order is divided into two suborders. The Megachiroptera only consists of one family and only one species (*Rousettus aegyptiacus)* is found in Europe (Schober & Grimmberger 1998). Of the 17 families belonging to the suborder Microchiroptera Rhinolophidae, Vespertilionidae and Molossidae are found in Europe (Schober & Grimmberger 1998). The Greater horseshoe bat, *Rhinolophus ferrumequinum*, is the largest European species in the family Rhinolophidae (Schober 1998). Together with the Noctule, *Nyctalus noctula*, and Serotine, *Eptesicus serotinus*, Greater horseshoe bats are among the biggest European bats. *R. ferrumequinum* have a mass between 16 g and 30 g, depending on the time of the year. The wingspan is between 33 cm to 40 cm and the forearm length between 51-61 mm. Males are about 2% smaller than females (based on forearm measurements), and weigh 2-15% less (Ransome 1990). *Rhinolophus ferrumequinum* can reach an age up to 30 ½ years (Caubère *et al.*1984, in Schober & Grimmberger 1998).

1.3.2 Distribution

Bats are widespread and found almost everywhere in Europe and even in most parts of Scandinavia (Schober & Grimmberger 1998). Populations of many bat species have decreased recently (Stebbings 1988).

Rhinolophus ferrumequinum prefers mild and warm regions. The Mediterranean and Balkans are the main areas of Greater horseshoe bats distribution (Schober 1998) (see Figure 2). *Rhinolophus ferrumequinum* is an endangered species today and is almost extinct in Germany (Schober & Grimmberger 1998). The most northern colonies are found in south west England. In Germany there is only one known small nursery colony and a few hibernating sites.

The number of *R. ferrumequinum* decreased in England from 19th century. Today there is an estimated number of about 5000-6000 (Harris *et al.*1995). However the Greater horseshoe bat population seems to be increasing again (Ransome 1990).

Figure 2 Distribution of *Rhinolophus ferrumequinum* in Europe (from Schober 1998)

1.3.3 Ecology

In Chiroptera communal roosting is very common and bats may originally have inhabitated caves. As human civilisation expanded some bats started to roost in buildings. In northern areas of its distribution *Rhinolophus ferrumequinum* roosts in buildings during the summer time, and hibernates in caves and mines. In southern Europe Greater horseshoe bats colonise caves all year round (Schober 1998). Together with breeding females and their offspring, sub-adult males and females can also be found in maternity roosts (see below). Adult males usually live solitarily mines and buildings surrounding the maternity roost (Schober 1998), but they are also found in the maternity colony from time to time (see below). Seasonal migration, especially between summer and winter roosts, is very common in bats. Some species like *Nyctalus* or *Pipistrellus* undergo seasonal migrations of up to 1700 km (Altringham 1996). Distances between summer roosts and hibernation sites in Greater horseshoe bats are usually between 20 to 30 km (Schober & Grimmberger 1998).

Most European Chiroptera are insectivores. The diet of *Rhinolophus ferrumequinum* seems mainly to consist of beetles, moths, dipterans and Hymenoptera. Prey spectrum differs over the seasons. Prey found in droppings and below their roosts suggest that *R. ferrumequinum* prefers large prey (Jones 1990; Pir 1994).

1.3.4 Reproduction and Offspring

Bats in temperate climatic zones are mostly monoestrous. As mating usually occurs in the autumn, female bats either use delayed ovulation and fertilisation or delayed implantation to time the birth of their offspring (Altringham 1996). Unlike other small mammals they only give birth to a small number of pups, most often only one. The rearing of offspring is carried out exclusively by the females. There is only one reproductive cycle per year in Greater horseshoe bats. Their common mating time is in autumn from September to November but pairing continues through hibernation and spring (Ransome 1990). Fertilisation takes place after hibernation. Females normally give birth for the first time at about the time of their third birthday, and males produce sperm in either their second or third spring (Ransome 1990). Females give birth to only one offspring at a time. Depending on the climate pregnancy can last up to 75 days. The usual gestation period is 6 to 8 weeks. Juveniles are born without fur and with eyes closed. On the date of birth their mass is about 5 – 6g. At the age of seven days they open their eyes and already have fur (Schober 1998). Juveniles of Greater horseshoe bats are capable of flying at an age 17 days old and are fully grown within 60 days of birth (Ransome 1991). They leave the roost at about 28 days of age. Even though they start to learn foraging from this time they are still fed by their mothers up to an age of about 45 days (Jones *et al.* 1995)

1.4 Aims and Hypotheses of the Project

1.4.1 Aims of this Study

Little is known about bats as ectoparasite hosts. One aim of this study was to analyse parasite-count data collected over the last 4 years to receive information about ectoparasite distribution in the colony. Interesting topics were ectoparasite distribution over the seasons and in different groups of the bat society.
In the other part of this study grooming behaviour in Greater horseshoe bats is investigated. One aim was to find out whether allo-grooming between adult bats occurs and if so whether more often within than between matrilines. Social grooming is already described in other Chiroptera species (e.g. Wilkinson 1986). Relatedness in the colony is known (Rossitter *et al.* 2000). Time spent self maintenance in juveniles and lactating females has been of major interest as well.

1.4.2 Hypotheses to evaluate in this Study

- Parasite load in juveniles will vary according to their age: Infants might be more defenceless against ectoparasites in several ways than fully-grown juveniles.
- Parasite load will be highest in adult and juvenile bats in poor condition: Bats in poor condition might be defenceless against ectoparasites in several ways.
- Parasite load will be lowest in adult males: Adult males do not live in the maternity roost and are less likely to be infested.
- Parasite load in lactating females will be higher compared to pregnant or non-breeding females: If grooming reduces parasite load this will have an impact on lactating females. They will have less time for self-grooming than pregnant and non-breeding bats.
- Overall parasite load will be highest in summer: Ectoparasites reproduce in summer, a higher number of mites will lead to a higher infestation level.
- Percentage infestation will rise with number of parasites in the roost: As more parasites are in the roost as more likely will it be to be infested.
- Juveniles will receive less allo-grooming by their mothers as they grow older: Juveniles will gain the ability to groom themselves with age and will therefore receive less allo-grooming by their mothers.

- Younger juveniles will perform less self-grooming compared to older juveniles: Young juveniles will not have the same ability of self-grooming as older juveniles and they will be allo-groomed by their mothers.
- Time spend self-grooming in lactating bats will rise as their offspring grows older: Lactating bats will devote more time self maintenance as offspring grows and needs less care by its mother.
- Time spend grooming will be inversely related to parasite load: If grooming is effective for removing ectoparasites, individuals will carry less ectoparasites as more time they groom themselves.

2 Material and Methods

2.1 The Colony

The colony breeding roost of about 100 Greater horseshoe bats and about 30-40 infants born in every year is situated in the attic of the Woodchester mansion (see Figure 3) near Stroud in Gloucestershire. The mansion was abandoned in an unfinished state in the late 1860s and is still unoccupied by man. This site has been studied for more than 40 years (Ransome 1990). Today all bats are ringed. A population genetics study has been carried out over the last years (Rossitter *et al.* 2000). From both the ringing studies and the genetic investigations, matrilineal relatedness is well known.

Figure 3 The Woodchester Mansion (Picture from Ransome 1990)

2.2 Parasite Load

2.2.1 Counting Parasites

Parasites on the bats were counted 18 times from 1996 until 1999. In every year, bats were caught for parasite counts only between four to six times to minimise disturbance. Table 1 shows on which day in which month of each year bats were caught. On these days all bats were caught with a hand net from their roosting places at about 7:00 hrs and packed individually in fabric bags. After measuring and examining, the bats were weighed and the forearm length was taken, parasites on the wings and the tail membranes were counted. Parasites were counted by extending the wing- and tale membrane onto a photography light-box. Wing and tail membranes were stretched carefully on the pane and screened for parasites. Bats were recognised by their ring number. As the parasites stick on very well, they do not fall off when the bats are measured. All parasite counts were carried out by the same person and the parasites were not removed. Mainly one species of parasite was found, the wing-mite *Eyndhovenia euryalis* (>95%, Jones personal observation) determined after Rudnik 1960.

Table 1 Capture dates in the four years of this study

	1996	**1997**	**1998**	**1999**
May			7.	13.
June	4.	3.	9.	
July	21.	13.	12.	10.
August	25.	3. 24.	3. 24.	2.
September	5.		14.	3.

2.2.2 Database

A data base containing 1113 data sets was created with Microsoft Excel 97. Data sets were combined by using Microsoft Access 97. Table 2 shows the information each data-set contains about a bat. The database was evaluated with Microsoft Excel 97, Microsoft Access 97, Sigmaplot 5.0 and Minitab 12.

2.2.3 Age Determination in Juvenile Bats

Day 0 infants can be identified because the umbilical cord is still attached. Older infants are aged from a growth curve of known age infants.

2.2.4 Droppings

The droppings R. Ransome uses for his investigation were collected from the fabric bags bats were kept in (see 1.2.1 Counting Parasites).

Table 2 Information every data set contains about a bat

Reference number	Every bat receives a reference number when it is caught the first time in its life. This number stays the same during a bat's life.
Ring number	The ring number of the bat when it is caught. Ring numbers can change during lifetime. New born juveniles first get coloured plastic rings, which are later changed to the usual metal rings.
Date	Capture date
Mother Reference number	Where known the reference number of the mother bat is filled in.
Age of the bat	Age in days if it was a juvenile Age in years when it is a sub adult or adult bat
Birthday	Only mentioned for juvenile bats, was counted backwards when age in days was known
Days of lactation	Only lactating females (age in days of the juvenile was used as days of lactation for the mother bat)
Body-mass	Every bat was weighed on every capture, mass in g with an accuracy of one decimal place
Forearm length	The length of the forearm was not measured every time in adult bats, so if this value was missing in the list it was filled in from former captures. The length in mm with an accuracy of one decimal place
Sex/Age Status	The population was divided in the following groups: - Juvenile-when bat was born in the same year - Lactating female-when on the same date or later on the juvenile of this year was caught* - Pregnant female-when an offspring bat was caught later on in the year* - Non breeding female-when no offspring were caught* ** - Adult male-two year or older males with visible testes - Sub adult male-one to two year old males without visible testes
Number of pups	Information on how many offspring a female bat had inclusive of the year of the capture.
Sex	Gender of the bat
Parasites	The sum of parasites counted on wings and tail membrane
Mean d^2	If known (see 3.3.10)

- *when there was any doubt, e.g. a bat had swollen nipples but no juvenile was caught, this row was left empty
- **apart from two exceptions all non-breeding bats were sub-adult

2.3 Grooming Behaviour

2.3.1 Recording Videotapes

To investigate the grooming behaviour in Greater horseshoe bats, video recordings of the colony were used. The colony has been continuously observed over the last years with an infrared light and an infrared sensitive video camera. Tapes are only recorded at night-time as there is not much activity during daytime in the roost (personal observation). Using video equipment allows observation with minimal disturbance. Studies in Mexican free-tailed bats, *Tadarida brasiliensis mexicana* (Mistry & McCracken 1990) showed no behavioural change responding to this kind of light. Video recording produced in average about up to 10 hours coverage per night including pre-emergence period and post-return period. As Greater horseshoe bats always hang freely, behaviour is “readily” to observe. Tapes for this investigation were recorded during 1997, 1998 and 1999. A sketch of observing bats in the attic is pictured in Figure 4.

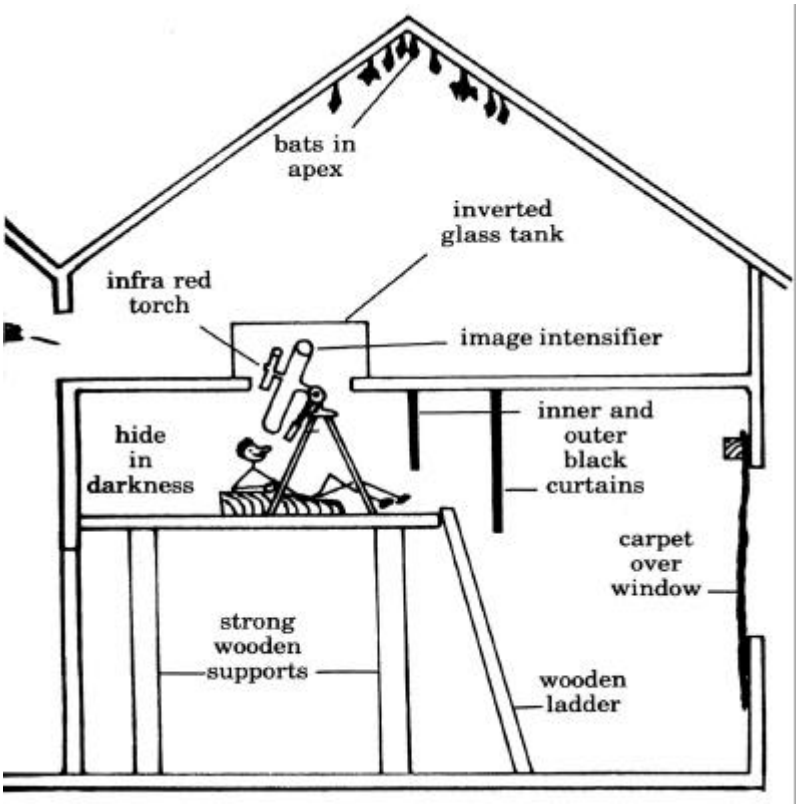

Figure 4 Observing bats in the attic of Woodchester Mansion. For the observation a custom-made time lapse infra-red video system (Omega Nightvision Ltd) is used (picture from Ransome 1990)

2.3.2 Marking Adult Bats

The metal rings all bats are banded with can only be used to identify a captured bat. To distinguish bats individually on the video some bats were marked. Table 3 shows how many bats were marked and on which dates. In 1997 and 1998 a radio-tracking study was carried out. These were the occasions when bats also were marked for video recordings. In both years, the radio-tracking study consists of three sessions. When bats were caught they were tagged and every radio transmitter (0.7-1.3g, Holohil, Carp, Ontario) was individually painted with black and white patterns, so bats could be distinguished on the video screen. Figure 5 shows examples of the black and white patterns used. Tags were attached between the shoulder blades, using Skinbond Surgical Adhesive, after having clipped the fur. Also light plastic discs (r=0,6 cm) painted in the same pattern were glued onto the back of the head. Radio-tags lasted from a few days up to four weeks and plastic discs usually last between three and four days.

Table 3 Radio tracking data: Dates when and how many bats where marked

Date	13.7.97	3.8. 97	24.8.97	12.7.98	3.8. 98	24.8.1998
Number of marked bats	4	7	5(1)*	8(1)*	10(1)*	9
Number of bats in the roost	85	79	46	84	92	59
Percent of bats marked	~ 5%	~9%	~11%	~10%	~11%	~15%

* in brackets is the number of marked non-breeding females

Figure 5 Examples for used patterns to mark bats individually

2.3.3 Marking and Observing Juveniles

Juveniles were not marked on the same occasions as adult bats. One idea to get data from juveniles was to use the video recordings and to measure the forearm length on the screen and determine age. Therefore, data from juveniles at different age could have been collected. The problem with this method was that the attic had a slope and the measurements taken were not accurate enough to determine age. In September 1999 eight juveniles between 55 and 77 days of age were marked with plastic discs (see above), glued on the back of their heads. This was to collect independent data of grooming behaviour in older juveniles. Unfortunately the juveniles moved to a place in the attic that was not monitored by the video-camera and later on the camera fell over, so only few data were collected from this session. As juveniles are born without fur, one can distinguish between juveniles with and without fur. Juveniles without fur are between zero and seven days of age. However, these young juveniles could not be observed individually as they were always attached to their mothers when monitored.

2.3.4 Observed Arena

The video image covered an arena of about 63cm x 42cm. To focus on always as many bats as possible, conditions were not kept constant. Area and size covered were changed from time to time. Figure 6 gives an example what a video image looked like. I watched the videos in upside down format, also shown in Figure 6, as this appeared to me making the observation much easier.

Figure 6 Example of the observed arena

2.3.5 Analysing Videotapes

Data were collected with focal-animal sampling (Altmann 1973) and analysed with the OBSERVER software from Noldus. With this software, it is possible to create behaviour classes containing several behaviour elements. Behavioural observations can be recorded with the computer. As bats were marked individually, one could monitor a single bat's behaviour and the time it spent performing this behaviour. In Table 4 the observed behaviours for analysing grooming behaviour are described.

Table 4 Behaviours and definitions observed for analysing grooming behaviour on videotapes.

Behaviour classes	Behaviour elements	Definition	Remarks
Presence	Present	Bat is full visible in arena	
	Absent	Bat is not visible in the arena	
Grooming*	Self-grooming	Bat is grooming itself	
	Allo-grooming offspring	Bat is grooming its infant	
	Allo-grooming	One bat is grooming another bat which is not its infant	Was not observed
	Non grooming	Bat does not groom	

2.3.6 Analysed Time

Radio tracking data from the begin of my work in summer 1999 showed that lactating bats returned from foraging to the Woodchester mansion in the middle of the night. They than stayed there between 1 to 3 hours before leaving to forage again. Radio tagged bats without offspring were also monitored in this study coming back to the roost during night-time. In addition viewing videos beforehand suggest that bats return in the middle of the night (shown in Table 5). The defined standard observation time for grooming behaviour was four hours after sunset (data for sunset from the Whittacker almanac 1997,1998 and 1999) and lasted for one hour.

Table 5 Two video observation nights: Number of adult bats visible in the image scanned in half hour intervals

Time	Estimated number of adult bats being present	
	16./17.7.98	26./27.8.98
20:00 h	15	
20:30 h	14	none
21:00 h	14-15	none
21:30 h	6	2
22:00 h	none	3-4
22:30 h	1	4
23:00 h	0	7
23:30 h	9	8
00:00 h	10	9
00:30 h	8	10-11
01:00 h	4	9
01:30 h	2	13
02:00 h	2	16
02:30 h	0	20
03:00 h	0	25
03:30 h	5	lots
04:00 h	0	30
04:30 h	4	>30
05:00 h	10	15
05:30 h	>35	13
06:00 h	>35	25-30
Number of bats habitating the roost on this date	~ 84	~ 60

These two nights where also analysed to describing night time budgeting in Greater horseshoe bats. Further behaviours observed in this context are shown in Table 6. Behaviour classes were inspired by Winchel & Kunz (1993).

Table 6 Behaviour observed in addition to grooming behaviour; relevant categories

Behaviour	Definition	Remarks
Resting	Motionless rest	
Alert/active	Head or body raised in alert position or in motion	
Moving	Moving about the roost	
Offspring attached	A bat hangs onto another bat the other way round the two are motionless or swinging back an forwards	This behaviour was observed during this study.
Touching-noses	Bats touching the nose of the other bat	

2.4 Statistics

For statistical analysis the programs Minitab for Windows, release 12.1 and Sigma Plot for Windows Version 5.0 were used. The test for normal Distribution was in all cases the “Anderson-Darling Normality Test”. If not mentioned otherwise data points are independent (only one data point per bat) and parasite data is not normally distributed (see 3.4. Parasite Distribution, Figure 4).

Correlation coefficients:
r = Pearson
r_s = Spearman’s rank

3 Results

3.1 Time-Budgeting

In order to get a broad overview of behaviour and time budgeting in Greater horseshoe bats, video-recordings made during the nights of the 16th July 1998 and of the 27th of August 1998, were analysed. Each recording took place between 20.00 hrs and 6.00 hrs. Data from nine different bats were collected. Results are presented in Table 7. Self-grooming was more often observed in the night of the 27th of August 1998. Allo-grooming the offspring was only observed on the night of the 16th July 1998, where offspring was between 6 to 24 days old. During all other observation it became apparent that no distinctive pattern could be found to show how bats time budget their nights.

Table 7 Night time-budgeting in nine adult females observed in two nights

Bat No	Age in years	Age of offspring in days	Month	Time Present in minutes	*Self-grooming	*Allo-grooming	Times touching nose	*Resting	*Active	*Moving	*Kid-attached
1	5	6	July	1,4	6,7	42	0	0	14	26	0
2	6	12	July	33	1,9	1,2	0	82	5,3	4,6	0
3	8	18	July	72	0,5	22	0	0	0	0	72
4	10	24	July	76	33	0	0	45	0,8	0,4	18,8
5	2	non	July	79	31	0	5	55	8,0	1,5	0
6	2	non	July	0,7	0	0	0	0	0	100	0
7	4	41	August	177	12	0	13	32	2,8	1,7	47
2	6	53	August	273	27	0	4	60	2,1	1,4	8,8
8	10	54	August	9,1	0	0	0	0	4,0	20	0
3	8	59	August	9,2	75	0	0	25	0	0	0
9	8	59	August	7,3	39	0	0	33	17,6	0	0

* % of time being present

3.2 Grooming

3.2.1 Allo-grooming (within a matriline family and an other matriline group)

Allo-grooming between adult marked females was not observed, this also applied to unmarked bats. Allo-grooming was exclusive to mothers grooming their offspring only.

3.2.2 Grooming in Adult Females

Up to ten adult female bats were marked in any one session, where most of them where lactating. Table 8 shows the length of time observed bats were present within an observation hour. During this time their grooming habits and time taken to carry out this activity was recorded; time spent self-grooming and time spent grooming their offspring. Observations from 20 nights resulted in 14 conclusive recordings.

Table 8 Adult females: age of offspring and time spent grooming

Age of juvenile in days	% Time being present in observation hour	% Time spent self grooming in time being present	% Time spent allo-grooming juvenile in time being present
1	16,87	0,00	33,70
14	91,99	13,04	10,50
15	34,74	0,00	6,22
17	20,27	37,72	3,07
24	0,50	0,00	0,00
32	14,58	4,72	0,00
37	24,64	27,71	0,00
44	4,19	90,95	0,00
45	96,88	23,55	0,00
51	35,71	0,00	0,00
51	96,88	0,00	0,00
No offspring	13,20	89,50	0,00
No offspring	11,20	65,00	0,00

As offspring grows, lactating bats will spent less time at the roost during night time activity. However, no significant correlation between time present and age of offspring was found (r = 0,135; P > 0,6 n=5). The criteria for this analysis also considered that lactating bats may devote more time to self-grooming as juveniles grow. But this was not the case. The time individual mothers spent self-grooming against a growing scale in the age of her offspring showed no correlation: r = 0,067; P > 0,05 , n= 7; (see also Figure 7 below).

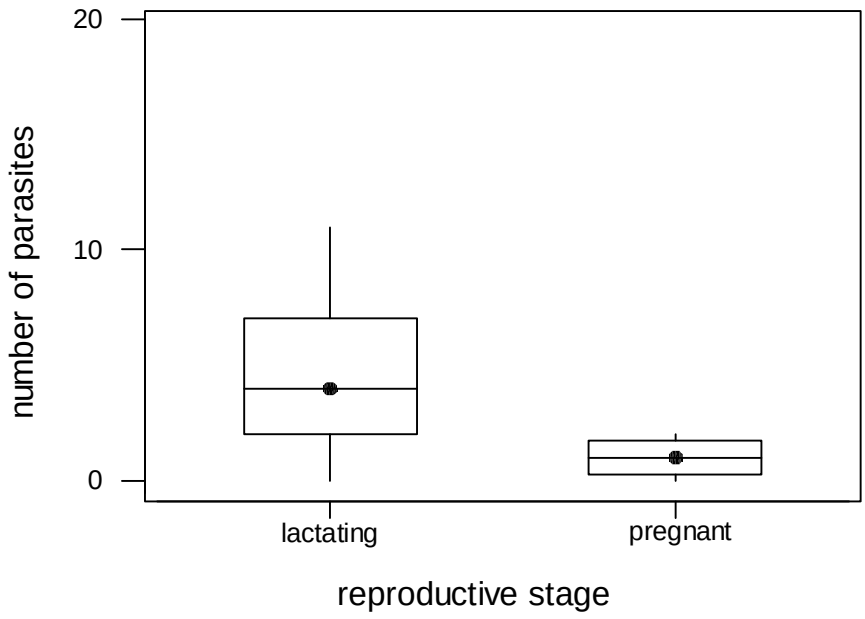

Figure 7 Lactating bats: Time mothers spent self-grooming against age of their offspring

Significantly, it was found that the amount of time mothers spent allo-grooming their young did correlate to offspring age: r=0,743; P < 0,01; n=10. In this case a scale of decreased attention was observed and expected, as the offspring grew (see also **Figure 8** below).

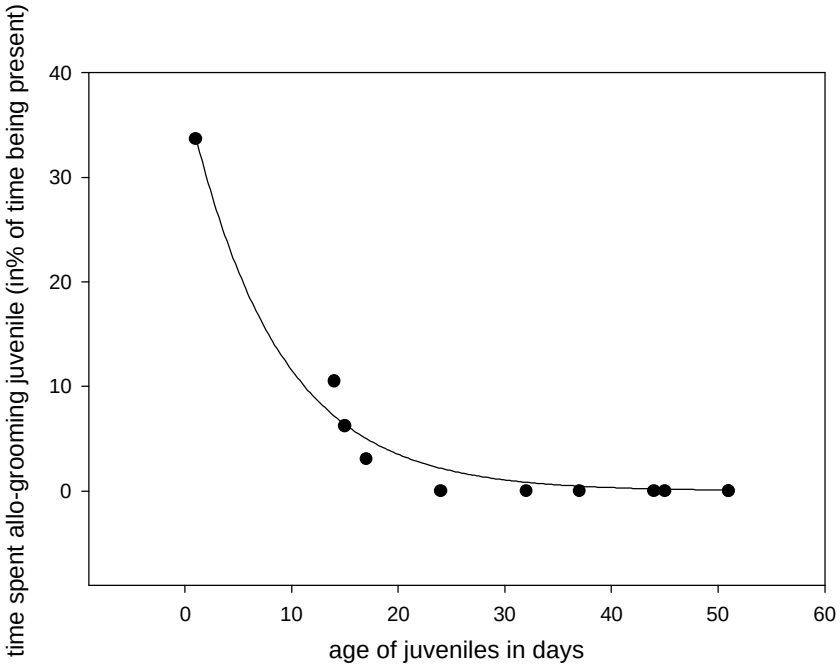

Figure 8 Lactating bats: Time mothers spent allo-grooming offspring against age of their offspring *;* non-linear regression

The time mothers spent allo grooming their offspring can be described with the equation:

$f(x) = ae^{-bx}$; with x = age of juvenile and f(x) = time receiving allo-grooming

(b>0); a = 38,2248; b = -0,1195; (Df=1, SS=1013, F=442, P<0,0001, R=0,99)

Time spent self-grooming in reproductive and non-reproductive females during the same time of the year (14th July 1997 and 16th July 1998) was compared, but no significant difference was found; Reproductive females n = 3; Median = 0; Non-reproductive females n = 2; Median = 77,25; P > 0,05 (adjusted for ties).

3.2.3 Juveniles

Juvenile grooming behaviour was also considered. Table 9 shows the finding on grooming behaviour in juvenile bats of 60 days and older.

Table 9 Older juvenile bats: Age, sex and time spent self-grooming

Age in days	sex	date	% of time being present spent self-grooming
60	Male	8.9.99	42,95
68	Female	7.9.99	95,05
71	Female	6.9.99	15,13
78	Male	9.9.99	62,11

Juveniles aged between 60 to 78 days showed no significant difference in their grooming habits when compared with infants between 7 to 20 days old; (Wilcoxon-Mann-Whitney Test; Old Juveniles N = 4 Median = 52,53 Young N = 2;Median = 36,92;P= 0,4875). Whilst observing the grooming habits of juveniles a difference in the attention paid to the grooming process was noticed. One young juveniles, for example groomed a wing over and over for some time, suggesting it was not satisfied with the cleanliness (or whatever the qualitative intention) of the wing and so continued over again until it was satisfied with the result. The grooming habits of older juveniles was similar to that of adult bats.

3.2.4 Other Social Interactions

Allo-grooming between adult bats throughout the series of observation sessions was not observed. However, adult bats had another kind of physical contact, which can only be described as touching noses. This point of contact between bats was fleeting, lasting on average one second. This difference of social contact between adults in contrast to their offspring was also observed by Kulzer who kept Greater horseshoe bats in captivity (personal observation). Nose touching was also observed in a study of lesser long-nosed bats (*Leptonycteris curasoae*) (Fleming *et al* 1998).

Other social interactions included mothers who continued to roost with their young apparently beyond necessary parental care. As juveniles grew into independent bats they continued to attach themselves to their mothers as late as 53 days old. Mother and young pair with the juveniles attached upside down, they can be seen hanging and sometimes swinging together.

3.3 Parasites

3.3.1 Composition of Data

Between 1996 and 1999 more than one thousand parasite-count data from two-hundred different bats were collected. More than half (119) of these bats were females. On average a bat was caught five times. Individual female bats were caught up to 16 times, whereas male bats were only caught up to 8 times. Twenty bats were caught only once, half of which were juveniles and might not have survived their first year. Table 10 shows the composition of all catches. As can be seen more than half of the data was collected from lactating or juvenile bats, because the investigated site is a maternity roost. Interestingly, sub-adult females were caught almost twice as often as sub-adult males. This suggests that sub-adult females are more likely to stay in the roost than sub-adult males. Parasites were mainly found on the wing membranes. Of the 4083 counted parasites in this study only 41 (1%) were found on the tail membrane whereas 2089 (51%)were counted on the right wing and 1953 (48%) on the left wing.

Table 10 Composition of captured bats by sex, age and reproductive status

Status	Times caught	% Of total
Adult Male	39	3,5
Pregnant Bats	73	6,6
Lactating Bats[1]	288	25,9
Juveniles	372	33,4
Sub-adult female	161	14,5
Sub-adult male	86	7,7
Unknown[2]	94	8,5
Total	1113	100

[1] Lactating: bats were lactating when they were caught or earlier in the year

[2] All except for five

Table 11 reflects how many bats were caught each year. Even with capture dates in May and September (months when bats may arrive from or are about to leave for their winter roost) the mean number of bats caught in each capture increased in 1999. In the same year the highest number of different bats were caught as well. This suggests that the colony size in Woodchester Mansion is about to increase again, as it decreased from the early 60s (Ransome 1990).

Table 11 Number of capture sessions per year and number of bats caught

Year	Number of Bats caught	Number of captures	Mean bats per Capture	Number of different bats
96	218	4	54,5	87
97	252	4	63	93
98	325	6	54,2	104
99	318	4	79,5	120

3.3.2 Parasite Distribution

Figure 9 shows the parasite distribution on bats for all catches. The distribution is not normal but right skewed with a minimum of 0, a maximum of 36, and a median of 2 parasites per bat.

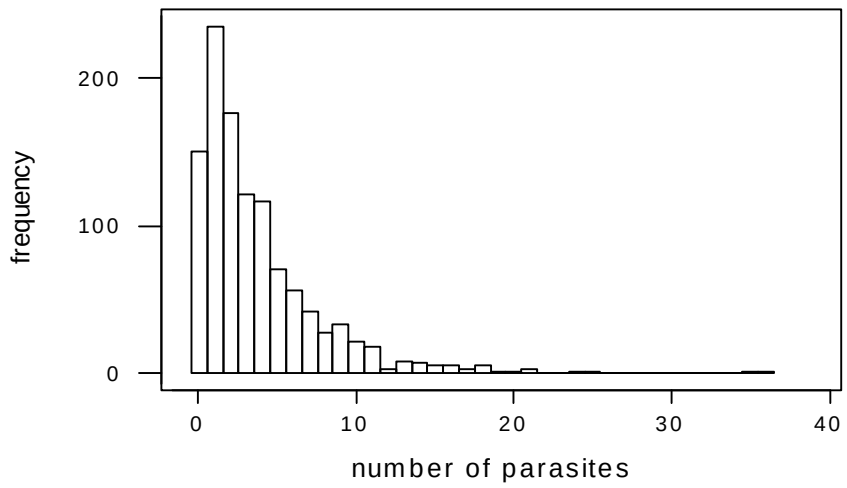

Figure 9 Distribution of parasites found on individual bats in all catches (n=1113)

3.3.3 Infestation of the Colony

Table 12 gives an overview of the colony's infestation percentage. Infestation is understand as one or more parasites found on a bat. In the same table the median number of parasites found on a single bat are given. There is a positive correlation between the infestation percentage of the colony and the median parasite-load on bats: $r_s = 0,835$; $P < 0,001$; $n = 18$. This means that as more parasites are present in the colony, it is very likely that each bat will be infested with at least one parasite. Most often more than 80% of the bats were found to be infested, but at least approximately three quarters of them were always infested. All the bats were infested on only one date.

Table 12 Overall infestation of the colony and median number of parasites per bat on each capture date

Date	% Of bats infested	Median number of parasites per bat	Date	% Of bats infested	Median number of parasites per bat
04.06.96	71%	1,0	07.05.98	76%	2,0
21.07.96	77%	2,0	09.06.98	83%	2,0
25.08.96	84%	3,0	12.07.98	85%	3,0
05.09.96	100%	3,0	03.08.98	96%	4,0
03.06.97	95%	4,0	24.08.98	97%	4,0
13.07.97	89%	4,0	14.09.98	88%	2,5
03.08.97	95%	4,0	13.05.99	72%	1,0
24.08.97	93%	3,0	10.07.99	77%	2,0
			02.08.99	89%	2,0
			03.09.99	83%	1,0

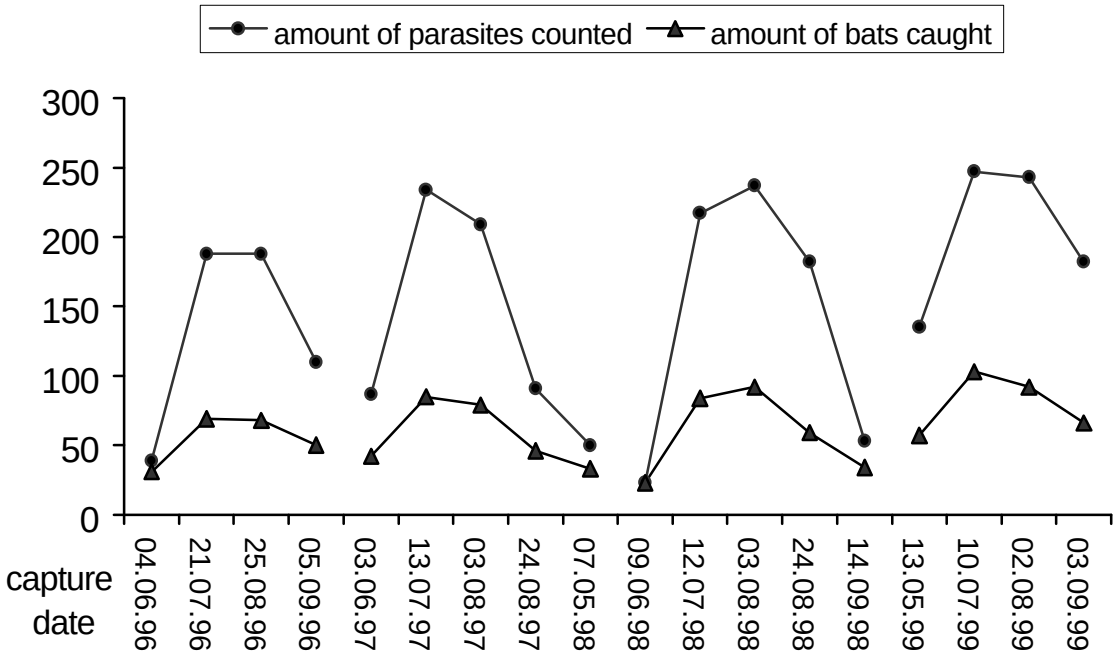

Figure 10 All capture dates: Number of parasites and number of bats

A strong positive correlation (r_s=0,979; p < 0,001; n=18) was found between the number of bats caught and the number of parasites counted (see Figure 10).

Table 13 below contains the infestation percentage of bats in different life states. Between 85% and 100 % of juveniles and lactating bats were infested. Lower infestation rates were sometimes found in other groups.

Table 13 Percentage infestation of bats grouped via different life stages on all capture dates

Capture Date	JUV %	LAC %	PRG %	SUF %	SUM %	AMA %
04.06.96			60%(10)	90%(10)	57%(7)	50%(2)
21.07.96	93%(27)	85%(27)	0%(1)	38%(8)	33%(6)	
25.08.96	89%(27)	92%(25)		57%(7)	60%(5)	
05.09.96	100%(23)	100%(14)		100%(3)	100%(4)	100%(1)
03.06.97			92%(12)	92%(12)	100%(9)	100%(5)
13.07.97	97%(30)	93%(28)		73%(11)	100%(7)	40%(5)
03.08.97	96%(28)	96%(26)		92%(12)	100%(6)	67%(3)
24.08.97	96%(24)	92%(12)		100%(6)	67%(3)	
07.05.98			83%(23)	25%(4)	67%(3)	
09.06.98			100%(5)	78%(9)	80%(5)	50%(2)
12.07.98	100%(28)	97%(29)	75%(4)	54%(13)	75%(4)	
03.08.98	100%(32)	97%(31)		100%(15)	75%(4)	71%(7)
24.08.98	100%(27)	92%(25)		100%(3)		
14.09.98	85%(26)	100%(2)		100%(5)		
13.05.99			83%(18)	73%(11)	60%(10)	0%(1)
10.07.99	97%(33)	90%(29)		57%(14)	63%(8)	33%(9)
02.08.99	100%(36)	93%(27)		67%(12)	50%(4)	
03.09.99	84%(31)	85%(13)		75%(8)	0%(1)	50%(2)

JUV= juveniles; LAC = lactating bats; PRG = pregnant bats; SUF = sub-adult females; SUM = sub-adult males; AMA = adult males The number in brackets is N

3.3.4 Parasite Load in Relation to Age/Sex Class

The population can be distinguished as adult non-breeding females, juvenile bats, pregnant or lactating females, sub adult male or female bats, adult male bats. This differentiation makes sense as these groups have a different way of living. Adult males usually roost solitary, although occasionally, they are caught in the colony. To compare parasite load of bats at different stages three different dates where chosen. As adult male bats are rarely caught I concentrated on dates when several where present. Results are presented in Table 14 below. On all three dates a significant difference between parasite load of the different groups were found. Noticeable is that adult males carried the least parasites on all dates studied. This may result from mainly living a solitary life outside the colony, where contact with parasites is low.

Table 14 Results of the Kruskal-Wallis test to compare parasite load in relation to age/ sex class

	3.6.97	13.7.97	3.8.98
	Median	Median	Median
Sub adult females	7,5 (n=12)	1,0 (n=11)	3,0 (n=15)
Sub adult males	4,0 (n=9)	2,0 (n=7)	1,0 (n=4)
Adult males	2,0 (n=5)	0,0 (n=5)	1,0 (n=7)
Pregnant females	4,0 (n=12)		
Lactating females		4,5 (n=28)	4,0 (n=31)
Juveniles		6,0 (n=30)	8,0 (n=32)
P(adjusted for ties)	P = 0,028	P = 0,000	P = 0,000

3.3.5 Changes throughout the Years of Counting

To see whether the parasite load in the colony is the same in the different years for July data from each year was compared. July is the only month where bats were caught in all four years. For the other months at least one year is missing. In terms for the colony it was found that in July the parasite load was not the same in every year. Results suggest more parasites in 1997 and 1998. This finding is consistent with what was found when only comparing parasite load in juveniles (see 0.) Comparing parasite load in the colony without juveniles lead to similar results as shown in Table 15.

Table 15 Comparison of parasite load in July for the four years of parasite counts in the colony, with and without juveniles bats (results of Kruskal-Wallis Test)

Year	Median number of parasites (Juveniles included)	Median number of parasites (Juveniles excluded)
1996	2 (n=69)	1 (n=42)
1997	4 (n=85)	3 (n=55)
1998	3 (n=84)	2 (n= 56)
1999	2 (n=103)	1 (n=70)
P (adjusted for ties)	P < 0,001	P < 0,001

3.3.6 Season and Parasite Load

Comparing the median number of parasites on bats in each year on all dates I found a significant difference in 1996, 1998 and 1999. In 1997 no significant difference in the median parasite load was found between the different months. For the remaining years it was consistently found that the median parasite load was lowest in the earliest date for each year. Results are shown in Table 16.

Table 16 Median number of parasites on bats on every capture date (Kruskal-Wallis Test)

Year	Date	Median (n)	P (adjusted for ties)	Year	Date	Median (n)	P (adjusted for ties)
1996	4.6.96	1 (31)	<0,001	1998	7.5.98	2 (33)	<0,001
	21.7.96	2 (69)			9.6.98	2 (23)	
	25.8.96	3 (68)			12.7.98	3 (84)	
	5.9.96	3 (50)			3.8.98	4 (92)	
1997	3.6.97	4 (42)	>0,1		24.8.98	4 (59)	
	13.7.97	4 (85)			14.9.98	2,5 (34)	
	3.8.97	4 (79)			13.5.99	1 (57)	<0,002
	24.8.97	3 (46)			10.7.99	2 (103)	
					2.8.99	2 (91)	
					3.9.99	1 (66)	

Similar results are found when all data was brought together. Results are shown in Table 17. Six occasions were distinguished May, June; July, early and late August and September. Parasite load in the colony was not found to be the same on all occasions. Particularly in May, the Median was just one mite per bat, whereas at the other times it differed between two and three. For both statistics always all data was used.

Table 17 Median number of parasites for all four years distinguished in 6 seasons

	May	June	July	early August	late August	September
Median	1(n=121)	3(n=65)	2(n=341)	3(n=262)	3 (n=262)	2(n=150)
Kruskal-Wallis Test P < 0,001 (adjusted for ties)						

3.3.7 Juveniles

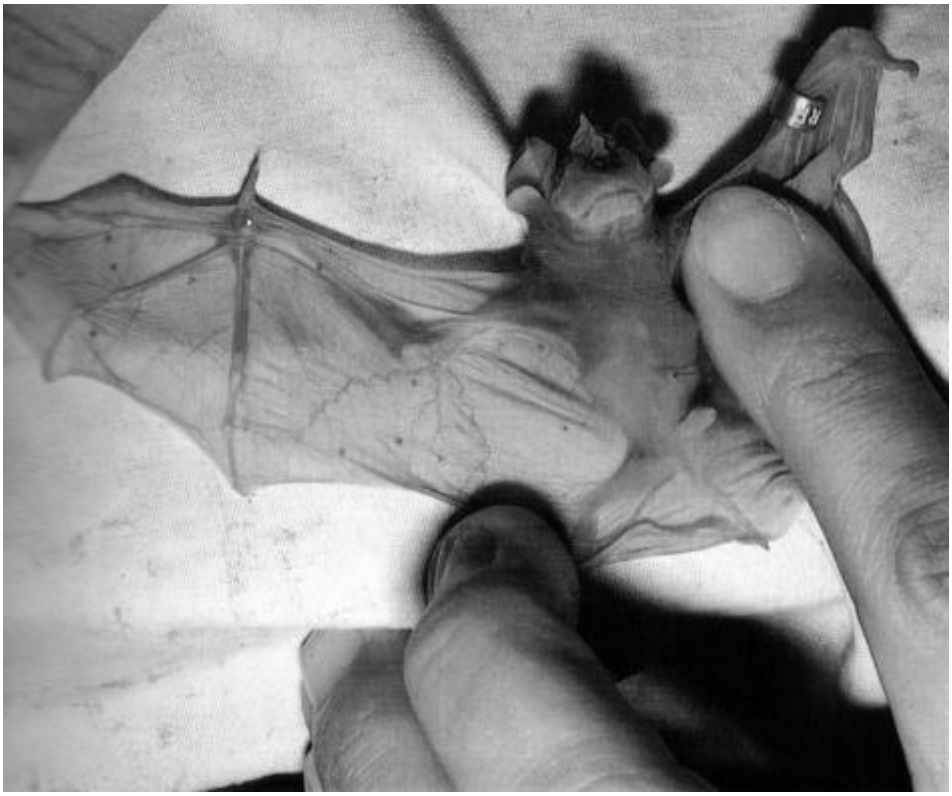

Figure 11 A male Greater horseshoe bat of a view days old highly infested with wing-mites (picture from Ransome 1990)

Why is it interesting to closely study juveniles and their parasite load? As soon as the juveniles are born there are obviously more hosts available for the mites. The life cycles of the host and parasite might be synchronised (Foster 1969). Juveniles are dependant on their mothers. Their immuno-competence might not be fully developed and their ability to groom themselves may be limited (Marshall 1982; McLean &Speakman 1997). Thus, infants could be preferable hosts for parasites. Figure 11 illustrates a young male juvenile with parasites. Even though bat juveniles are born without fur, *Eyndhovenia euryalis* was only found on the wings in every developmental state of juveniles.

Table 18 shows juveniles birth dates. All were born over a period of approximately one month each year. There was up to two weeks difference between the years when bats begin their birth period. The difference of the mean birthday was also up to two weeks. Ransome (1973) describes two phases during gestation period of Greater horseshoe bats which affect the timing of birth.

Birth dates of bats in the Woodchester colony are usually normally distributed (Ransome 1994). An example of the distribution of birth dates for 1999 is shown in Figure 12.

Table 18 Birth dates of bats in each year of this study

	1996 (N=32)	1997(N=31)	1998(N=32)	1999(N=38)
First born	28. June	13. June	24. June	18. June
Last born	31. July	13. July	20. July	13. July
Mean birthday	14. July	30. June	6. July	30. June

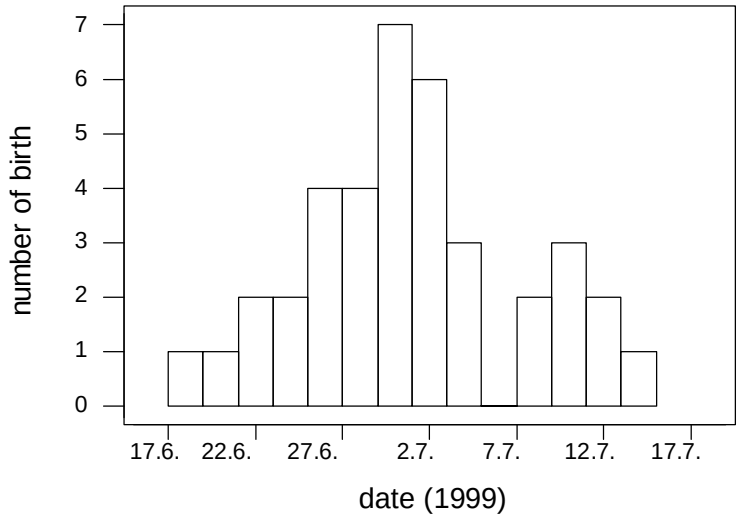

Figure 12 Distribution of birth dates for bats born in 1999 only

3.3.7.1 Juvenile Age and Parasite Load

As mentioned above, it was at the outset of the project that new-born infants may be more vulnerable to ectoparasitism than older juveniles. Figure 13 shows the ratio of parasite load against juvenile age. Only one data per juvenile is randomly picked from all juvenile data-sets. Until 32 days old, all pups were carrying parasites. From the age of 45 days beyond, most juveniles were infested with only 4 or less mites. There is a slight negative correlation between the number of parasites against the age of juveniles : r_s =-0,345 ($P < 0,001$; $n = 131$). 45 days is suggested to be the time lactation lasts (Jones *et al*.1995).

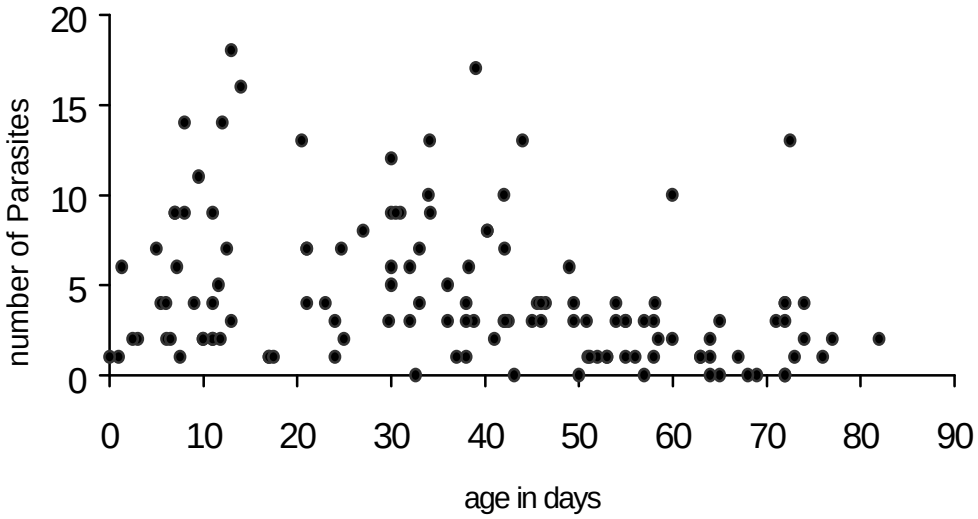

Figure 13 Parasite load against age of juveniles

Figure 14 shows the number of parasites counted on 10 bats (6 ,4), all born in 1997. These ten individuals generally follow the same pattern in as described above, i.e. that parasite load reduces with age.

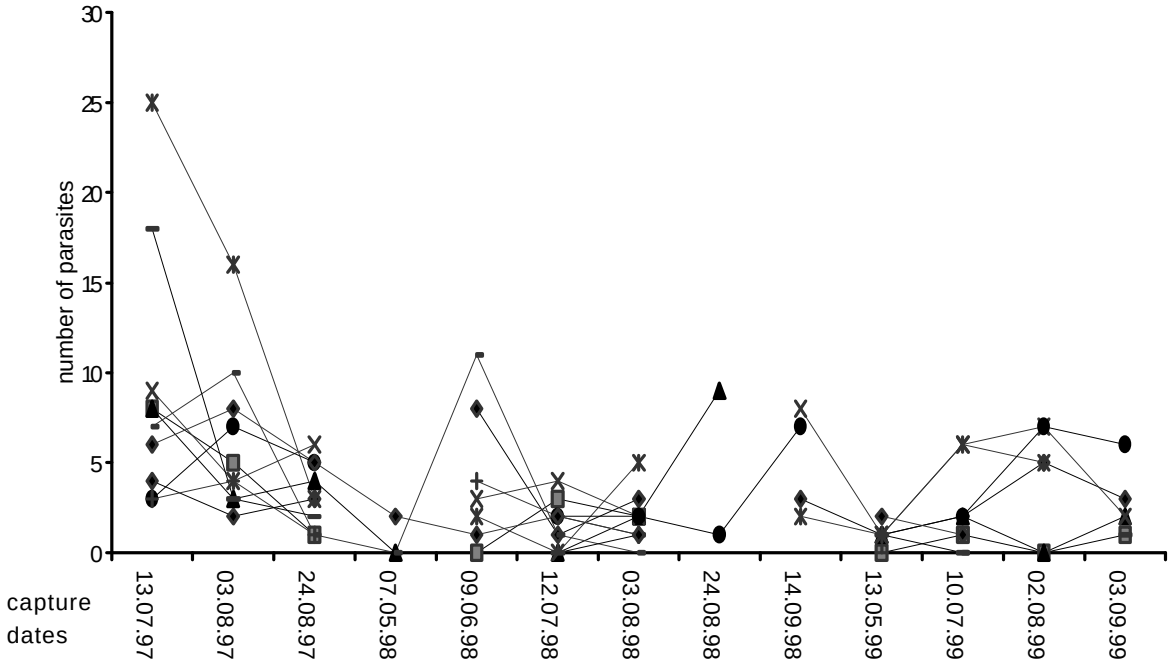

Figure 14 Parasite load of ten individual bats (6 ,4 ; all born in 1997) on several dates

3.3.7.2 Differences in the Quantity of Parasites infesting Male and Female Juveniles

On average the sex ratio for new-born bats is equally balanced but an increase in the male juveniles bat population was observed in 1986 and 1987 at the Woodchester colony (Ransome & McOwat 1994). This imbalance in the population proposed an investigation into the volume of parasites infesting the male and female juveniles. A different could result from a bias in parental care investment towards one sex. This could have been possible as mothers might invest different amount of time for maintenance on different gender. As with other mammals such as the red deer (*Cervus elaphus*) where mothers invest more time or attention to one sex over the other (e.g. Clutton-Brock *et al*. 1982). Parasite load in male and female juveniles was compared on 13 different dates. On non of these dates a significant difference in parasite infestation between male and female juveniles was found. (see Table 19 below). Consequently parasite load of male and female juveniles were combined for subsequent analysis.

Table 19 Juveniles: Results of Wilcoxon-Mann-Whitney test comparing parasite load in males and females during this study

Date	Median number of parasites		
	Female juveniles	Male juveniles	P (adjusted for ties)
21.07.1996	3 (n=12)	4 (n=15)	> 0,05
25.08.1996	3,5 (n=12)	2 (n=15)	> 0,05
05.09.1996	3 (n=11)	3 (n=12)	> 0,05
13.07.1997	8 (n=15)	6 (n=15)	> 0,05
03.08.1997	5,5 (n=16)	8 (n=13)	> 0,05
24.08.1997	3 (n=13)	2 (n=10)	> 0,05
12.07.1998	4 (n=13)	9 (n=15)	> 0,05
03.08.1998	6 (n=15)	9 (n=17)	> 0,05
24.08.1998	3,5 (n=12)	3 (n=15)	> 0,05
14.09.1998	2 (n=13)	2 (n=13)	> 0,05
10.07.1999	4 (n=18)	3 (n=15)	> 0,05
02.08.1999	3 (n=20)	4 (n=16)	> 0,05
03.09.1999	1 (n=19)	1 (n=12)	> 0,05

3.3.7.3 Juveniles Parasite Load compared to other Bats in the Roost

As mentioned above, juveniles are probably the favoured host for parasites at least as long as they are not fully grown and fully developed. On 13 dates the parasite load of juveniles and the other adult bats in the roosts were compared (see Table 20). This method eliminates the possibility that results are influenced by the season. Results showed that groups of juveniles, including infants up to 52 days old always had a significant higher parasite load than the other bats. In older juveniles results showed no difference in median number of parasites carried compared to the other bats. On one particular occasion these juveniles even harboured less parasites than the other (adult) bats. In conclusion these results strongly suggest that it is age in juveniles and not season what determines parasite load.

Table 20 Results of Wilcoxon-Mann-Whitney test comparing juvenile parasite load and parasite load of all other bats in the roost for all four years

		Median number of parasites			
Year	Date	Juveniles	All other bats	Age of Juveniles in days	P (adjusted for ties)
1996	*21th of July*	*4 (n=27)*	*1 (n=42)*	*0 to 23*	*< 0,001*
	25th of August	3 (n=27)	3 (n=41)	25 to 58	> 0,05
	5th of September	3 (n=23)	4 (n=26)	36 to 69	> 0,05
1997	*13th of July*	*6 (n=30)*	*3 (n=55)*	*0 to 31*	*< 0,001*
	3rd of August	*6,5 (n=28)*	*3 (n=51)*	*21 to 52*	*< 0,001*
	24th of August	3 (n=24)	2,5 (n=22)	42 to 73	> 0,05
1998	*12th of July*	*6,5 (n=28)*	*2,0 (n=55)*	*0 to 20*	*< 0,001*
	3rd of August	*8 (n=32)*	*3 (n=60)*	*14 to 42*	*< 0,001*
	24th of August	3 (n=27)	4,5 (n=32)	35 to 63	> 0,05
	14th of September	2 (n=26)	7,5 (n=8)	55 to 86	< 0,01
1999	*10th of July*	*4 (n=33)*	*1 (n=70)*	*0 to16*	*< 0,001*
	2rd of August	*3 (n=36)*	*1 (n=54)*	*19 to 38*	*< 0,01*
	3th of September	1 (n=31)	1 (n=31)	51 to 70	> 0,05

3.3.7.4 High Parasite Load and Juvenile Mortality

Does a high parasite load lead to juvenile mortality? As parasites are shown to weaken the fitness of their hosts (Brown & Brown 1986) parasites might reduce fitness of juveniles as they seem more likely to have higher parasite load than other bats in the colony. With this understanding it was interesting to investigate whether juveniles with a high parasite load are more likely to die than other juveniles. An investigation of juvenile mortality in the colony found that from 1984-1988 28.1% of all juveniles reached there second year (Ransom 1990).

As seen above, there is a decrease in parasites carried per bats when they reach an age of 45 days, so as a result juveniles were divided into two groups. One group of juveniles between 0 and 44 days of age and the other from 45 to 82 days old. Only the data concerning juveniles born between 1996 and 1998 was used, as there was no recapture data for those born in 1999.

Table 21 Overview and definition of parasite load in juveniles

Juvenile age in days	Median number of parasites	High parasite load	Times high parasite load	Minimum/Maximum numbers of parasites
0-44 (n=240)	4	9 and above	55 (42)*	0/36
45-82 (n=143)	2	5 and above	20 (18)*	0/13
Ó			75 (52)*	

* in brackets number of different cases

Table 22 below shows, more juveniles survived their first year between 1996 and 1998 than between 1984 and 1988. Always about 60 % of juveniles survived at least their first year irrespective of the amount of parasites they carried during the year since they were born.

Table 22 Survival rates of juveniles grouped by different parasite load

	Number of juveniles	Number of juveniles which survived at least the first year	% of juveniles which survived at least their first year
All Juveniles born between 1996-1998	95	54	56,8 %
Juveniles with high parasite load	52	30	57,6 %
Juveniles with no high parasite load*	43	24	55,8 %
Juveniles with low parasite load**	15	8	60 %

* juveniles which at every observation carried less than 9 parasites at an age of 44 days or under and less than 5 Parasites at an age of 45 days and older
** juveniles which at every observation carried less than 5 parasites at an age of 44 days or under and less than 5 Parasites at an age of 45 days and older

3.3.7.5 Differences in Parasite Load throughout the Years of Counting

In assessing juvenile mortality, juveniles with a high parasite load were mostly found in 1998 and 1997, it was interesting to investigate, whether there were differences in average parasite load between years. For this reason data from July of the four years observed was compared Results are shown in Table 23. July was chosen as there is no other month when data was collected in all four years. Even though the capture date is later in 1997 the juveniles are not older than in the other years as the mean birthday is later in 1997 compared to the other years.

Table 23 Median number of parasites on juveniles during July from 1996-1999

(Kruskal-Wallis P < 0,05 (adjusted for ties))	1996	1997	1998	1999
Median number of Parasites in July	4,0 (n=27)	6,0 (n=30)	6,5 (n=28)	4,0 (n=33)
Juvenile age in days	Up to 23	Up to 30	Up to 18	Up to 22

The parasite load in juveniles differed during the July month of 1996 to 1999. With most parasites present on juveniles in 1997 and 1998. The number of new born bats did not differ significantly during the compared years which also applied to the colony size. There may be other external circumstances that could cause more parasites on juveniles in these years such as weather or the roost's microclimate.

3.3.7.6 Could Juveniles Parasite Load be affected by their Mother's Parasite Load?

Figure 15 Female Greater horseshoe bat with offspring (Picture from Ransome 1990)

Parasite load of mother-young pairs is interesting to investigate for two reasons. Mother and juvenile are often found within close physical proximity (see Figure 15) This is because mothers attending her young, when they nurse their pups, groom them or keep them warm. So the interchange of parasites is more likely to occur between mother young pairs than between other bats in the roost, simply because of their proximity to each other. In Addition to close physical contact, an inherited determination for parasite infestation can be a reason to find a positive correlation between mothers and their juveniles. With Barn swallows, a positive correlation is found between parasite load of fathers and their offspring, even when reared in a another birds nest (Møller 1990). There was a significant positive correlation between mother and infant parasite load: r_s =0,364 ($P < 0,001$; n=120) (see also Figure 16 below). Correlation coefficient found between parasite load of young juveniles (up to an age of 20 days) and their mother was r_s = 0,408; ($P < 0,001$, n=110).

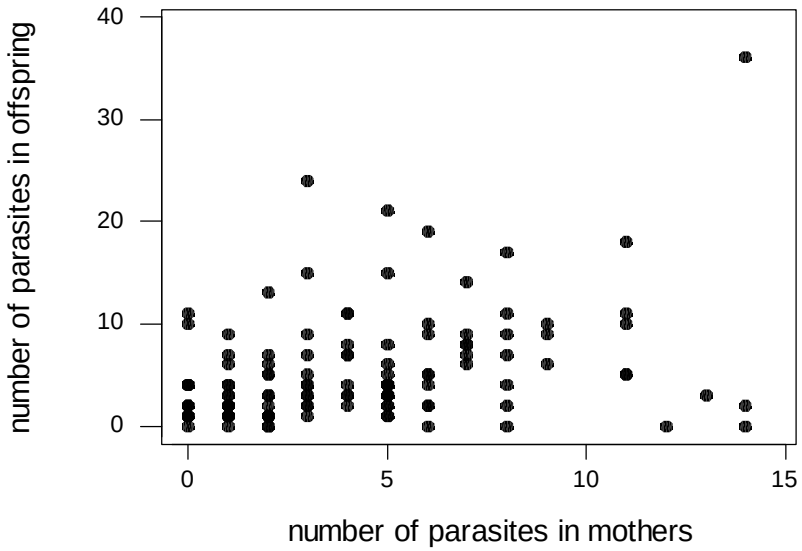

Figure 16 Parasite load in mothers against parasite load of their offspring (all ages)

3.3.7.7 Body Condition and Parasite Load

Body condition could influence parasite load and vice versa. As juveniles grow older they gain weight (see Figure 17 below). I describe how much a juvenile bat is expected to weigh at a specified age. Young bats in weak body-condition could possibly harbour more parasites than bats in good condition, as weak bats might not be so healthy and not as strong to fight parasites. Bats found in weak body condition often carried many parasites, but it is not clear whether the high parasite load was the cause of the poor condition (Brown 1994).

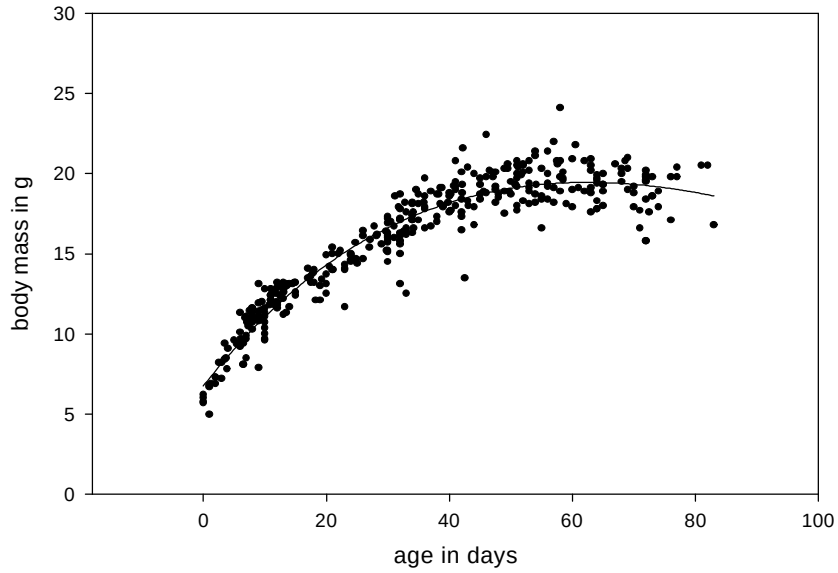

Figure 17 Body condition in juveniles: body mass versus age in days

As can be seen, body mass in juveniles steadily increased up to 60 days old and then decreased slightly from that age on. Mass versus age in juveniles is best described with the equation:

$$f(x) = -\left(y0 + ae^{(-bx)} + cx\right)$$

a = 44,7945; b = 0,016886; c = 0,26368; y0 = -51,5303

with x = age and f(x) = body mass

DF=3 SS=5178 F=1275 P=<0,0001 R=0,95

From the equation above, I calculated an expected body mass from a given age. Figure 18 shows parasite load versus difference from the expected mass. Both juveniles in good and in poor condition harboured less parasites than juveniles in average condition.

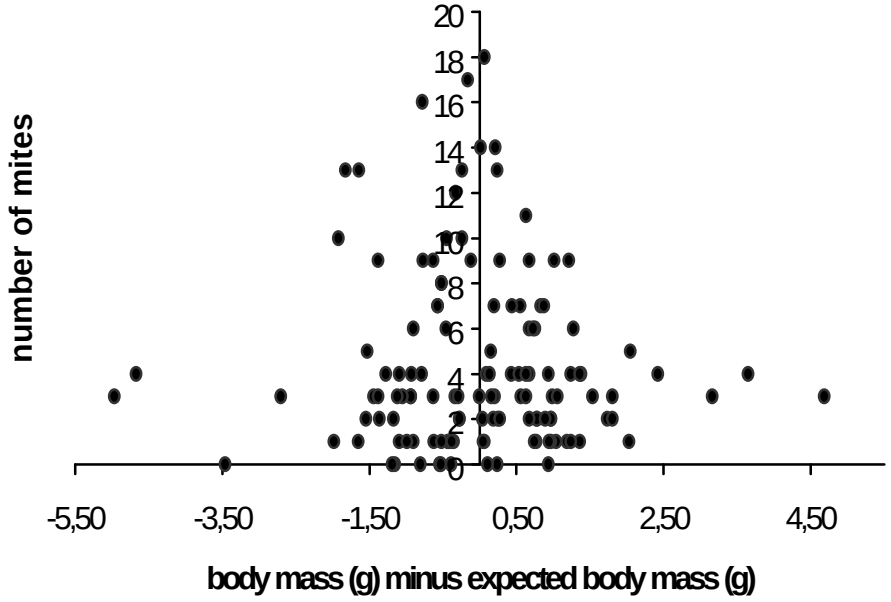

Figure 18 Number of parasites versus body condition in juvenile bats

Bats in good body condition are mixed age. I found no correlation between age of juveniles and the difference I found to their predicted body-mass (r_s= - 0,065; P > 0,05) (see Figure 19)

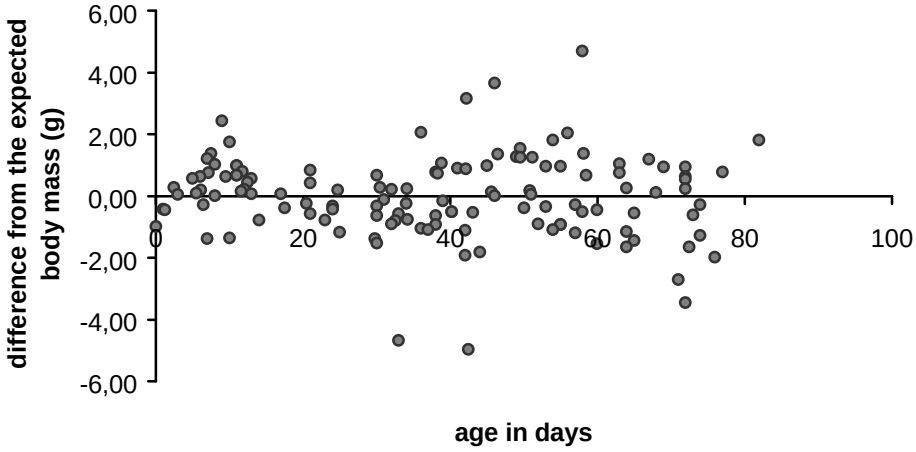

Figure 19 Differences from the expected body mass versus the age of juveniles

3.3.8 Pregnant and Lactating females

3.3.8.1 Pregnant Bats

Pregnancy changes weight and body physiology. Pregnant female Greater horseshoe bats gain up to 50% of their body mass. Hormone release changes during pregnancy and this might influence other physiological functions and can lead for example to a reduced immunocompetence (Christe *et al*. 2000). Odour may also differ. These natural changes in the bodies physiology provoked analysis of parasite load during the gestation period. Parasite load during gestation time is shown in Figure 20. These results do not suggest a direct connection between parasite load and time of gestation: r_s = -0,246; (P> 0,05; n=39)

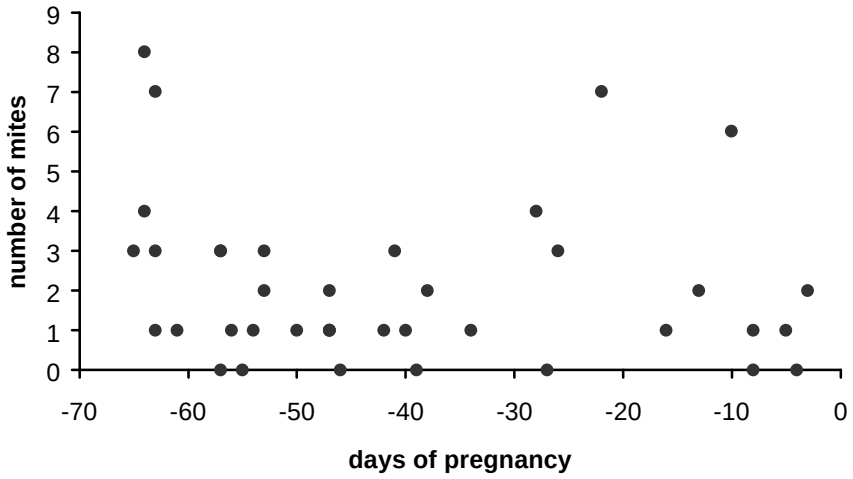

Figure 20 Parasite load during gestation time: 0=day of birth

3.3.8.2 Lactating Bats

Lactating bats have their juveniles to look after: breastfeeding, grooming and keep them warm. Time spent on self maintenance might be reduced by time devoted to the care of their offspring. Lactating bats may also spend more time in the roost looking after their juveniles. McLean and Speakman (1997) describe that lactating brown long-eared bats (*Plecotus auritus*) spend less time grooming than do non-reproductive bats. If grooming has an influence on parasite load, this could lead to more parasites on lactating bats. Figure 21 below shows this particular stage of lactation which seems not to have an influence on parasite load. (r_s = -0,198; P > = 0,05; n = 48)

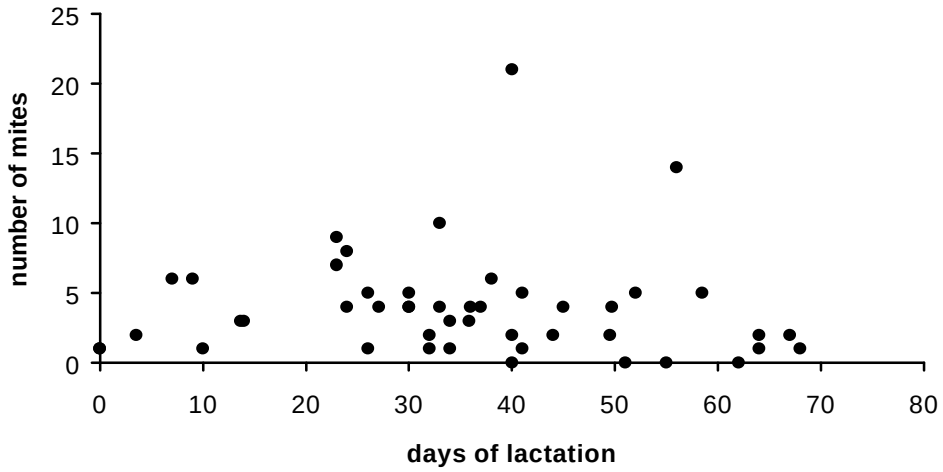

Figure 21 Parasite load during lactation in adult bats.

3.3.8.3 Comparison of Parasite Load in Pregnant and Lactating Bats

Although days of pregnancy and days of lactation do not seem to have a direct influence on parasite load, data of pregnant and lactating females was compared for any difference. The only date (12.07.98) where both pregnant and lactating females were present was chosen to compare parasite load. Parasite loads were compared on the same date to eliminate the possibility that season has an influence on the results. (Wilcoxon-Mann-Whitney test Lactating (N = 29,Median = 4,0) Pregnant (N = 4 Median = 1,0) $P < 0,05$ (adjusted for ties)) (see also Figure 22) This result suggests that there is a difference between the number of parasites pregnant and lactating females carry.

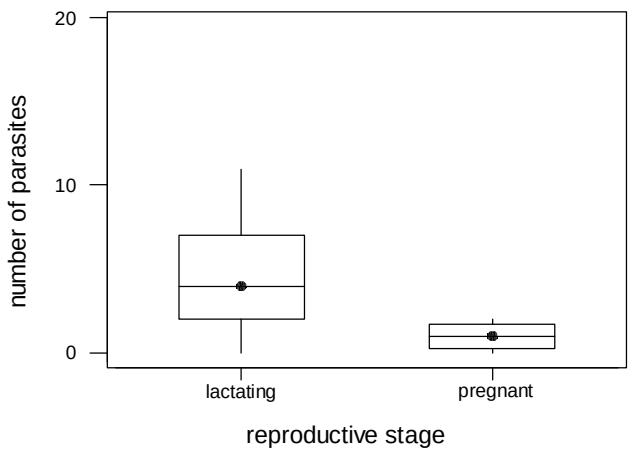

Figure 22 Box plot median number of parasites in pregnant and lactating females on the 12.7.98

3.3.8.4 Comparison of Breeding and Non-breeding Females / Sub-adult Females

Parasite load in pregnant, lactating and non-breeding females was compared (see Table 24) In three out of four occasions I found no difference in parasite load between pregnant and non-reproductive females. On one particular occasion (3.6.96) non-reproductive females carried more parasites than pregnant ones. On all dates in July, where offspring was aged between born and 31 days, lactating females carried significantly more parasites than non-reproductive females. Mothers with older infants did not always carry more parasites than non-reproductive females.

Table 24 Adult females: Results of Wilcoxon-Mann-Whitney test comparing parasite load in pregnant, lactating and non-reproductive bats

Date	Median number of Parasites per bats				Age of offspring/ days of lactation
	Pregnant	Lactating	Non-reproductive	P (adjusted for ties)	
4.6.96	1 (n=10)		2 (n=10)	> 0,05	
21.7.96		2 (n=27)	0 (n=8)	< 0,01	0-23
25.8.96		3 (n=25)	1 (n=7)	< 0,01	25-58
3.6.97	4 (n=12)		7,5 (n=12)	< 0,05	
13.7.97		4,5 (n=28)	1 (n=11)	< 0,01	0-31
3.8.97		4,5 (n=26)	2,5 (n=12)	> 0,05	21-52
24.8.97		3,5 (n=12)	1,5 (n=10)	> 0,05	42-73
7.5.98	3 (n=23)		0 (n=4)	> 0,05	
09.6.98	2(n=5)		2(n=9)	> 0,05	
12.7.98		4 (n=29)	1 (n=13)	< 0,01	0-20
3.8.98		4 (n=31)	3 (n=15)	> 0,05	14-42
10.7.99		2 (n=29)	1 (n=14)	< 0,05	0-16
2.8.99		2,5 (n=26)	1 (n=12)	< 0,01	19-38
3.9.99		1 (n=13)	1 (n=8)	> 0,05	51-71

3.3.8.5 Do more experienced Mothers have less Parasites than less experienced Ones?

The idea behind this question was that less experienced mothers might be less successful in self-maintenance (e.g. self-grooming) whilst caring for their offspring at the same time. If grooming reduces parasite load, then less experienced lactating females might be more likely to host more parasites than experienced ones. Inexperienced mothers were defined as lactating females having their first offspring. Experienced mothers where classed as those who had born their second or more infant. To compare these two groups several dates were chosen when 5 or more inexperienced females were caught. From the investigated 7 dates, only on one occasion was a significant difference between the parasite load of inexperienced and experienced mothers found (see Table 25). These results suggest experience in rearing offspring is not likely to influence parasite load in lactating females.

Table 25 Results of the Wilcoxon-Mann-Whitney Test to compare parasite load in experienced and not experienced mothers

	Median number of parasites		Wilcoxon-Mann-Whitney test
Date	Inexperienced	Experienced	P (adjusted for ties)
21.7.96	2 (n=5)	1,5 (n=22)	>0,05
25.8.96	3,5 (n = 6)	3 (n = 19)	>0,05
3.8.98	3 (n=5)	4,5 (n=24)	>0,05
24.8.98	4 (n=5)	4,5 (n=18)	>0,05
10.7.99	2 (n=9)	1,5 (n=18)	>0,05
2.8.99	5 (n=8)	1,5 (n= 16)	<0,01
3.9.99	2,5 (n=6)	1 (n=5)	>0,05

3.3.9 Body Condition and Parasite Load in Adult Bats

A good and common measure for body condition in bats is the ratio of forearm length to body mass. Using this method, varying physical sizes of individual bats is then balanced. The lower the value the better the bat condition. I also found that adult bats gain mass with age, shown in Figure 23. Data is independent. Only one data point of each bat was randomly chosen from the adult bat category. Pregnant bats were not considered.

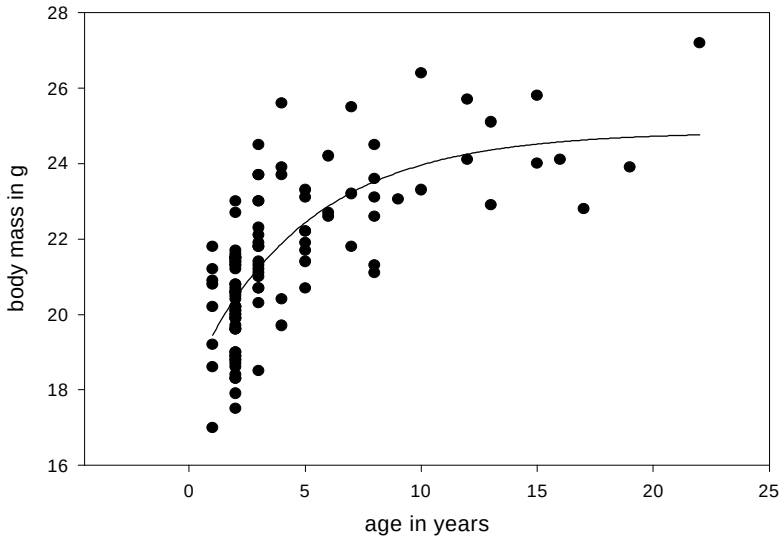

Figure 23 Body mass versus age in adult bats

The gain of body mass in adult bats as they age is described with the equation:

$$f(x) = y0 + a \times \left(1 - b^{x}\right)$$

with x = age and f(x) = body mass; (y0=18.2460;a=6.5940;b=0.8176)

DF=2; SS=243, MS=122; F=61.5; P<0.0001; R=0,73

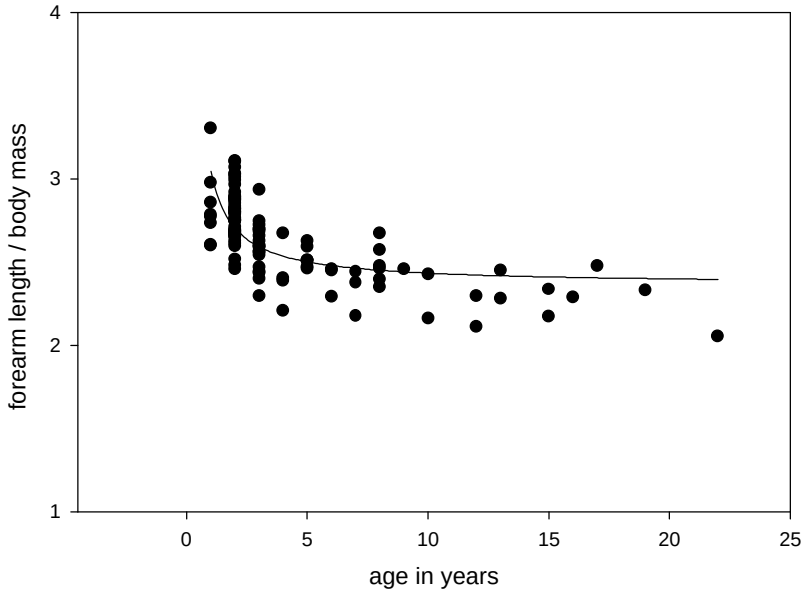

Figure 24 Body condition in adult bats is defined as forearm length / body mass versus age in years

I described body condition in adult bats with a function of the ratio: forearm length/body mass versus age. This resulted in the equation:

$$f(x) = y0 + \left(\frac{a}{x}\right) + \left(\frac{b}{x^2}\right)$$

With x =age and f(x) = forearm length/body mass

y0 =: 2,18; a = 1,72; b = -1,05

DF=2; SS= 3,6; F=75,6; P<0,0001; R = 0,76

With the equation above, I calculated an expected value for the ratio of forearm length / body mass for a given age. Then I took the difference between the calculated value and the real value. I applied the difference versus the parasite load, shown in Figure 25. If the difference is positive, bats are in a better condition than expected and vice versa.

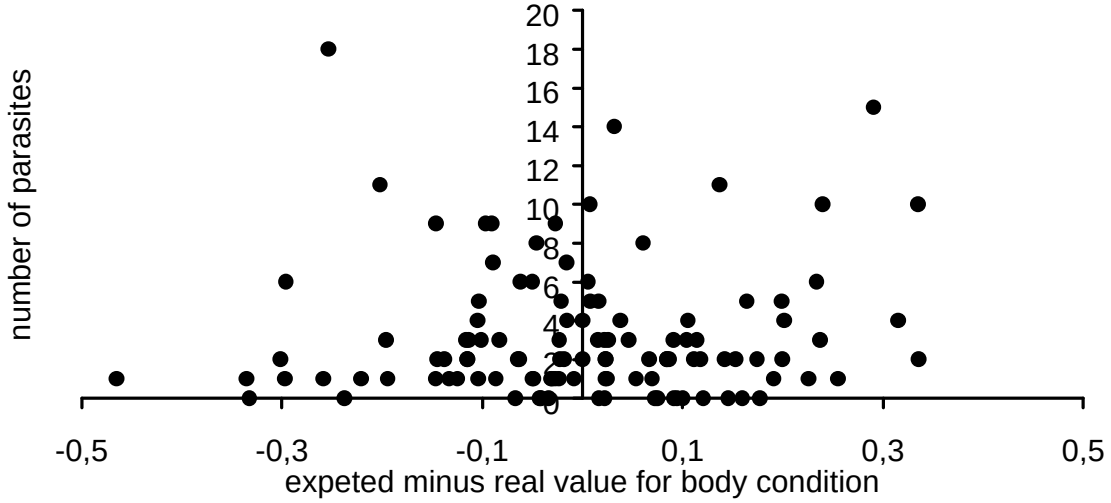

Figure 25 Body condition and parasite load in adult bats

I did not find a significant correlation between body condition and parasite load in adult bats (r_s= 0,051; P> 0,5 n=111).

3.3.10 Parasite Load and Heterozygosity

In barn swallows it has been proofed that parasite resistance is inherited, a genetical predestination for parasite resistance was found (Møller 1990). In addition Coltman *et al.* 1999 found evidence that parasites might mediate against inbred in the soay sheep and heterozygosity in genes might be a measurement for genetical fitness. Within in the scope of the genetic population study in the colony seven microsatallites were investigated (Rossitter *et al.* 1999) and mean d^2 determined.

mean $d^2 = \frac{1}{n}\sum_n (i_a - i_b)^2$,

i_a and i_b are the length in repeat units of alleles a and b at locus i, and n is the total number of loci at which an individual was scored (here 7). Mean d^2 can explain variations in fitness-related traits. Individuals with high mean d^2 are assumed to be fitter because they are heterozygous at more loci throughout the genome (Pemberton *et al.*1999). In Figure 26 parasite load versus d^2 is shown.

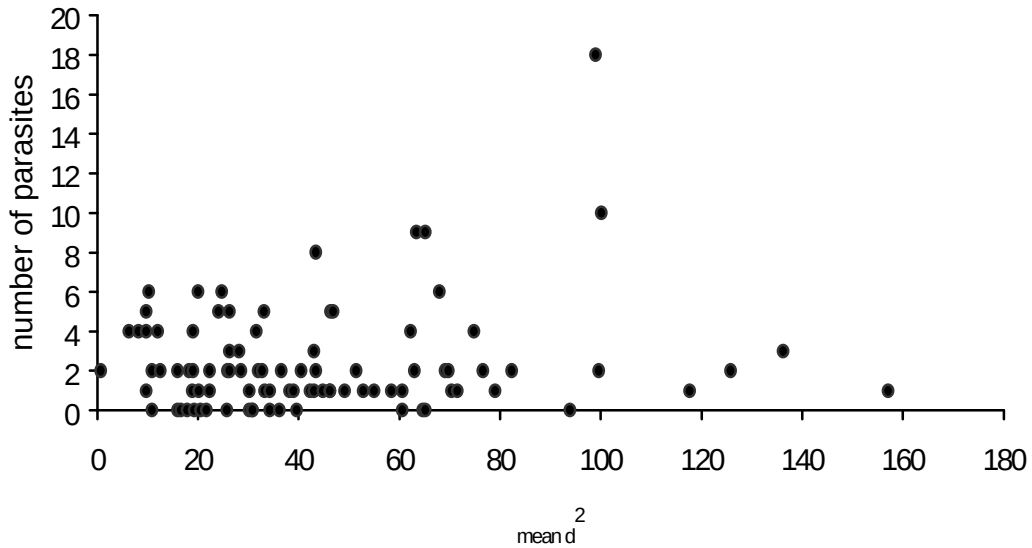

Figure 26 Adult bats: Parasite load against mean d^2

I found no significant correlation between mean d^2 and parasite load in adult bats (r_s= 0,011; P > 0,5, n=92)

3.4 Parasite Load and Grooming Behaviour

No experiment was performed to prove that grooming reduces the number of carried wing mites. Mites which are found in droppings of Greater horseshoe bats are parasitic to the beetles that bats eat. Parasitic bat mites are never found in droppings of Greater horseshoe bats (Ransome personal observation). To find a correlation between parasite load and time spent grooming the number of parasites counted on the date that was closest to the grooming observation was then used. Grooming observation were about two or three days later than parasite counts. No significant correlation between the two parameters was found: r_s = -0,132; P = 0,666;n=12 (see Figure 27)

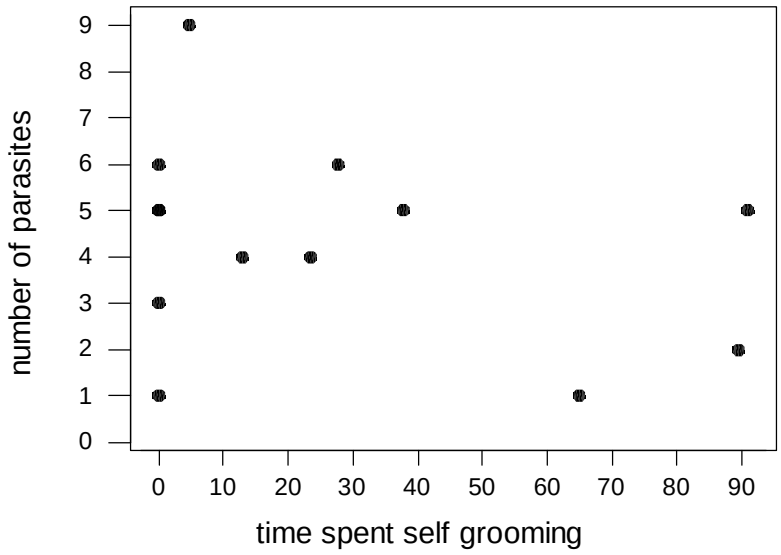

Figure 27 Number of parasites against time spent self-grooming in adult female bats

Again, to find a correlation between parasite load and time received allo-grooming the number of parasites counted on the date that was closest to the grooming observation was used. A significant negative correlation was found between parasite load of known juveniles and the time they received allo-grooming by their mothers: r_s = -0,968; P < 0,05 n=4 (see Figure 28 below).

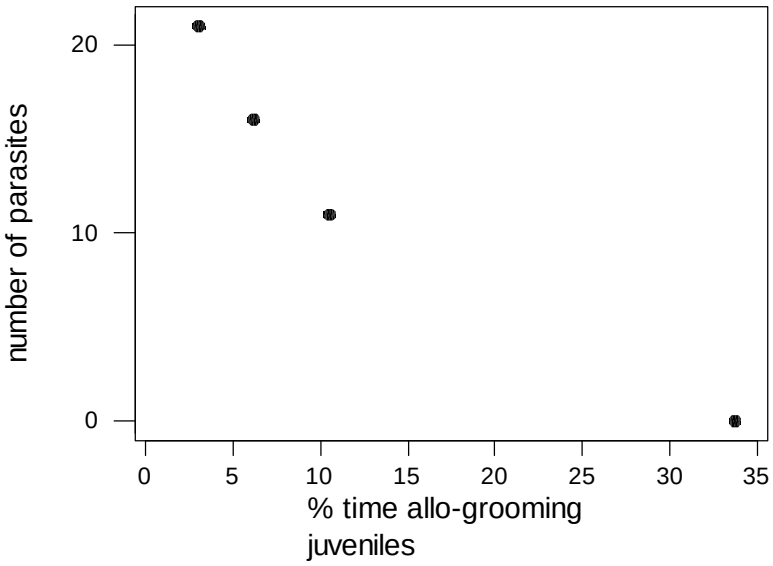

Figure 28 Juveniles: Number of parasites against time received allo-grooming from their mothers

4 Discussion

I first discuss grooming behaviour in Greater horseshoe bats. Few studies have previously been conducted on grooming behaviour in free living colonies of bats. The results of this study give new information about grooming behaviour and social interactions in a Greater horseshoe bat colony.
Second, I focus on parasite infestation in the researched colony. Bats as parasite hosts as well as the parasites themselves are little researched. This part resulted from analysing data collected counting parasites on Greater horseshoe bats over the last four years. This data collection resulted in a unique data base containing more than 1100 data-sets on more than 200 different bats. I believe this is a strong data base from which to draw relevant conclusions about host and parasite interactions.
Finally I synthesise my results and discuss the prediction that grooming serves to reduce the level of parasite infestation.

4.1 Grooming

Greater Horseshoe bats show a typical organisation for temperate bats (Bradbury 1977). Females are philopatric and form nursery colonies. Males are predominantly solitary and roost mostly underground throughout the year (Ransome 1990). Their mating system is polygynous.

4.1.1 Allo-grooming

One main interest of this study was to investigate allo-grooming in and between the matrilines of the researched colony. Allo-grooming is widely described in Chiroptera (*Vampyrum spectrum* and *Desmodus rotundus, Phyllostomus hastatus, Phyllostomus discolor* and *Myotis bechsteinii*) (Wilkinson 1985, 1986, 1987; Vehrencamp *et al.* 1977; Kerth 1997) as well as in many other mammals (e.g. Hutchins & Barash 1976, Mooring & Hart 1995). On the other hand there are also bat species known which do not perform social-grooming, such as *Leptonycteris curasoae* (Fleming *et al.* 1998), *Carollia perspicillata* (Williams 1986; Wilkinson 1986) and *Artibeus jamaicensis* (Wilkinson 1986).
Despite extensive observation in this study I did not observe any conspecific grooming in *Rhinolophus ferrumequinum*. Allo-grooming exclusively took place

between mother and young pairs. In this respect *Rhinolophus ferrumequinum* appears to resemble non-cooperative bats such as *Carollis perspicillata* (Fleming 1988) or *Leptonycteris curasoae* (Fleming *et al.* 1998).
In the common vampire bat social grooming is linked to food sharing. It is used to monitor other bats' potential for giving and receiving blood (Wilkinson 1986). Communal nursing together with social grooming are found in *Phyllostomus hastatus* (Mc Cracken & Bradbury 1981). In *Rhinolophus ferrumequinum* so far juvenile "milk thieves" were observed (Ransome 1990) but no communal nursing behaviour or food sharing, occurs.

Myotis bechsteinii is an insectivorous European bat like *Rhinolophus ferrumequinum*. In contrast with the Greater horseshoe bat, Bechstein's bats are known to allo-groom each other (Kerth & König 1997). An important difference between this two species, I believe, is their group living. Colonies of Bechtstein's bats are more stable. The roost in the Woodchester mansion is habitated by almost the same bats year after year. However immigration from and emigration to other colonies does occur. In a six year period 12 bats were shown to emigrate and five bats were known to have immigrated (Ransome 1990). Studies in a population of *Myotis bechsteinii* (Kerth *et al.* 2000) have shown that even bats from colonies living only 2 km apart do not mix and only one female in five generations might dispers.
Allo-grooming seems often be linked to other co-operative behaviour and might even be used to support and encourage this. But co-operative behaviour is often linked to reciprocity (Russel 1983) which is not guaranteed in a colony where bats emigrate and immigrate. I believe that the Greater horseshoe bat groups are not stable enough to favour allo-grooming. Wilkinson (1986) observed four bat species all belonging to the same family. The two species forming more stable groups than the other two showed social grooming behaviour where as the other two did not. He also found that the bats with similar group stability but different levels of relatedness showed co-operative behaviour (Wilkinson 1987). This suggest that for bats group stability is more important than relatedness to evolve co-operative behaviour.

4.1.2 Grooming in Adult Females

I hypothesised that juveniles will receive less allo-grooming from their mothers as they grow older. In addition I expected that the time spend self-grooming in lactating females will rise as their infants become older. I did find a strong negative correlation between infant age and the time mothers spent grooming them. Mothers were not observed grooming offspring older than 18 days. Grooming provides tactile stimulation which initiates physiological events for growth and development (Schanberg *et al.* 1984). Saliva may contain antibacterial agents (Hart & Powell 1990) and being groomed might be especially important for infants in which immuno-competence has not fully developed (Christe *et al.* 2000).

In comparison no significant correlation was found between juvenile age and the time mother spent self-grooming. So a reduction of time devoted allo-grooming offspring did not lead to an increase in time spent self-grooming. I found no significant difference between breeding and non-breeding females in the time dedicated self-grooming. However, this last finding might be a result of the small sample size of non-breeding females.

My finding concerning maintenance of juveniles corresponds with a study by McLean and Speakman (1997) about the brown long-eared bat. They too found a decline in grooming dedicated to juveniles as lactation proceeded, as well as finding that the course lactation had no effect on self-grooming in females. However, they did find lactating bats groomed less overall than non-breeding ones. Lactating females might also reduce time grooming offspring as juveniles bats improve their ability in self-grooming (see below; McLean & Speakman 1997)

4.1.3 Grooming in Juvenile Bats

As described above, juveniles received less grooming as they got older. No significant difference was found in the time spent self-grooming between juveniles in different age groups. One reason for these findings could be that the sample size was too small. But the observations here do suggest a difference in the quality of grooming with age. In observation young juveniles appeared less effective in self-grooming than older juveniles.

4.1.4 Suggestions for further Studies

For further studies I would suggest the following to optimise observation. It would be advisable to mark more bats. About 100 observation hours lead only to about 14 data points for adult bats. It would be very advantageous to mark bats to obtain reliable data on sex, age and reproductive status. It would be very interesting to find a non invasive method to collect grooming data from pregnant females and infant bats.
I found the reflecting colour-rings used on some bats in the Woodchester colony were not very useful. One problem was, that it is not possible to distinguish between brown and black rings in infrared light. Another problem was that on many occasions the rings were not properly visible. I found the plastic-discs employed in this study much more practical.

4.2 Parasites

4.2.1 Parasite Load throughout the Colony

The infestation levels in the colony with *Eyndhovenia euryalis* was always relatively low. The median parasite load never exceeded 4 mites, and on almost half of the capture dates it was between one and two. Every year the number of mites increased in July and August and decreased towards September. The number of mites increased with the number of bats in the roost. This could be an indication of reproduction activity of the mites and it also suggests that reproduction is linked to the number of bats in the roost and to reproduction in the bats. Foster (1969) showed that ectoparasites are likely to synchronise their life cycle with their host's life cycle. Also Christe *et al.* (2000) found evidence for synchronised lifecycles in *Myotis myotis* and its mite *Spinturnix myoti*. Parasites may adjust their lifecycles to their hosts due to the increased number of hosts available (Christe *et al.* 2000).
In 1 out of 18 occasions all bats in the colony were infested. It happened on the fifth of September 1996 a date when overall number of parasite in the colony had already decreased for that year. 100% infestation in the colony on a late date could be due to cold weather. Bats might cluster together as they do for thermo-regulation (Neuweiler 1993) and close physical contact makes parasite transfer and infestation more likely despite a decreased number of parasites. Indeed September 1996 had an average

temperature of 14.8 °C. The average for the other years was between 15,6 °C and 16,9°C (Totterdown, Bristol, UK; Weather-data).
On all other occasions about three quarter or more of all bats were infested with wing mites.
In other studies all bats belonging to one colony were infested in the summer month (Schmidt 1994; Deunff & Beaucournu 1981). Some possible reasons why this is different in this colony follow.
In the Woodchester colony *R. ferrumequinum* seem to carry less spinturnicid parasites in comparison to other bat species and other *R. ferrumequinum* colonies. Not only the percentage infestation but also the overall infestation appears to be lower. Schmidt (1994) found on juvenile and adult females *Myotis myotis* an average of 10 to 17 *Spinturnix myoti* during summer. Deunff & Beaucournu (1981) found an average infestation of *Rhinolophus ferrumequinum* with *E. euryalis* during summer time between 4 to 8 mites per bat in his study. Colony size can also have an impact on the parasite level on individuals in a group (Mooring & Hart 1992).

Several factors can influence the amount of parasites carried by an individual. Size is a factor. With a body of about almost 1 mm diameter *E. euryalis* is a relative large mite. It is known that smaller ectoparasites can be found in larger numbers on an individual than do larger ones (Marshall 1982). Also the numbers of other parasites species hosted can also influence the number of parasites on a host's body. As no other parasites were counted in this study and wings were exclusively inhabitated by *E. euryalis* I cannot comment further on that point.
Ectoparasites themselves can be infested with parasites which might lead to changes in mortality (Walter 1996).
The so called "dilution" effect, living in a large group makes it more unlikely for an individual to be attacked by parasites, only helps against flying, bloodsucking ectoparasites (Mooring & Hart 1992). Where as for permanent parasites it was found that group size is more of a disadvantage (Mooring & Hart 1992).
Finally, ectoparasites have to contend with two kinds of climates. The microclimate on bats body as well the outside weather conditions influence their lives (Walter & Procter 1999).

4.2.2 Juveniles

Even though a negative correlation between age and parasite load was found in juveniles, there were many exceptions where very young juveniles only carried one or two parasites. The number of parasites carried by juvenile bats falls significantly with an age of about 45 days. This age corresponds with the time lactation ends in Greater horseshoe bats (Jones *et al.* 1995). In addition I found young juveniles harbouring significantly more parasites than the other bats in the roost. As juveniles grew older this difference disappeared. On one occasion I even found older juveniles carrying fewer parasites than the other bats in the roost. These findings are consistent with Gannon & Willig (1995) who found that ectoparasite loads of *A. jamaicensis* and *S. rufun* varied with age, with juveniles harbouring significantly higher numbers.

I observed that levels of infestation had no impact on the survival rates during the first year of a juveniles life. The survival rate in general is increasing. From 1984 - 1988 28.1 % of juveniles survived their first year (Ransome 1990) from 1996-1999 these number rose to 58%. This may be due to a newly build in incubator (Ransome personal observation).

In this study the percentage infestation of juveniles and lactating bats was always more than 80%, where the levels of infestation in other groups were less on several dates. Parasite load in infants correlated positively with their mothers' parasite load.

Some possible reason for why *E. euryalis* may prefer mainly young juveniles are as follow:

Immunological responses to ectoparasites have been shown to protect the host or even reduce parasite load (Allen 1979). Immuno-competence in infants might be not fully developed (Christe *et al.* 2000) and young juveniles may be easily accessible hosts. Grooming is supposed to be an effective behaviour against ectoparasites and self-grooming develops with age (Mc Lean & Speakman 1996). As mentioned above, ectoparasitic mites have to cope with the microclimate on a host's body as well as general weather conditions. Mites may therefore prefer young juvenile hosts to avoid exposure to external climate conditions and remain in a more stable environment.

Most parasite load seems to be divided between mothers and their offspring. These suggest that *E. euryalis* depends on vertical transmission. This would explain why no influence on survival rate of juveniles was found. Parasites depending on vertical

transmission rely on host reproductive success and are therefore less harmful (Tompkins *et al.* 1996).

Parasite load in juveniles decreases when lactation ends and the close mother-offspring connection loosens. Juveniles start to leave the roost when they are between 28-30 days old. They also start to eat insects at that time (Jones *et al.* 1995), however change of diet and leaving the roost may not be the main reasons why parasite load drops in juveniles.

4.2.3 Pregnant and Lactating Females and Reproductive Status in Adult Females

Neither a correlation between stage of pregnancy and levels of parasite load nor one between lactation time and parasite load were found. But lactating females had a significantly higher parasite load than pregnant and non breeding/sub-adult females. In addition no difference was found in parasite load between pregnant and non-breeding females. Data collected on the same date was compared so that the results were not influenced by season. My findings do not agree with the study of Christe *et al.* (2000) in *Myotis myotis* and the mite *Spinturnix myoti*. Christe *et al.* (2000) found pregnant females carrying more parasites than non-breeding females and they also found a reduction in the number of parasites during lactation time. Again, *Myotis myotis* in this study is higher infested with wing mites than it is *R. ferrumequinum* in my study.

4.2.4 Years and Seasons

Average parasite load varied during the years of this study. In July juveniles carried more parasites in 1997 and 1998 than in the other two years. The same was true for all other bats in the colony and the overall percentage infestation and the percentage infestation in the different groups was higher in July 1997 and 1998 than 1996 and 1999. No factors measured in this study are likely to give an explanation for these findings.

On the dates compared, juveniles were between 0 and 30 days old. As showed above a change in the levels of parasites carried by juveniles occurs when they are older than 44 days. So age of juveniles is not likely to be the reason for this finding. Also mentioned above, weather conditions also have an impact on a mite's life. Weather data of July in all four years do not suggest an influence of weather for this finding. Average temperature was less in July 1998 (17.7°C) but similar for the other

years (1996=19.2°C; 1997=19.3°C; 1999=19.5°C). Average precipitation was less in 1999 (0,3 mm) but similar for the other three years (1996=1,7mm, 1997=1,8mm, 1998=1,8mm) (Totterdown, Bristol, UK; Weather-data).

4.2.5 Differences between different Groups of Bats

Adult males carried less parasites than other bats on the dates compared. Spinturnicidae live exclusively on their host's body (e.g.Rudnik 1960) and might be transferred from one bat to the other. Adult bats live most of the time dispersed and spend only little time in the Woodchester roost. I assume this makes it more unlikely for them to become infested.

I also believe *Eyndhovenia euryalis* may prefer bats living in a group for several reasons. Many hosts at the same place give the parasite opportunity to change hosts if necessary. It also increases the likelihood of finding a mating partner and raises the probability that offspring will survive if enough hosts are available. In addition parents and offspring do not have to compete for resources.

4.2.6 Body Condition

A high parasite load is often found on ill and weak bats (Brown 1994). The question here always is, does this parasite load result from weak condition or do parasites weaken their hosts. I predicted that there would be a correlation between parasite load and body condition; with bats in poor condition carrying more parasites than bats in good condition. This assumption was not decisively proven in my study. I found no correlation, either for juveniles or for adults, between body condition and parasite load. Instead results in juveniles suggest that *Eyndhovenia euryalis* prefers "average" bats. Neither juveniles in poor nor ones in very good condition where found highly infested but bats with not much difference from the expected body mass were. Lee and Clayton (1995) found significantly heavier nestlings in louse-infested nests than in louse free nests of the swift *Apus apus*. So they suggest that a nestling in good condition might be better capable to support more lice than a weak nestling. A similar situation may be true for *Eyndhovenia euryalis.* Results so far suggest that *E. euryalis* is mainly vertically transmitted. Parasites known to be transmitted from parents to their children (and therefore dependant on their hosts reproductive success) are understood to be less harmful to the host than horizontally transmitted parasites (Tompkins *et al.* 1996). So it might be for this reason that *E. euryalis* might

not be very destructive to *R. ferrumequinum* and prefers host juveniles in normal conditions.

4.2.7 Further Studies

Further investigations might collect mites from bats and determine sex and development stage of both mites and the bats they were collected from. Among other things this could evidence the assumption above on vertical transmission. I did not have any influence on the dates when data were collected and I would advise more careful date selection. In my study I only had continuous data for July to compare between the years but not for any other months. It would also be useful to record climatic conditions inside the roost, as well as the weather conditions outside, because these could have an impact on parasite infestation as well (see above).

4.3 Grooming Behaviour and Parasite Load

4.3.1 Do Mites get groomed off?

In the impala it was shown that grooming is an effective behaviour for removing ectoparasites. Also self-grooming in Chiroptera is suggested to reduce ectoparasite load (Marshall 1982; Wilkinson 1986).

As far as I know, no experiments have been performed yet to prove whether grooming reduces the level of ectoparasite infestation in bats, and this was also not possible within the scope of this study.

An indirect proof that mites are groomed off would be to find them in their droppings. Pir (1994) found digested mites in Greater horseshoe bat droppings which he could not determine. Ransome (personal observation) determined parasitic beetle mites in droppings collected from the fabric bags bats were kept in. Bats might have eaten them incidentally with beetle prey. He never found a single mite of the species *Eyndhovenia euryalis* even though beetle mites passed through the digestive system almost unharmed.

Spinturnicidae stick very persistently on a bat's wings (Brown 1994). Grooming wings in Greater horseshoe bats mostly consists of licking them with the tongue (Kolb 1982). Taking into account that their body is well adapted to stay on a bat's wings (Fain 1994) I assume that the tongue is not very effectively for *Rhinolophus*

ferrumequinum to remove *E. euryalis* off its wings. The best way to remove this parasites would be by scratching or biting them off with the teeth, so I expected to find *E. euryalis* in Greater horseshoe bat droppings.

4.3.2 Parasite Load and Grooming Behaviour in Juveniles and Lactating Females

Some results found in this study are contradictory. On the one hand juveniles generally carried fewer parasites as they grew older, on the other hand juveniles received less grooming from their mothers as they became older. This finding does not indicate that grooming reduces parasite load in juveniles bats. In contrast, it might imply that juveniles which received less grooming had fewer parasites. However it could be, of course, that juveniles may gain the ability of self-grooming with age (McLean & Speakman 1997) and would not rely on their mother's grooming anymore. In addition to this I did not find a difference between the amount of time older juveniles spent grooming themselves compared to younger juveniles.

However, for four observed juveniles I found a strong negative correlation between the time they received grooming from their mothers and number of parasites they carried. But in contrast to the general findings about parasite load in juveniles here parasite load decreased with age; the youngest one carried least parasites whereas the oldest had the highest infestation.

I did not find a correlation between parasite load and time spent grooming in lactating females. In addition to this I did not find a difference in the time breeding and non breeding females dedicated self-grooming. But I did find a difference in the levels of infestation with *E. euryalis* in breeding and non-breeding females. This finding does not support the assumption that grooming reduces parasite load. However, McLean and Speakman (1997) found a difference in the amount of self-grooming between these two groups in *Plecotus auritus*. Because of the small sample size of non-breeding females I cannot deny the possibility that something similar would be found in a study with more observed bats.

As I showed so far the infestation with *E. euryalis* appears to be considerably low and seems to have no major impact on the Greater horseshoe bat. So it might be possible that the cost of grooming (Mooring & Hart 1995) them off would be higher, than the fitness reduction they cause.

4.3.3 Further Investigations

To research the direct impact on grooming on parasite load, an experiment equivalent to Mooring *et al.* (1996a) on the impala should be done. One really should prove that grooming is a useful behaviour in reducing infestation levels. Further studies on the effect of time devoted to grooming on parasite load, parasite counts and video-observation should be co-ordinated so that they will take place on the same date. This may lead to more reliable results than the ones obtained in this study. Furthermore the question of whether *E. euryalis* provoke itching and might be therefore have an influence on grooming behaviour can then be answered.

4.4 Other Social Interactions

4.4.1 “Touching Noses”

As already discussed above, allo-grooming did not take place between adult bats. The only kind of interaction I observed between adult bats was something I would describe as “nose touching”, similar to what was observed in the Lesser long-nosed bat (*Fleming et al.* 1998). This behaviour also was observed in captive Greater horseshoe bats (Kulzer personal observation). I observed it only 22 times during about 100 observation hours and never between marked bats.

4.4.2 Mothers roosting with their weaned Offspring

Another interesting observation made during this investigation was that even though juveniles were apparently no longer groomed by their mothers at an older age, breeding bats were observed swinging for long periods of time with other bats which I assumed to be her offspring.

4.4.3 Further Investigations

As social interaction was rarely observed I believe that for further studies it is worth investigating these two behaviours more deeply. I suggest, as individuals in this colony are well known, to start by identifying the actors. By marking mothers and their offspring it could be proved whether it is a breeding bat and her juvenile offspring swinging together. And with more bats marked it would surely be interesting to research between which bats “nose touching” interactions take place.

I believe that this colony is so thoroughly researched in many ways, it would be a great loss not to use this information to continue this work in some of its aspects! I also believe this should be done in a more co-ordinated manner than some data for this study were collected.

5 Summary

This study investigated grooming behaviour and parasite load in the Greater horseshoe bat.

Among the findings in this study it was that allo-grooming does not occur between adult bats. Even though *R. ferrumequinum* forms long-term roost societies these groups are not really closed. Female bats immigrate to and emigrate from the colony and this could perhaps prevent co-operative behaviour developing like social-grooming between adult bats because reciprocity is not ensured.

It was also found that age of offspring was related to the amount of time lactating females groomed their offspring, although there was no effect on the time lactating bats dedicated to grooming themselves.

E. euryalis infestation levels always were at considerably low levels.

The main hosts were young juveniles and their lactating mothers. The parasite load of mother and offspring correlated positively. There is some evidence that lifecycles may be synchronised in this parasite-host-community. Juvenile parasite load decreased when lactation ended and younger juveniles always carried more parasites than the other bats in the roost. This difference vanished when juveniles grew older.

Lactating bats were always more infested with mites than non-breeding females. This findings suggest that the close physical contact between mother and offspring seems to be an in important part in the *E. euryalis* lifecycle and that vertical transmission occurs at substantial levels in these this mites. Infestation on adult males was always considerably lower than other bat groups. Adult bats are usually solitary and therefore are unlikely to be infested.

I found no evidence that *E. euryalis* harmed its host *Rhinolophus ferrumequinum*: Infestation with *E. euryalis* had no impact on the survival rate of juveniles. In addition to this I found no correlation between body condition and parasite load. In juvenile bats it seemed that bats in average condition were more likely to have high infestation levels of mites than bats in very good or very poor condition.

The results on the impact of grooming on parasite infestation are controversial. I found no correlation between parasite load and time spent self-grooming in lactating females; but a strong negative correlation between time spent allo-grooming offspring and parasite load of offspring and as the time mothers spent allo-grooming their

offspring decreased with age of offspring, juvenile infestation level increased. The general trend was for young juveniles to have a high parasite load which decreased with age. Further research on this area might produce more conclusive results.

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