Ecology and Dispersal of the Bedbug

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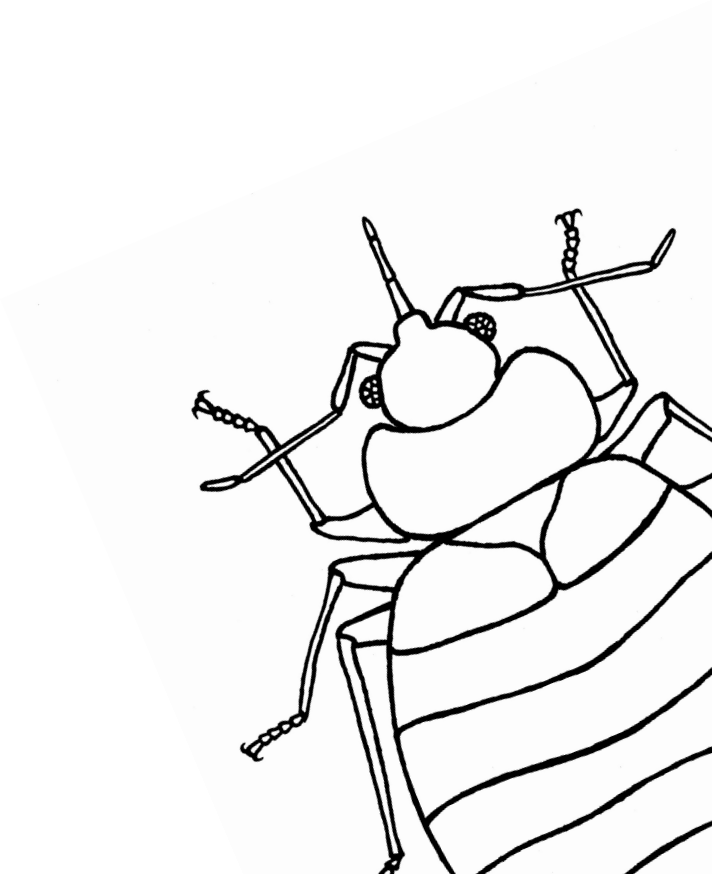

Abstract

The global bedbug resurgence has left the scientific community racing to fill large gaps in our understanding of the biology and ecology of this forgotten pest. Studying the ecology of a species so closely associated with humans has inherent difficulties, necessitating the development of laboratory arenas that replicate natural infestations. The arena developed herein provides bedbugs with the opportunity to exhibit natural foraging, hiding and dispersal behaviours on a scale that reflects their natural environment.

Using this arena I test hypotheses relating to; 1) how bedbugs use harbourage space, and 2) the factors affect their dispersal. My research revealed that harbourages in the vicinity of the host are used first and peripheral harbourages only form as the infestation develops. The preferential use of harbourages adjacent to the host is explained by the finding that feeding frequency was negatively correlated with distance from the host. However, despite this advantage of residing in close proximity to the host, bedbugs form discontinuous harbourages, leaving regions of unoccupied space. This suggests that there are factor(s) that limit harbourage density.

Female dispersal was unaffected by males presence, suggesting that sexual harassment does not drive dispersal in the bedbug. However, variation in the distribution of the sexes across harbourages suggests that females may be able to avoid males through harbourage selection.

Increased harbourage availability significantly delayed the onset of dispersal, suggesting that competition for harbourages near the host is a factor driving dispersal from the natal infestation. Given that a host is an almost unlimited food source and that the cost of dispersing is likely to be high, it is not immediately apparent why bedbugs choose to actively disperse. However, theoretical models show that where relatedness is high, dispersal always occurs to reduce competition. The high cost of dispersal may therefore be offset by kin selection.

To my wife Alexia and my children Zach and Joe

for their love and support during the preparation of this thesis.

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Mike Siva-Jothy has nurtured my scientific career for more than 12 years. He has supervised me from my days as an undergraduate, through two research assistant positions, one MPhil and finally through a doctoral degree. For all this and much more I am eternally grateful.

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Nothing brings you closer to someone than suffering the trials and torments of a PhD alongside them. Adam Dobson has been a shoulder to cry on, a companion to celebrate with, and a constant source of emotional and academic support throughout the duration of my post-graduate study. I will sorely miss working alongside him.

Over the years many people have come through our lab. They have all been a pleasure to work with and have greatly contributed to the stimulating and enjoyable working environment. Jens Rolf, Oliver Otti, Toby Fountain, Louise Heaton, Quentin Geissman, Joe Gallagher and YuPing Chen all deserve a particular mention for devoting significant amounts of their time to helping me with various aspects of the experimental design, data collection, analysis and proof reading that went into this thesis. Thank you.

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The foundation of this thesis is the fieldwork, which underpins the laboratory studies on which the research is primarily based. Locating bedbug infestations was only possible with the help of David Cain (Bed-Bugs Ltd.). When it comes to the subject of bedbugs, David is one of the most enthusiastic people I know. His dedication and appreciation of the need for research sets him apart from the rest in his field. Few pest controllers want the hassle of being shadowed by researchers, who slow down the treatment process and generally get in the way, however David has supported my project every step of the way and for this I am extremely grateful.

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Terms and Definitions

These terms are given specifically in the context of this thesis and the bedbug system.

Active dispersal – the process of an individual moving away from the natal infestation using its own locomotory system(s).
(Other authors do not distinguish local movement, between harbourages in the vicinity of the same host with active dispersal to a new infestation (and new host).)

Artificial host – the artificial feeding system I developed for use in my bedbug laboratory arenas.
(It is comprised of vertebrate blood treated with an anticoagulant and presented behind a membrane through which the haematophage can feed. Depending upon the sensory biology of the insect it may also be necessary to provide host cues such as heat, carbon dioxide or skin secretions to facilitate feeding.)

Bedbug - the single species *Cimex lectularius* L.
(Other authors use the term to refer to either the Cimicidae, or to those members of the Cimicidae that are associated primarily with humans.)

Conspecifics – other members of the same species.

Feeding status – the time since feeding, which is manifested by the amount of undigested blood present in the bedbug's gut. The distension of the abdomen relative to body size can be used as a metric for feeding status.

Foraging – the process of leaving the harbourage in search of a vertebrate host.

Harbourages (in context of natural infestation) – cracks or crevices in which one or more bedbugs reside between foraging trips. This is typically where eggs are laid and moulting occurs.

Harbourages (in context of laboratory arena) – the regions of the 10 mm wide, 3 m long paper strip under which one or more bedbugs reside. Where aggregations of bedbugs are separated by ≥1 cm, they are considered to occupy two separate harbourages.

Harbourage fidelity – the tendency of an individual bedbug to return to the same harbourage after foraging.

Host – a warm blooded vertebrate from which a bedbug is able to gain a blood meal.

Infestation – a population of bedbugs living in the abode of one (or more co-localised) host(s).

Mating status – the time since mating, which is manifested by the ability of a female bedbug to lay fertile eggs while in sexual isolation.
(Mating status may have important consequences for the success dispersal decision.)

Passive dispersal – the process of moving away from the natal infestation utilising the locomotory system of another organism (or other forms of naturally occurring kinetic energy in the environment).

1 Introduction

"The global rise of bed bugs early into the 21st century seems to be the culmination of numerous phenomena. Perhaps the most telling of these is the lack of understanding of the ecology and biology of the pest, which is essential for control."
(Doggett *et al.* 2004)

1.1 Conceptual Framework of this Thesis

The aim of this thesis is to develop our understanding of the biology and ecology of bedbugs in a pure science context, but with a view to informing control strategies. The findings of this thesis therefore have implications for our understanding of fields such as dispersal ecology and group living, as well as implications for the successful management and eradication of this emerging pest. For this reason the implications of the research will be discussed from a control perspective separately at the end of each discussion section.

The research presented in this thesis was conducted in three phases. The first phase involved the collection of observational data from natural infestations (Chapter 2). This data was used to inform the design of a laboratory arena that replicated the bedbugs' natural ecology, in the second phase (Chapter 3). The third phase of the research utilised this laboratory arena to conduct a series of manipulations to investigate the ecology of the bedbug and define some of the biological parameters that affect its dispersal (Chapters 4, 5 & 6).

1.2 Historical Context

1.2.1 The Arrival

The first record of bedbugs in the UK was published by Thomas Moufet (1634) (referenced in Usinger 1966), and consists of an account is of two noble ladies who were bitten while staying in Mortlake in 1503. Southall's "A Treatise of Buggs" (1730) gives details of the arrival and spread of bedbugs in the UK, along with some control measures. According to Southall, "[at the time of publishing] *not one seaport in England is free from* [bedbugs]*, in inland towns buggs are hardly known*.". This early pattern of infestations could be interpreted as evidence for repeated colonisation events from overseas source populations. However this may equally be a reflection of the population density of English coastal towns of this time period compared to inland towns, or a result of better transport links via the shipping routes resulting in a greater movement of people and goods.

By the 1930s (and probably long before) bedbug infestations throughout the UK were common (Busvine 1957). In fact, by the early 1930s a 'Royal Commission on Bed Bugs' was established. Data from Busvine (1964) showed that in one English town in 1934 more than 10% of homes were infested with bedbugs, while in London, the Royal Commission's report stated that in some areas up to 100% of homes had bedbug infestations (Ministry of Health 1934).

1.2.2 The Decline

Following the 1930s bedbugs in Britain declined, initially sharply and then more steadily, until the 1980s and remained at very low levels into the latter part of the 1990s (Busvine 1957, 1964, Boase 2008). Although evidence from the Environmental Health Departments of ten cities within the Greater Manchester area shows that a handful of reservoirs of bedbugs, primarily in Manchester and Salford, persisted at relatively high levels throughout the later part of the 20th century (Dunn 1993). These reservoirs may have played a significant part in the recent upsurge throughout the UK.

The organochlorine insecticide Dichloro-diphenyl-trichloroethane (DDT) is widely cited as being significant in, or even responsible for, the post 1930s decline in bedbugs (Busvine 1957, 1964, Doggett & Russell 2008, Mumcuoglu 2008). However, its widespread introduction following World War II coincided with many other factors also likely to have been involved in the bedbug decline (Pinto *et al.* 2007, Boase 2008).

These include major improvements in hygiene, sanitation and housing (Pinto *et al.* 2007). Busvine's (1964) own study of houses in an English town shows that bedbugs had already declined by 80% prior to the introduction of DDT in about 1945 (Figure 1.1) (see also Boase 2008).

In the UK one of the most significant factors likely to be involved in the dramatic decline in bedbugs was the 1930 Housing Act. After it was passed, work began clearing the slums and building properly planned houses with access to light, water, ventilation and sanitation.

In 1936 the findings of the Royal Commission on Bed Bugs contributed to the passing of the Public Health Act, making it the "*duty* [of the local authority]…*in the case of verminous premises*…[to take] *such steps as may be necessary for destroying or removing vermin*." (Public Health Act 1936, Boase 2008). Local authorities were now accountable for controlling pests in their areas, and had new powers of entry into infested premises, so that control measures could be carried out.

There is currently no published data on the abundance of bedbug infestations on mainland Europe, prior to the mid 1940s. It is therefore difficult to know if Europe experienced the same pattern of decline as occurred in the UK. In 1948 an extensive survey of public buildings and domestic premises (mainly flats) in Berlin (Germany), showed that bedbug infestation rates were as high as 40% in the city centre, decreasing to around 2% in the suburbs (Busvine 1957). In Denmark it has been mandatory to have a survey for bedbugs whenever a person wants to move house since 1945. Records of the findings from these surveys in one district between 1945 and 1955 provide some of the best data we have of the occurrence of bedbugs anywhere in Europe for that time period (Figure 1.2).

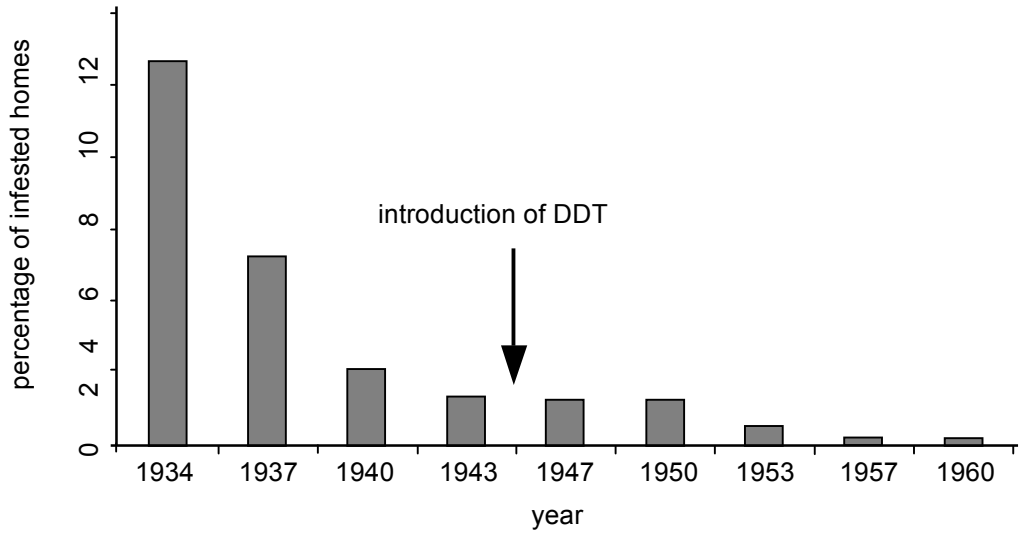


Figure 1.1 Decline in bedbugs through the 20th Century in an English town (modified from Busvine 1964 and Boase 2008).

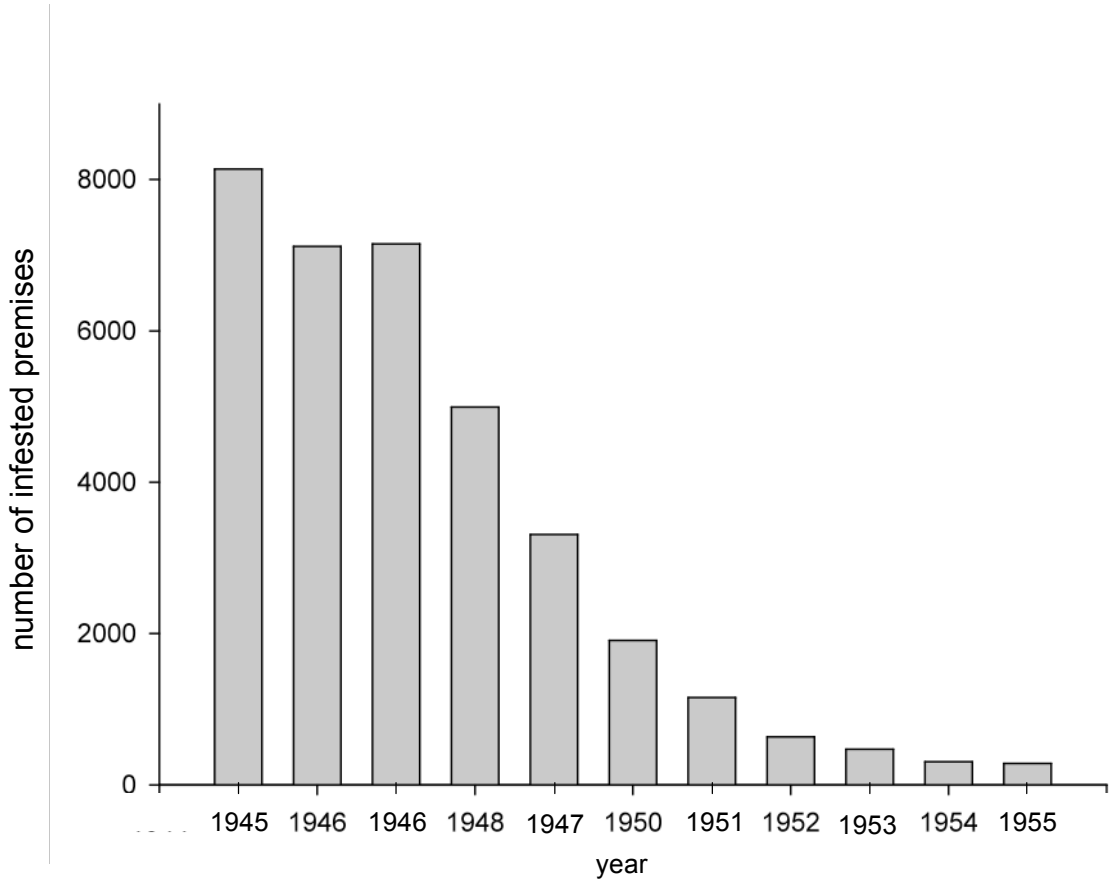


Figure 1.2 shows the number of bedbug infested premises found in one district of Denmark between 1945 and 1955. The total population is about 600 000 living in a total of about 225 000 habitations (mainly flats) (from Busvine 1957).

1.2.3 The Resurgence

Since ca. 2000 many reports have shown a dramatic increase in the number of new bedbug infestations in the UK (Boase 2001, Richards *et al.* 2008), Australia (Doggett *et al.* 2004, Doggett 2008), Asia (Hirao 2010, Lee *et al.* 2008, Tawatsin *et al.* 2011) and the US (Potter *et al.* 2008). Although, records since 1950 from the advisory service of the Danish Pest Infestation Laboratory (DPIL), show that bedbug enquiries, as a proportion of the total number of enquiries received, rose from 0.5% to 2.5% between 1960 and 1985, indicating that the resurgence may have begun in Denmark as much as forty years prior to the resurgence in the UK, Australia and the US (Kilpinen *et al.* 2008).

1.3 Overview of Previous Research

Virtually all of the descriptive biology of the bedbug was carried out in the first half of the Twentieth Century. Patton & Cragg (1913) first described the unusual mating behaviour of the bedbug and the tropical bedbug (*C. hemipterus*). They realised for the first time that the male copulatory organ, the paramere, is never introduced into the vagina of the female. Instead it is used to pierce the female's body wall, between the 5th and 6th abdominal sternites, so that the ejaculate is pumped into the body cavity *via* the "organ of Berlese" (later retermed the spermalege (Carayon 1959)). Through the 1930s and early 1940s Mellanby (1932, 1935, 1938, 1939a, 1939b), Johnson (1937, 1940, 1941) and Omori (1941) published the foundations of our current knowledge on the physiology, ecology and behaviour of both the bedbug and the tropical bedbug. In the 1950s and 1960s Carayon (1966) advanced our understanding of the reproductive physiology of the bedbug, and other cimicids. He examined the structure and function of the whole paragenital system and the process of insemination in detail, and described its significance in classification and in understanding how the group evolved. The "Monograph of the Cimicidae" (Usinger 1966) consolidated all prior knowledge and still forms the basis of our understanding of the group.

Following the publication of the monograph (Usinger 1966) there was a lull in bedbug research for about two decades, probably as a result of the pest's decline. However, interest in the group was revived when it was identified as a model organism for studying cryptic female choice (Eberhardt 1996) and later sexual conflict (Stutt & Siva-Jothy 2001). Since the 21st Century much of the new bedbug literature has focused

on the bedbug resurgence (Krueger 2000, Paul & Bates 2000, Boase 2001, 2004, Burgess 2003, Doggett *et al.* 2003, 2004, Doggett & Russell 2008, Poorten & Prose 2005, Potter 2005, Kilpinen *et al.* 2008) and their control (Temu *et al.* 1999, Cleary & Buchanan 2004, Doggett 2004, 2005, Potter 2005, 2006, Potter *et al.* 2006, 2007, Romero *et al.* 2007).

1.4 General Biology of the Bedbug

Bedbugs are true bugs (Order: Heteroptera), belonging to the family Cimicidae. Like all other members of this family they are obligate haematophages requiring blood from one of a range of vertebrate hosts in order to develop between instars and to reproduce (Figure 1.3). Along with *C. hemipterus* (the "tropical bedbug" found in the Old and New World tropics) and *Leptocimex boueti* (found in W. Africa) bedbugs are primarily associated with humans (Usinger 1966). However, wild populations have also been found on several species of birds and bats as well as rats, and under laboratory conditions they can be cultured on rabbits, mice and guinea pigs (Johnson 1941, Davis 1956, Adkins & Arant 1959, Usinger 1966, Reinhardt & Siva-Jothy 2007).

Bedbugs are negatively phototropic and hide in narrow crevices, typically within a few metres of the host (Butler 1893, Johnson 1941). For this reason host species are characterised by their spatial and temporal predictability and their tendency to aggregate in enclosed spaces such as caves or buildings (Reinhardt & Siva-Jothy 2007).

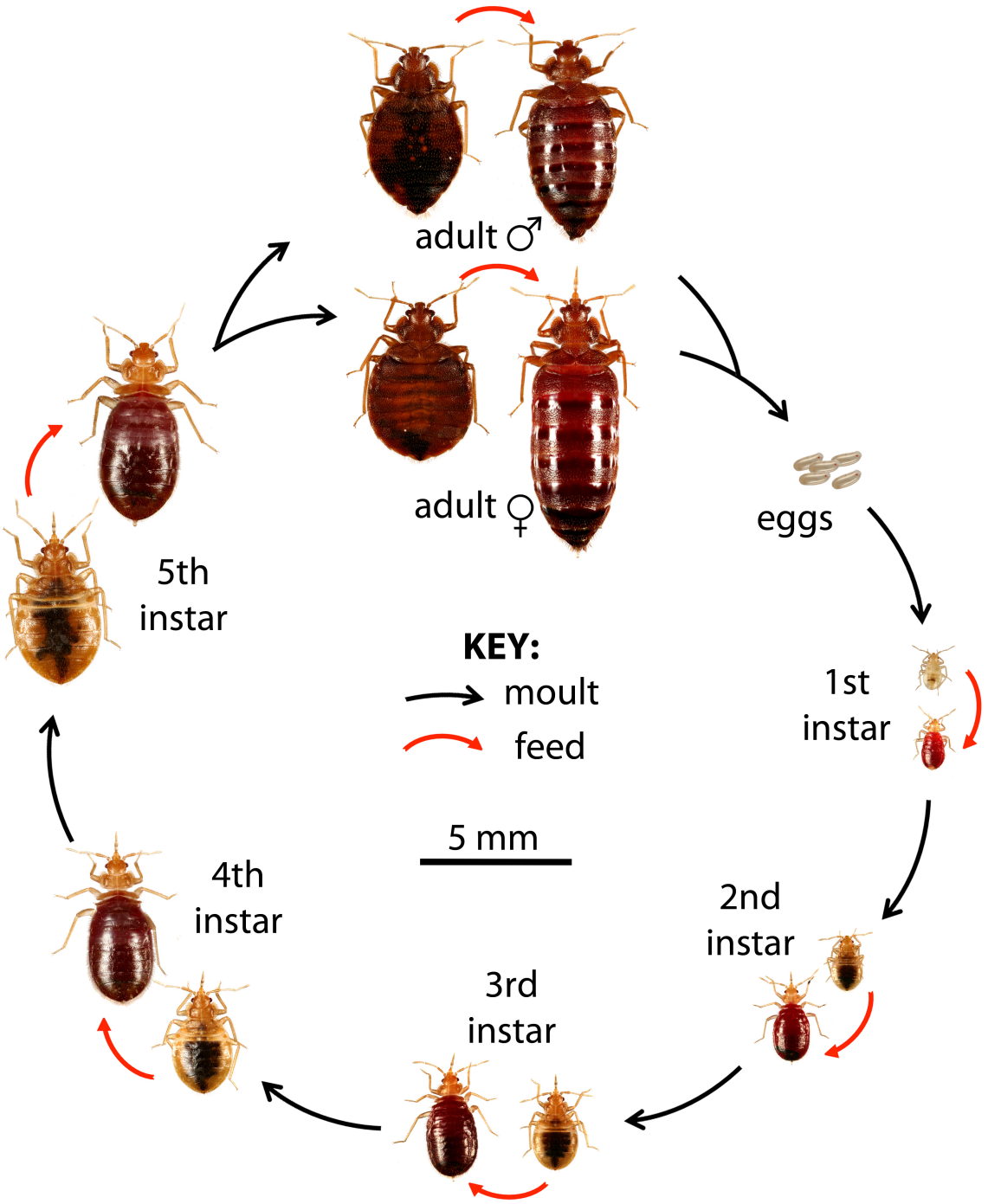


Figure 1.3 shows the lifecycle of the bedbug. With *ad libitum* food nymphs develop from one instar to the next in 5-7 days at 26°C, so the minimum duration of the lifecycle is approximately 6 weeks. In the absence of food most instars are capable of surviving many months, particularly at cooler temperatures.

1.4.1 Intraspecific communication and host detection

Bedbugs have a complex array of sensory receptors which are used in both communication and host location (Siljander 2006). They have been shown to respond to heat (Rivnay 1930, 1932b), humidity (Mellanby 1935), pheromones and kairomones (Levinson & Bar Ilan 1971, Levinson *et al.* 1974a/b, Rivnay 1932b), air movement (Kemper 1936, Johnson 1941), and carbon dioxide (Hase 1917 in Usinger 1966). The bedbug's ability to detect the host and its conspecifics is likely to be important in the selection of suitable harbourages, which may in turn be important in driving dispersal if suitable harbourages become a limiting resource.

1.4.1.1 Communication

Like nearly all Heteroptera, bedbugs have scent glands (Aldrich 1988). These glands produce both aggregation and alarm pheromones (Levinson & Bar Ilan 1971, Levinson *et al.* 1974a/b, Siljander 2006). Levinson & Bar Ilan (1971) identified the primary constituents of the scent glands as (E)-2-hexenal (73-92%) and (E)-2-octenal (8-27%). They showed that there was no aggregation effect of (E)-2-hexenal and (E)-2-octenal when applied to filter papers in choice-chamber experiments, but that these chemicals could cause rapid dispersal of aggregations if applied in the same proportions as they occur in the scent glands. It was thus concluded that (E)-2-hexenal and (E)-2-octenal were alarm pheromones. However, Siljander *et al.* 2008 identified and synthesised the aggregation pheromones and showed that (E)-2-hexenal and (E)-2-octenal were in fact two of ten essential components. They concluded that there was a threshold effect, whereby the aggregation pheromones become alarm pheromones when released at high concentrations.

The production of airborne aggregation pheromones is likely to be important in harbourage location after feeding (Siljander *et al.* 2008). Aside from reducing the search time and energetic costs associated with finding a harbourage, there is good evidence that bedbugs can also use pheromones to determine the demography of the harbourage occupants. In a dual-choice experiment Siljander *et al.* (2007) showed that both males and females preferred to aggregate on male-exposed paper discs compared to controls. Neither adult males nor adult females showed any significant preference for discs previously exposed to females or to nymphs over the control. By contrast, nymphs preferred nymph-exposed discs but showed no preference or avoidance of discs

previously exposed to adults of either sex. At first glance the responses of the adults seem counterintuitive: why should males or females choose male-dominated refuges? However, in none of the trials were bedbugs given the opportunity to choose *between* male-exposed and female-exposed discs. Thus, assuming that untreated discs were not identifiable as potential harbourages, it can only be inferred that both male and female bedbugs prefer male dominated refuges to residing outside a harbourage.

In laboratory cultures foraging is often triggered by the arrival of a freshly fed bug returning to the refuge (pers. obs.). It is not currently known what signals are received by the unfed bugs but they are likely to include visual, chemical and thermal stimuli. Although bedbugs do make "appetitive searches" in the absence of any external cues (Johnson 1941, Lehane 2005), there is clearly an advantage to knowing the host is present before leaving the safety of the harbourage. A freshly engorged conspecific is a very good indicator that the host is present and that successful feeds are currently taking place.

1.4.1.2 Host detection and location

Temperature is clearly an important cue in host location (Rivnay 1930, 1932b, Usinger 1966). However, the range over which bedbugs can detect body heat has been a matter of some debate. Rivnay (1932b) believed that bedbugs foraged randomly until they were within 3-4 cm of the host, at which point thermotaxis was initiated. However, Marx (1955 – in Usinger 1966) showed that bedbugs can detect the host from 150 cm away, and attributed the attraction to a combination of warmth and carbon dioxide. Bedbugs only use host-derived volatiles (kairomones) such a sweat and sebaceous gland materials for very short range host location (Rivnay 1932b). Immediately after feeding, the attractive cues become neutral or repellent, which ensures that the bedbug leaves the potentially hazardous feeding site as quickly as possible (Aboul-Nasr & Erakey 1968, Reinhardt & Siva-Jothy 2007).

1.4.2 Harbourage dynamics and availability

One factor likely to affect population growth in bedbugs is the availability of suitable harbourages. This factor was identified but not explored by Johnson (1941). There is currently very little known about the way bedbugs use harbourages. Whether or not they return to the same harbourage after feeding has not been determined. Similarly we do not know if access to harbourages is on a 'first come, first served' basis or if, for

example, larger bedbugs are able to displace smaller ones. Females may choose harbourages with fewer males, in order to reduce the costs of traumatic insemination (see Stutt & Siva-Jothy 2001). Similarly males may chose harbourages with more females in order to advance mating opportunities.

Mellanby (1938) showed that bugs starved for one week and then forced to run around for several minutes, paid a significant energetic cost; so much so that if a meal was not provided, the bedbugs died within a few hours. A bedbug travelling several metres from the harbourage to the host (and back) is therefore likely to suffer an energetic cost compared to one only travelling a few centimetres, particularly if the host is found to be absent and the journey is fruitless. Furthermore, Mellanby's (1938) experiments were carried out on unfed bedbugs, but adult bedbugs can take 4-5 times their bodyweight in blood in a single feed (Usinger 1966, Richard Naylor unpublished data). So although a feed will mitigate the effects of starvation, a bedbug that feeds to repletion and then has to walk for several minutes to find an available harbourage may pay significant costs, either directly in terms of reproductive output, or through requiring a shorter feeding interval to maintain egg production. It is not known if the energetic costs of travelling have a role in harbourage selection or what defines a suitable harbourage to a bedbug, however I will explore this in Chapter 4. Aside from the energetic costs, travelling between the host and the harbourage increases exposure to predators, primarily spiders (Usinger 1966), and increases the likelihood of being discovered by the host. Females that have to travel longer distances may also suffer from increased exposure to traumatic inseminations from males, which Stutt & Siva-Jothy (2001) have shown to be costly both in terms of longevity.

As well as distance from the host, harbourages may vary in quality. There is currently no data on what constitutes a 'good' harbourage. Bedbugs often show preferences for particular sites on a given design of bed (Pinto *et al.* 2007), but we do not know if, for example, harbourages are abandoned when they become too dirty or if harbourage requirements change with maturity. Given the tendency for bedbugs to defecate in their harbourages (Pinto *et al.* 2007), and that traumatic insemination routinely leads to the introduction of environmental microbes into the body cavity of the female (Reinhardt & Siva-Jothy 2007), it may be in the interests of the female to avoid particularly unsanitary harbourages. The dynamics of harbourage selection are potentially complex but their implications for pest management make this an important factor to consider, especially as it may also have a role in driving dispersal.

1.4.3 Influence of abiotic conditions

Like all insects the abiotic conditions of the bedbug's environment have a profound effect on its activity and reproductive rate. Richards *et al.* (2009) looked at the number of calls to pest control teams of London local authorities between 2000 and 2006 and showed that the highest levels of bedbug activity were recorded in the summer months (August-September), which is attributed to the warmer weather. Understanding the influence of the abiotic conditions is important for successful culturing and the development of a laboratory setup to house bedbugs under controlled conditions that reflect their natural environment. It is also important to understand the influence of environmental factors from a control perspective, since this will affect the rate at which an infestation grows and spreads.

1.4.3.1 Temperature

Of the abiotic factors affecting bedbug population growth, temperature is by far the most important (Usinger 1966). Temperature affects many aspects of their physiology and behaviour including feeding activity, development time and egg laying rate (Mellanby 1935, Johnson 1942). The mean total development time increases fourfold from 36.4 days to 127.9 days when bedbugs are cultured at 18ºC compared to 33ºC (Johnson 1942). Similarly, at 30ºC eggs hatch in 4 days, while at 23ºC eggs hatch in 7.92 days (Johnson 1942). At 13ºC bedbugs stop feeding and laying eggs altogether and any eggs that have been laid usually fail to hatch. (Jones 1930, Mellanby 1935). This temperature is referred to as the "developmental zero".

Johnson (1941) made the first attempts to model population growth in the bedbug. The major factor in his models was the affect of temperature, which cycled annually. Johnson's assumptions of annual temperature change were based on a survey of 5 houses in the London area, all of which showed a significant drop in temperature over the winter months. In fact 3 of the 5 properties that were surveyed had average temperatures below 13°C for approximately 6 months over the winter of 1935-36. Since the developmental zero of bedbugs is around 13ºC, Johnson's models of population growth in bedbugs were based on a relatively short reproductive season followed by a long period of stasis and winter die-off (Johnson 1941). However, today, room temperatures in houses are typically in the range 18-24°C. Consequently the primary assumptions of Johnson's (1941) models are no longer accurate. Seasonal affects are likely to be limited, since room temperatures in most houses are thermostatically controlled and are therefore unlikely to fall below the 13°C threshold. This allows

bedbugs to continue feeding and reproducing throughout the year, which may be an important factor in their resurgence.

The upper thermal death-point for bedbugs is 44ºC when exposed for 1 hour (Mellanby 1935). However, Chang (1974) showed that bedbugs cultured at 36ºC for two weeks suffered a 90% reduction in fecundity compared to those cultured at 27ºC, which was attributed to the loss of bacterial symbionts from their mycetomes (specialised organs containing symbiotic bacteria). It is therefore important to maintain culture temperatures well below 36ºC to avoid damage to the bacterial symbionts.

1.4.3.2 Humidity

Bedbugs have a number of behavioural and physiological adaptations, which allow them to resist desiccation in low humidity environments. An impermeable waxy cuticle, a very low rate of transpiration when inactive, and a tendency to aggregate to form water-conserving clusters helps them to reduced water loss (Benoit *et al.* 2007). They also have a very high tolerance to desiccation, surviving 30-40% loss of body water. Together, these adaptations allow them to survive for up to 2 weeks (in adults) at 0% RH (Benoit *et al.* 2007). Mellanby (1935) showed that when food is available, freshly fed bedbugs regulated the amount of superfluous water they excreted after feeding, according to the humidity of the environment. Thus, bedbugs cultured at lower humidities retained more water than those cultured at higher humidities, allowing them to resist desiccation for longer in drying environments.

Rivnay (1932a) showed that there was little or no effect of humidity (in the range 10-70% RH) on the rate of development of bedbugs, although Kemper (1936) noted that very high humidity often caused the death of laboratory cultures through encouraging fungal growth. The same effect may also occur in natural infestations if the density of bedbugs in a harbourage becomes sufficiently high. There may therefore be a selection pressure acting to limit the maximum density of bedbugs within a harbourage.

1.4.4 **Dispersal**

The demographic and genetic structure of populations can be greatly affected by dispersal (Denno & Peterson 1995, Lee *et al.* 2010, Strevens & Bonsall 2011), so understanding the phenomenon is of profound importance to the fields of population ecology, molecular biology and conservation among others. However, in many systems dispersal is, or until recently has been, immeasurable (Nathan 2001). Recent advances

in molecular techniques as well as the use of satellite tags, radio tags and transponders have led to improvements in our ability to detect and study dispersal (see Bilton *et al.* 2001, Nathan 2001). However, laboratory systems that allow the study of dispersal through careful manipulation of its influencing factors are scarce (but see Bengtsson *et al.* 1994, Strevens & Bonsall 2011 for examples). Consequently the first challenge of this research will be to develop a setup in which bedbug dispersal can be monitored and the factors affecting dispersal manipulated.

1.4.4.1 Active dispersal in bedbugs

Active dispersal can be driven by the absence of the host(s), such as in a vacated hotel room (Pinto *et al.* 2007), or by the application of pesticides such as synthetic pyrethroids, which have an excitatory affect on many insects (Barcay *et al.* 1990). However it also commonly occurs while the host is still present. It is not yet understood what triggers a bedbug to make the apparently risky decision to disperse away from an established infestation where there is a host providing an *ad libitum* source of food. The dangers associated with leaving a harbourage in search of a new host are likely to be high. Predation, primarily from spiders as well as several species of ant (Usinger 1966), which is virtually absent within the harbourage, is greatly increased once the bug is in the open. Furthermore, there is no guarantee that the dispersing bedbug will ever locate a new host, and yet active dispersal is a major factor in the spread of bedbugs throughout multiple-occupancy dwellings such as apartment blocks, hotels, hospitals and nursing homes (Pinto *et al.* 2007, Doggett & Russell 2008).

1.4.4.2 Passive dispersal in bedbugs

Unlike active dispersal, which is presumed to be limited to a few tens of metres, passive dispersal has the potential to transport bedbugs to and from anywhere in the world, hitchhiking on clothing, luggage, or other items (Boase 2001, Doggett *et al.* 2004, Pinto *et al.* 2007, Potter *et al.* 2008). Bedbugs have been widely reported on passenger aircraft (Doggett *et al.* 2004), trains (Busvine 1957), and ships (Rucker 1912, Doggett 2008). Arevad (1987, reported in Kilpinen 2008), has speculated that the resurgence in bedbugs after 1960 may have been due to an increase in the number of migrant workers and holiday makers from Southern Europe. Similarly Potter believes that the bedbug problem in the US was triggered by an increase in the number of migrant workers from Central America, many of whom were employed in the

hospitality industry, and may have been responsible for the accidental reintroduction of bedbugs into the hotels where they worked (Potter pers. comm.). However, Boase (2008) argues that an assumption of the importation hypothesis is that "there are large reservoirs of bedbugs in some countries, which are being exported to those countries experiencing an increase". He argues that there is no evidence that sufficiently large reservoirs exist. Furthermore, if foreign import of bedbugs was a key factor in driving the increase, then one would expect to find a large increase in the tropical cousin of the bedbug, *C. hemipterus*. Although this species has occasionally been found in the UK (Boase pers. comm.) and *is* now widespread in Northern Australia (Doggett et al. 2003, Doggett & Russell 2008), it is *not* the species primarily responsible for the global increase in reports of bedbugs (Boase 2008).

Little is known about those bedbugs that passively disperse. It is assumed that the dispersing individuals are a random sample of the population that have become accidentally associated with clothing or belongings, however in a close relative of the bedbug, the swallow bug (*Oeciacus vicuarius*), adults will actively enter a passive dispersal phase, clustering around the entrance of the nest in order to climb onto the returning swallow (Loye 1985). While the significance of the role passive dispersal has played in the current bedbug pandemic is still in debate, it has undoubtedly facilitated their spread.

1.5 Summary

Since the beginning of the 21st Century the occurrence of bedbug infestations in the UK has increased dramatically; a pattern reflected in the northern and southern temperate zones across the world. This recent trend has sparked a new wave of interest in bedbugs, largely from a control perspective. The reason for the recent upsurge is not clear and has been attributed to many different factors. However it is likely that there is no single underlying factor to which the bedbug pandemic can be attributed, but rather a combination of factors facilitating their reproduction, survival and dispersal. The 50 year near-absence of bedbugs combined with the suddenness with which they have re-emerged as a global pest has left chasms in our understanding of their biology and ecology; information which is essential for their control.

1.6 Thesis Outline and Core Questions

In Chapter 2 I will examine four case studies where dispersal is know to have occurred.

In Chapter 3 I will use this information to develop a laboratory arena that replicates the ecology of natural bedbug infestations.

In Chapter 4 I will use the laboratory arena (developed in Chapter 3) to answer the following questions about the way bedbugs utilise harbourages:

1) Does the number and distribution of harbourages increase with population size?
2) Are bedbugs faithful to particular harbourages or localities?
3) Does harbourage location influence the feeding frequency of the individuals within?
4) Is there a measurable energetic cost associated with commuting to the host?

In Chapter 5 I will use the laboratory arena (developed in Chapter 3) to answer the following questions about the factors affecting dispersal from an infestation:

1) Is dispersal driven by a lack of available harbourages?
2) Do females disperse to avoid sexual harassment from males?

3) Do females avoid males through harbourage selection within an infestation?

In Chapter 6 I will compare dispersing individuals with non-dispersing individuals to see if they differ in terms of:

1) Feeding status (time since feeding)
2) Mating status (time since mating)
3) Sexual harassment (number of copulatory wounding scars)
4) Body size

In Chapter 7 I will discuss the significance of my results.

2 Natural Infestations

2.1 Introduction

Studying bedbugs in the field is difficult because eradication normally begins immediately after an infestation is detected/reported. There are also ethical issues associated with allowing an infestation to develop and disperse to neighbouring properties for the purposes of research. Consequently I needed to construct a realistic laboratory arena that had no need for a human host. Any laboratory arena must enable bedbugs to behave in as natural a way as possible, allowing foraging, hiding and dispersal behaviours within an enclosure that is simple enough to manipulate and ensure repeatability of observed behaviours.

It was therefore necessary to examine natural infestations and measure the abiotic parameters, the size and duration of the infestations and the scale over which bedbugs move within and between infestations.

More than 95% of domestic infestations encountered in London are identified and treated before active dispersal to neighbouring properties begins (Cain, Bed-bugs Ltd., pers. comm.). Although actively dispersing infestations make up only a small proportion of the total, they are the most important from a control perspective as these infestations are responsible for producing the founders that potentially begin many new infestations. Since the primary focus of this thesis is to understand the factors affecting active dispersal in bedbugs, case studies focussed specifically on infestations where there was evidence for active dispersal.

2.1.1 Chapter aims

In this chapter I will determine the spatial and abiotic parameters necessary to construct a tractable laboratory-based arena setup. I will:

1) Measure the temperature and humidity in natural infestations.
2) Determine the minimum and maximum distances from the host that harbourages naturally occur.
3) Look for patterns in the spatial distribution of harbourages, relative to the host.

4) Identify aspects of behaviour and/or ecology that have direct relevance to the question of "what drives dispersal in bedbugs?".

2.2 Methods

All field studies were conducted in conjunction with D. Cain (Bed-bugs Ltd.). D. Cain is a former research biologist, with a background in molecular biology, who set up the first pest control company in the UK that specialises solely in the eradication of bedbugs. Although he no longer has direct ties with academia, he is internationally considered to be an expert in the field of bedbug behaviour and control.

Cain was asked to contact me when multiple adjacent infested dwellings had been identified. This is a good indication that the bedbugs are actively dispersing from one or more dwellings, as the probability of independent adjacent infestations is extremely small. Reliance on a pest control officer (Cain) for access to infestations typically gave me less than 48 hours notice and a window of approximately 30-60 minutes within which to collect data and samples before treatment to eradicate the infestation began.

For each infestation the abiotic conditions of the room were recorded and a scale plan of the premises was constructed, showing the nighttime location of the host, bedbug harbourages and any other key features that might influence the ecology and behaviour of the bedbugs. The population size of the bedbugs at each infestation was estimated and where it was known, the duration of the infestation was also recorded. However, this can be highly subjective, as people often do not notice the presence of bedbugs until the infestation is well established (pers. obs.).

If dispersal from the infestation was known to have occurred, this was noted, and where possible, neighbouring premises were surveyed so that comparisons could be made between recently colonised and more established infestations within the same building.

As well as marking the locations of the harbourages on the plan, the nearest and furthest harbourages were identified. For these harbourages the minimum distance a bedbug would have to travel to reach the host was measured as accurately as possible, taking terrain into account. Where bedbug harbourages were easily accessible, all individuals from within each harbourage were collected for analysis. Sex ratios and proportions of nymphs were established for each of these harbourages.

2.2.1 Statistics

All means are presented ± 1 standard error. Calculations were performed in Microsoft Excel 2008 for Mac.

2.3 Case Studies

2.3.1 **Case Study 1**

Large Victorian house in London W14, split into 5 flats, visited June 2007

The basement flat (Flat A) had no signs of an infestation. The ground floor flat (Flat B) had a very light infestation, with only a few bugs, all of which were found in the bed. The first floor flat (Flat C) had a moderate infestation that was believed to have been present for about six months, with no attempt made to treat it. There were approximately 100 bedbugs, all of which were found on the bed frame. The second floor flat (Flat D, Figure 2.1) was the most heavily infested flat, estimated to have had as many as 50 000-100 000 bedbugs present. It was therefore likely to be the source of the infestation. The flat was owned and occupied by an elderly man who horded newspapers. The newspapers were kept in stacks 50-80 cm high throughout his house (Figure 2.1 & 2.2). The duration of the infestation in Flat D was believed (by the occupant) to have only been 6-8 weeks. However, the number of bedbugs in the flat, along with the vast number of eggs, exuvia and bedbug faecal material, suggests this is likely to be a gross underestimate. The pest control officer (Cain – Bed-bugs Ltd.) estimated the duration of the infestation to have been approximately five years. This seems equally unlikely, as there was a notable absence of dead bedbugs within any of the harbourages, suggesting that the infestation had not persisted for much longer than the lifespan of a bedbug (6-12 months).

The occupants of the top floor flat (Flat E) (where the infestation was first reported) were first aware of bedbugs in their flat nine months previously. This was successfully treated at the time by a pest control company, but they had recently become re-infested, leading to the suspicion that an adjoining flat might be the source of the infestation. At the time of my visit the infestation in Flat E had 10-20 bedbugs, all of which were found on the bed frame.

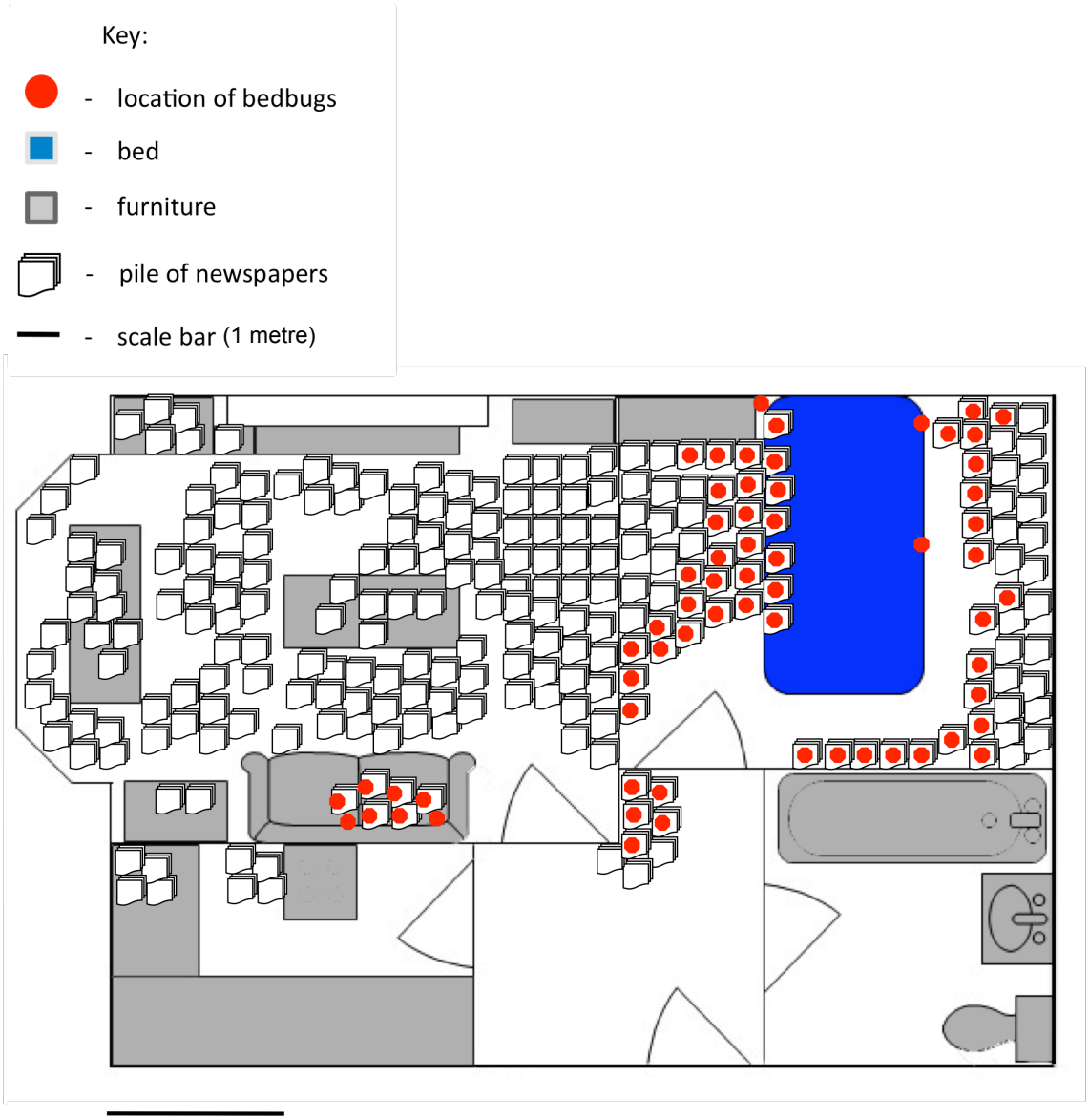


Figure 2.1 shows the room plan of **Case Study 1, Flat D** (2nd floor).

Figure 2.2 (a) shows stacks of newspapers piled up against the side of the bed. The debris on the bed is primarily exuvia (cast skins) from the bedbugs. (b) shows one of the newspapers from the side of the bed unfolded to reveal many bedbugs, exuvia and faecal material.

Table 2.1 summarises the main characteristics of the infestation in **Case Study 1, Flat D**.

Case Study 1: Flat D	
duration of infestation	1-5 years
total number of bedbugs present	50 000-100 000
dispersal to neighbouring flats	yes
number of hosts	1 (male)
number of harbourages	800-1000
minimum harbourage-host distance	< 10 cm
maximum harbourage-host distance	450 cm (220 cm) *
temperature in room	26˚C
relative humidity in room	43 %

* Host occasionally changed nighttime location from bed to sofa (in lounge) to avoid bedbugs. The bedbugs in harbourages on sofa were therefore believed to feed when the host slept on sofa rather than travelling 450 cm to the bed.

2.3.2 **Case Study 2**

Large Victorian house in London NW1, split into 4 flats, visited September 2008

This building was believed, by the tenants, to have been infested for approximately 2-3 months. The ground floor flat (Flat A) had a minor infestation with a total of eight adults and nymphs found closely associated with the bed. The first floor flat (Flat B) was the most heavily infested flat and is also believed to have been the initial source of the infestation, which had subsequently spread to other flats in the building. A total of 185 live bedbugs were found in Flat B, comprised primarily of adults (n=50) and first and second instar nymphs (n=123). The uneven demographic suggests that the infestation was in its second to third generation. The adults were likely to be the first generation of offspring from the initial coloniser(s), which have since reached maturity and produced the large number of small nymphs. Given that a single female can produce around 50 eggs in two weeks, and that nymphs hatch and develop through each of the 5 nymphal instars at approximately weekly intervals, this would make the infestation approximately 10 weeks old. This ties in closely with the estimate of infestation duration given by the tenants. A total of n=21 bedbugs were found in the second floor flat (Flat C); most of these were small nymphs, all of which were found on the frame of the divan-style bed. The top floor flat (Flat D) had one small area of faecal traces on the wall but no live or dead bedbugs were found.

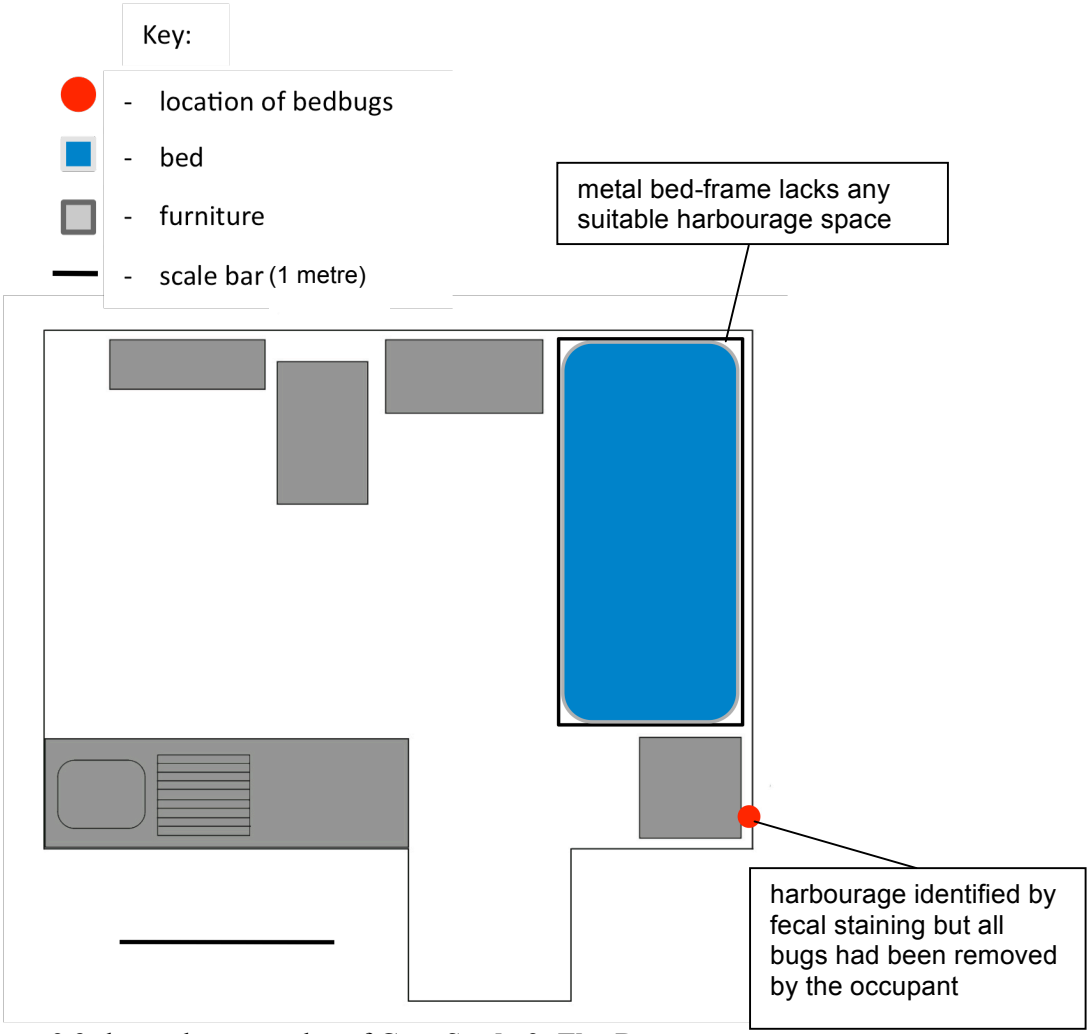


Figure 2.3 shows the room plan of **Case Study 2: Flat D**.

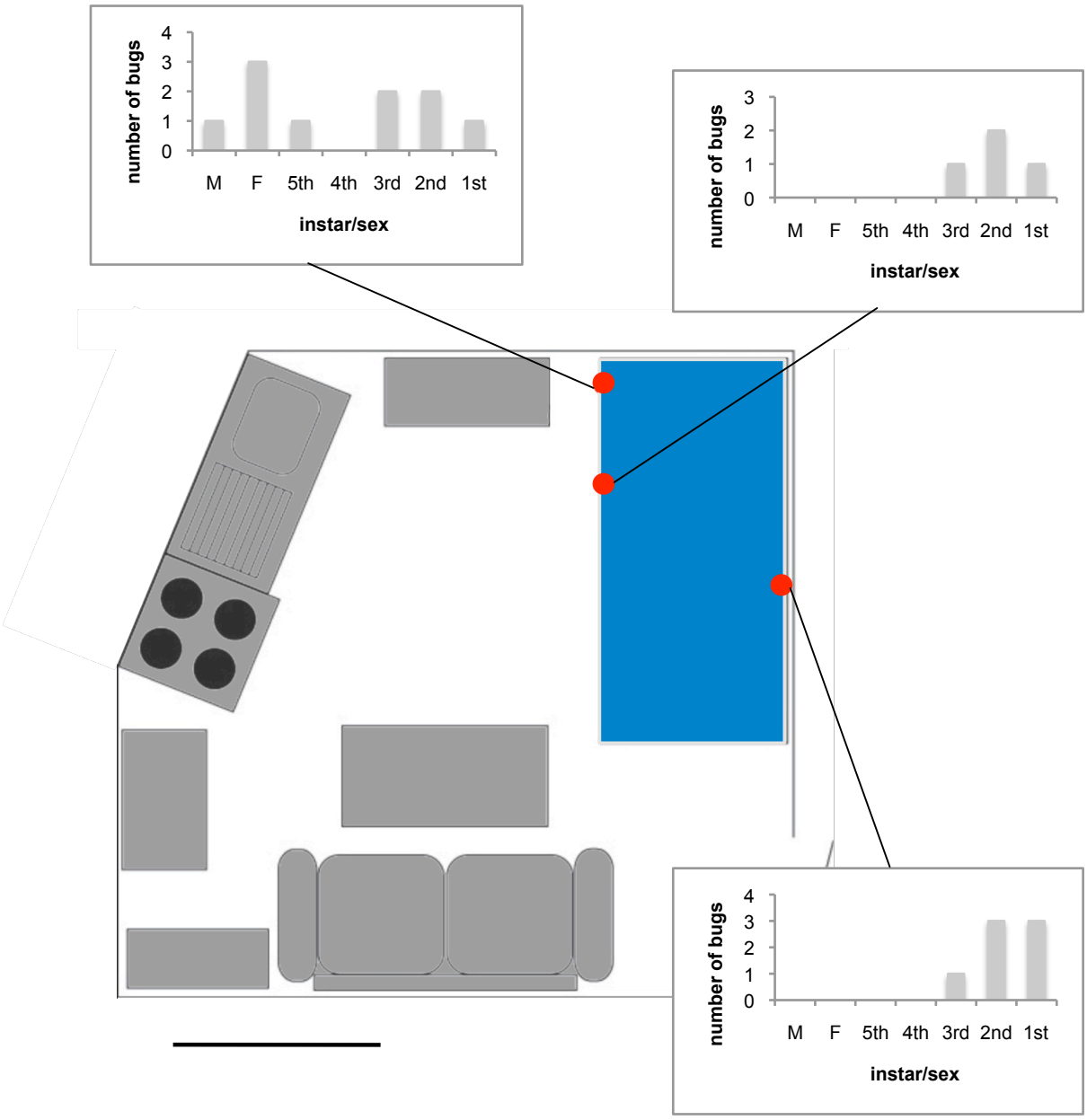


Figure 2.4 shows the room plan of **Case Study 2: Flat C** with the age structure of the bedbugs found within each harbourage in the flat overlaid.

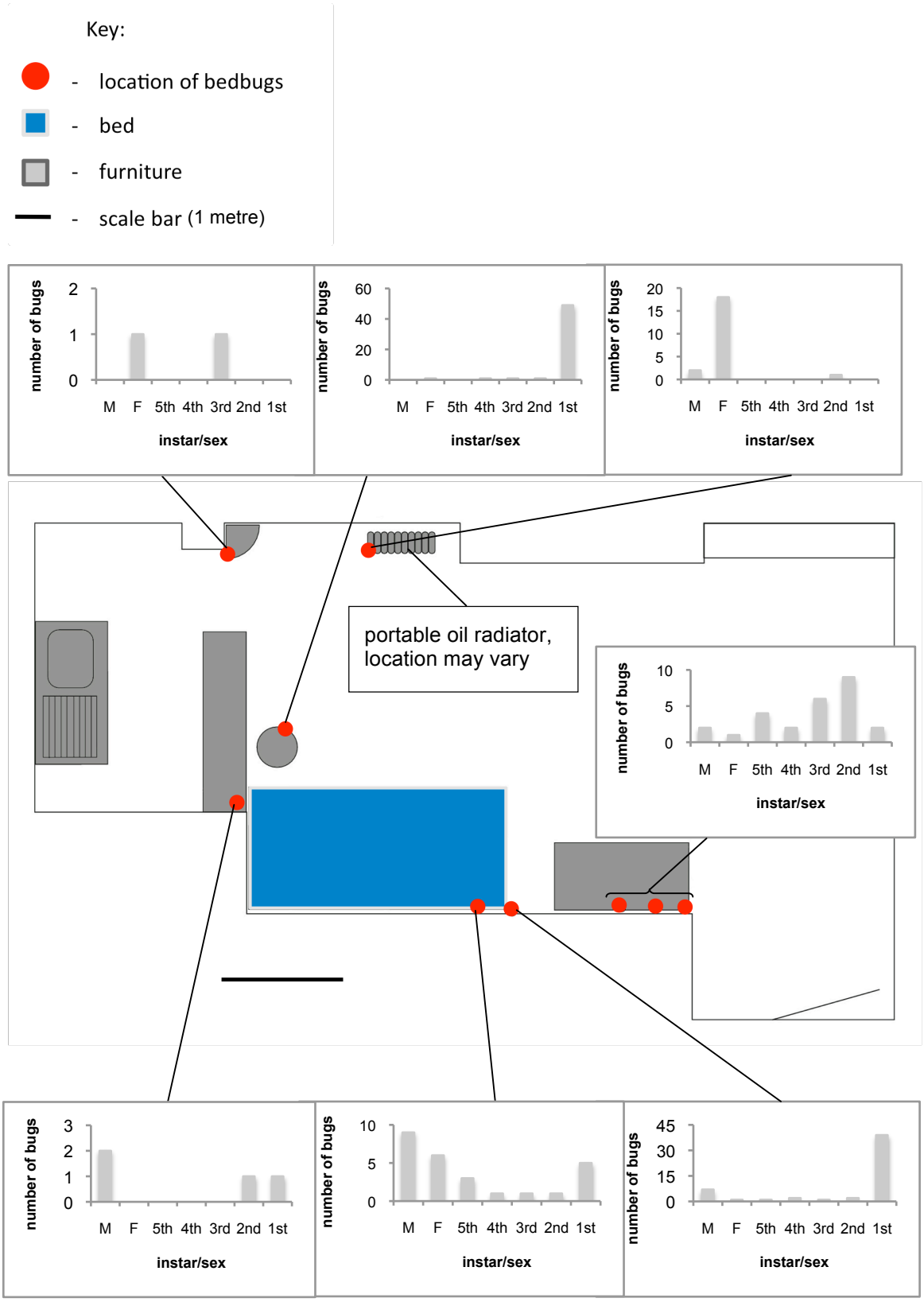


Figure 2.5 shows the plan of the room for **Case Study 2: Flat B** with the age structure of the bedbugs found within each harbourage in the flat overlaid.

Table 2.2 summarises the main characteristics of the infestation in **Case Study 2, Flat 3**.

Case Study 2: Flat 3	
duration of infestation	2-3 months
total number of bedbugs present	< 200
dispersal to neighbouring flats	yes
number of hosts	1 (male)
minimum harbourage-host distance	40 cm
maximum harbourage-host distance	245 cm
temperature in room	19˚C
relative humidity in room	26 %

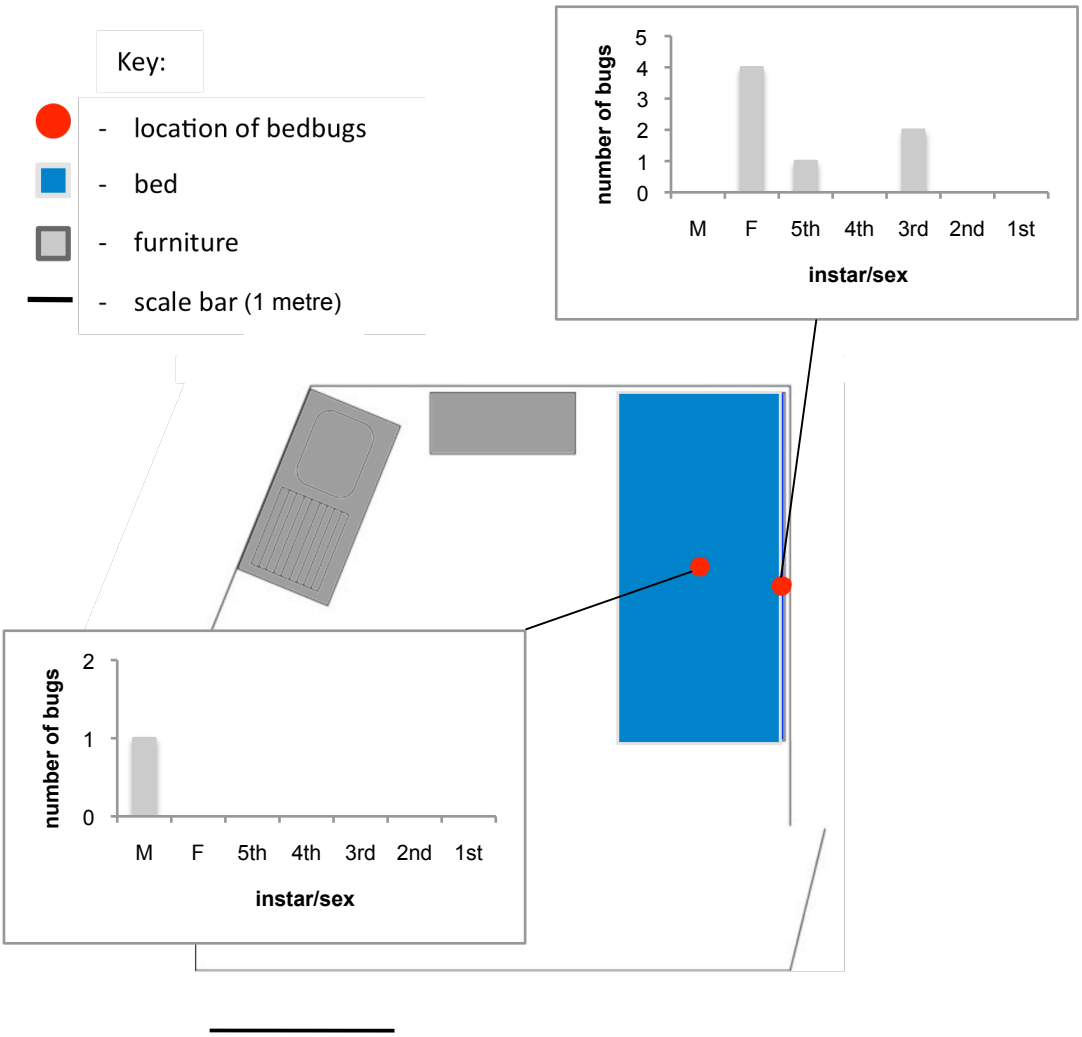


Figure 2.6 shows the plan of the room for **Case Study 2: Flat A**, with the age structure of the bedbugs found within each harbourage in the flat overlaid.

2.3.3 **Case Study 3**

Apartment in large housing complex in London SW11, visited November 2010

This infestation was identified when several adjoining flats had become infested and independently reported the problem. The tenant had been sleeping in the bedroom until four months before our visit (Figure 2.7). He had then been taken ill and spent the following three months in hospital. On his return to the flat he spent one night in his bed and had been severely bitten. Consequently he had slept in his armchair every night for the month leading up to my visit.

Neither the sofa or the armchairs had legs, so their bottom edges sat directly on the floor. This provided a long, continuous crevices at ground level, in which the majority of the bedbugs were found. Bedbugs were also found around the cushions of the sofa and armchairs (Figure 2.8) as well as crawling over the tenant.

There were several large bowls of water on the floor of the flat which the tenant used to brush bedbugs into when he caught them walking over himself. These bowls of water in conjunction with a general lack of ventilation probably contributed to an unusually high ambient relative humidity of around 75%.

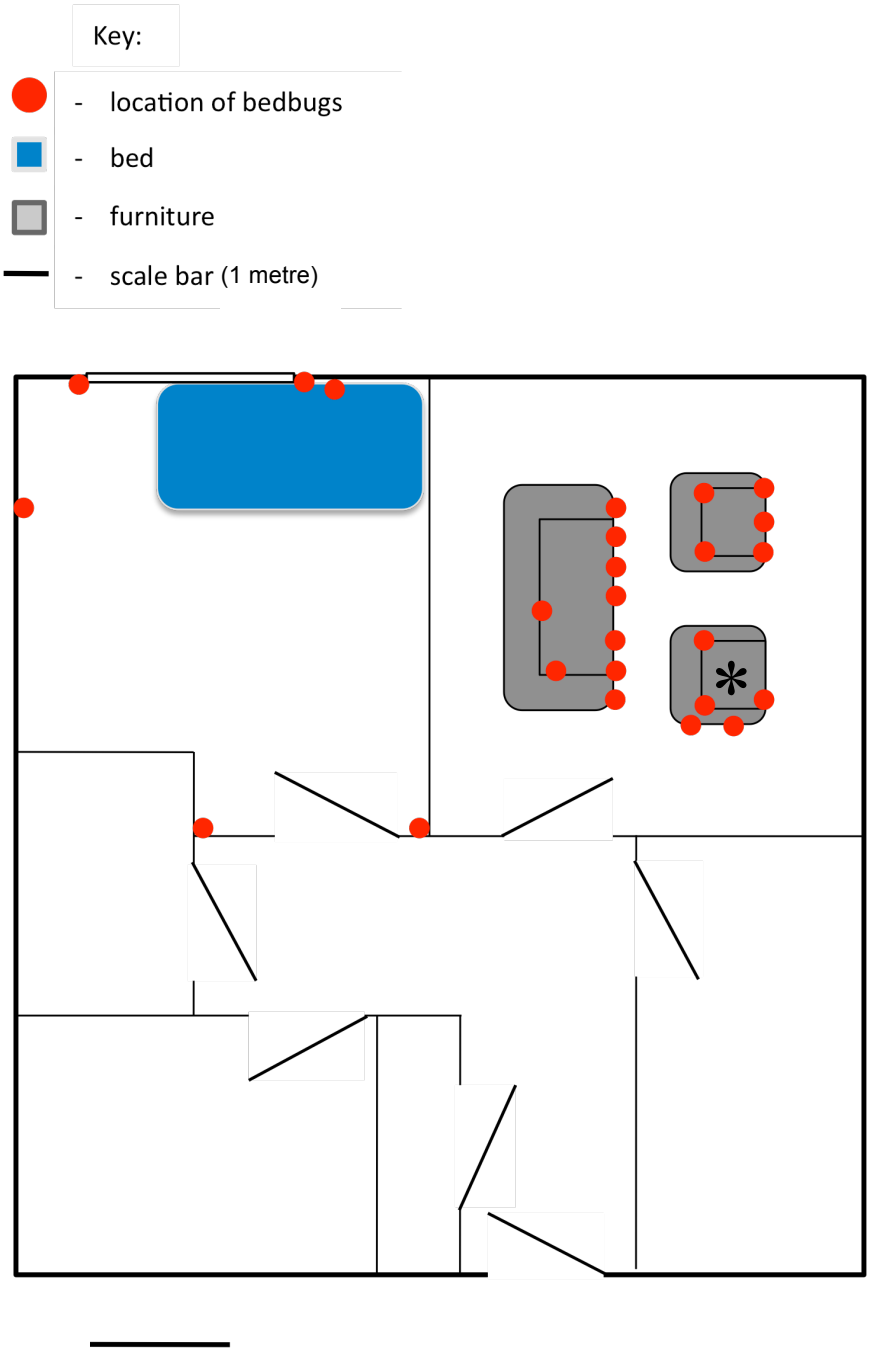


Figure 2.6 shows the plan of the room for **Case Study 3.** The armchair marked with "*" is where the tenant spent the majority of each day and slept for the month leading up to the visit.

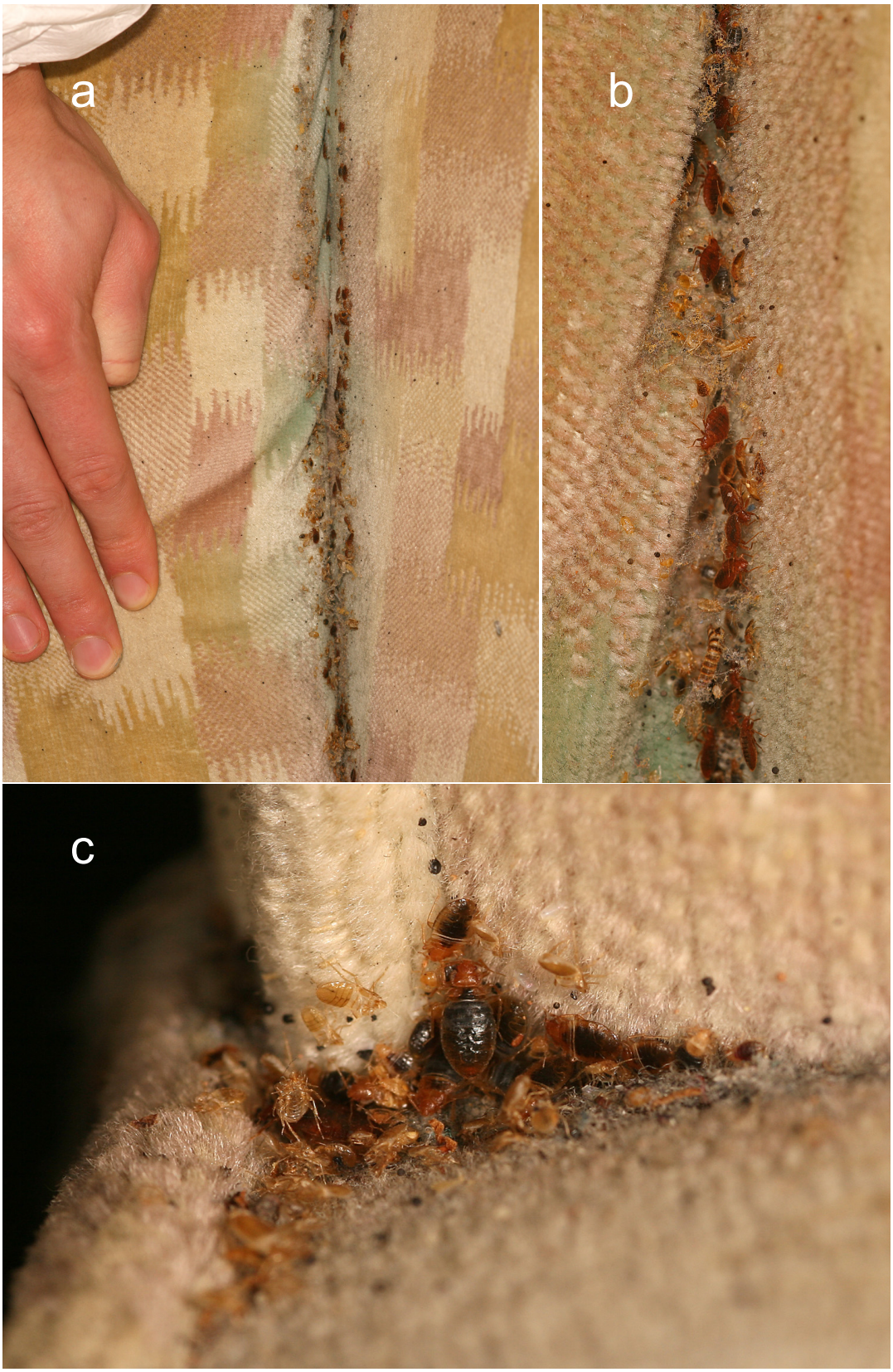


Figure 2.8 shows bedbugs and exuvia (cast skins) around the cushions of the armchair (a & b) and the sofa (c) of for **Case Study 3.**

Table 2.3 summarises the main characteristics of the infestation in **Case Study 3**.

Case Study 3	
duration of infestation	1-2 years
total number of bedbugs present	2500-3000
dispersal to neighbouring flats	yes
number of hosts	1 (male)
number of harbourages	25
minimum harbourage-host distance	0 cm
maximum harbourage-host distance	900 cm (175 cm)*
temperature in room	18˚C
relative humidity in room	75 %

* Host had moved nigh time location from bedroom to living room, to avoid bedbugs. Bedbugs in harbourages in bedroom appeared not to have fed since host had moved, and were therefore not travelling 900 cm to feed.

2.3.4 **Case Study 4**

Sheltered accommodation complex in London S3, visited November 2010

This single story complex comprised eight flats connected by a central corridor. Each flat had a sleeping area and a living area with only partial separation between the two (Figure 2.9). There was also a separate kitchen and a bathroom in each flat. Flat 2 was the most severely infested flat with 1500-2000 bugs (Figure 2.9). The majority of these bedbugs were located in the divan bed base (Figure 2.10); however there were also several hundred bedbugs inside the sofa. Although the occupant did not sleep on the sofa, he did spend the majority of each day there.

The only other flat in the complex with an infestation was Flat 3, on the opposite side of the corridor (Figure 2.11). The infestation in Flat 3 was comparatively minor, with only 42 bugs found in the entire flat. All of these were dead and most were found in the entrance to the flat, which had apparently been treated liberally with an unidentified insecticide.

In addition to the two flats, bedbugs were found in a number of sticky traps, which were located in the central corridor and adjacent to the beds in flats 2 and 3 (Figure 2.11). These sticky traps provide two valuable insights into bedbug dispersal. Firstly they confirm that bedbugs actively disperse along corridors, and are not just carried passively between flats on clothing, furniture and other movable items. Secondly these traps show that all instars and both sexes actively disperse (Figure 2.11).

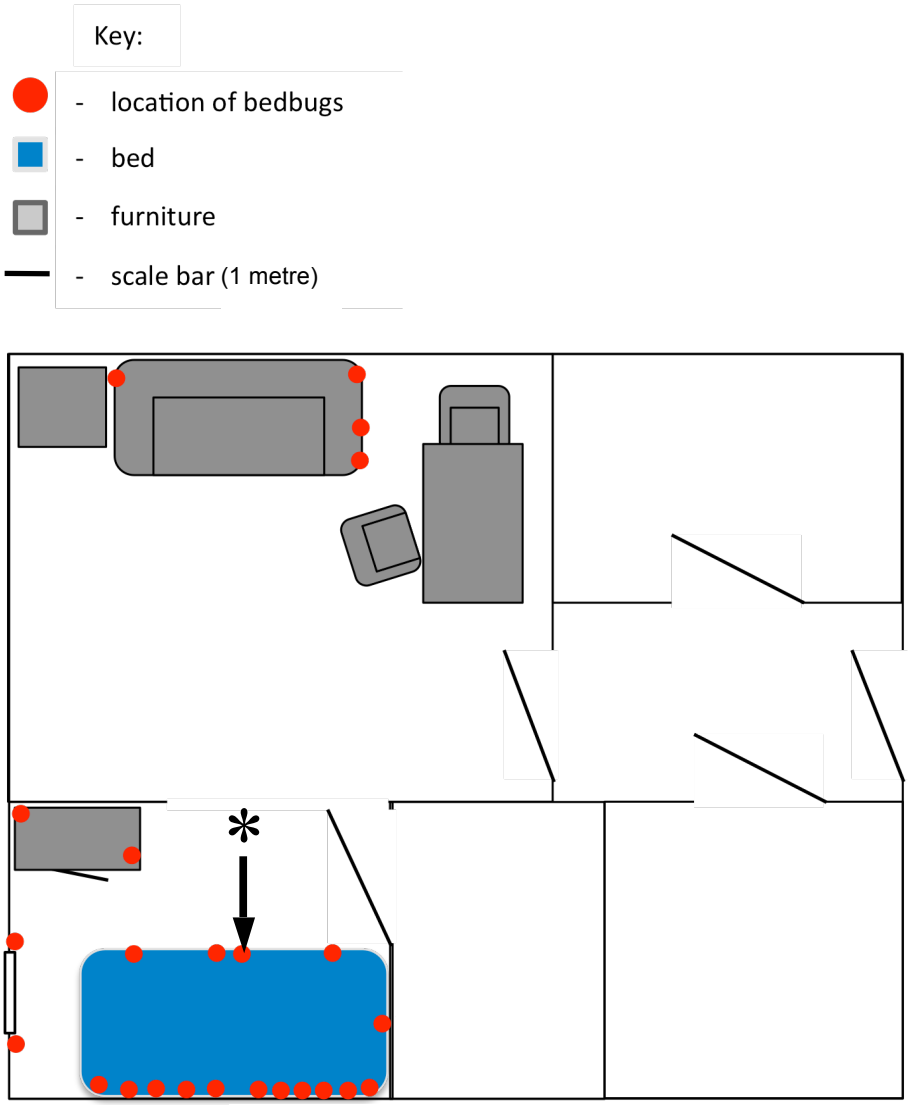


Figure 2.9 shows the plan of the room for **Case Study 4: Flat 2**. Harbourage marked with "*****" is shown in Figure 2.10a (below).

Figure 2.10a) shows an exposed harbourage of bedbugs with numerous faecal spots and exuvia on the wooden internal frame of the divan bed in **Case Study 4: Flat 2** (see * in Figure 2.9). **b)** shows a patchy distribution of harbourages along the bed frame in **Case Study 4: Flat 2**. The significance of this patchy distribution in continuous harbourage space will be discussed in Chapters 3, 4 and 5.

Table 2.4 summarises the main characteristics of the infestation in **Case Study 4: Flat 2**.

Case Study 4: Flat 2	
duration of infestation	1 year
total number of bedbugs present	1500-2000
dispersal to neighbouring flats	yes
number of hosts	1 (male)
number of harbourages	24
minimum harbourage-host distance	30 cm
maximum harbourage-host distance	225 cm
temperature in room	21˚C
relative humidity in room	28 %

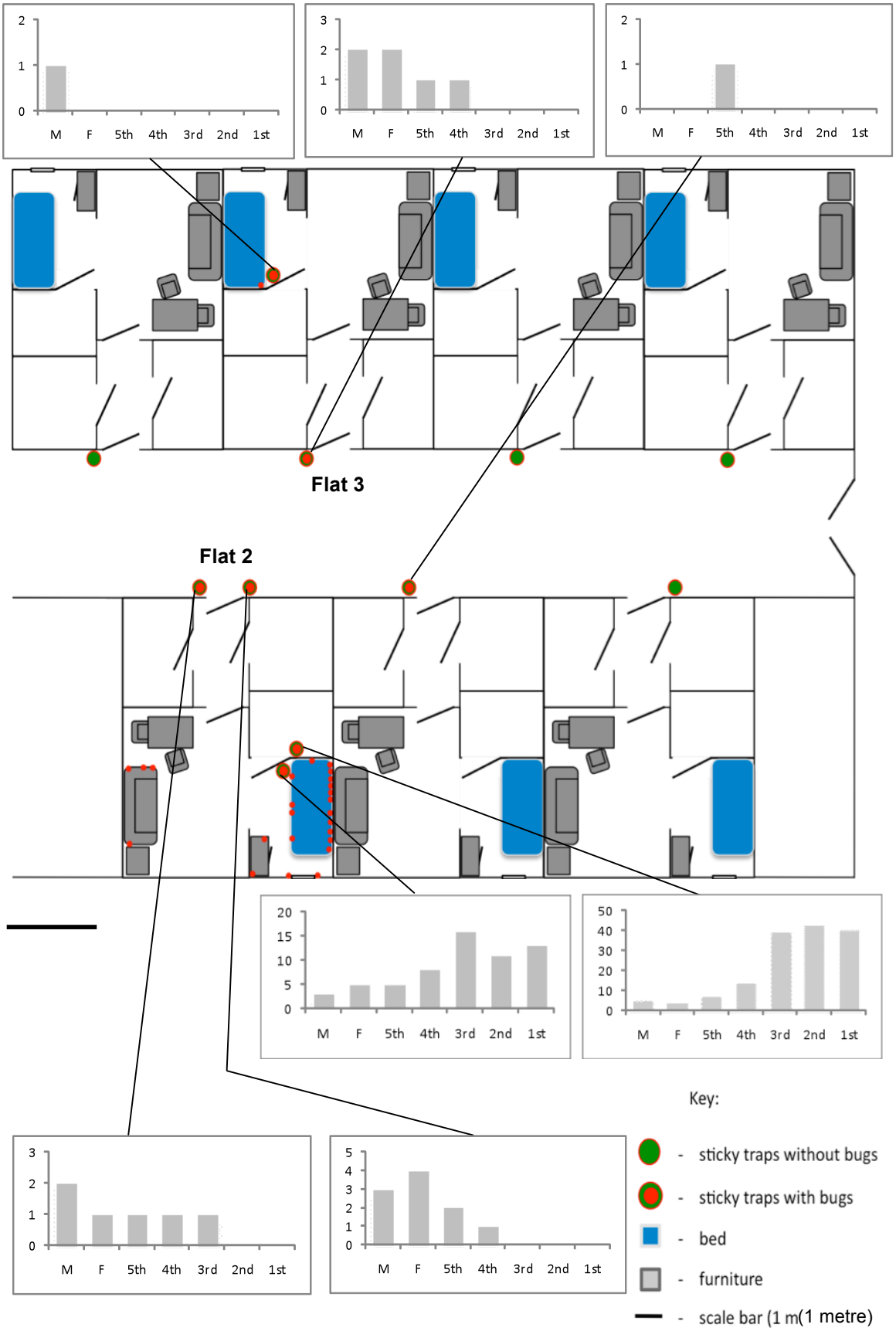


Figure 2.11 shows the age structure of the bedbugs captured in sticky traps in the flats and corridors of **Case Study 4** overlaid onto a plan of the apartment complex.

2.4 Discussion

2.4.1 Abiotic conditions

A laboratory arena capable of producing biologically meaningful data must provide bedbugs with abiotic conditions that reflect those of their natural environment. Excessively high or low humidity have been shown to influence the behaviour and survival of bedbugs (Kemper 1936, Johnson 1942, Benoit *et al.* 2007). Temperatures of 13°C or below have been shown to halt bedbug development and activity (Jones 1930, Mellanby 1935), while temperatures of 36°C and above have been shown to damage bacterial symbionts resulting in reduced fecundity and mortality (Chang 1974). The ambient temperature and humidity of the infested rooms ranged between 18-26˚C and 26-75% RH respectively (n=4). A laboratory setup with abiotic conditions within these ranges therefore reflects the natural conditions under which bedbugs thrive and disperse.

2.4.2 Distribution of harbourages and proximity to the host

To inform the design of the arena it was necessary to determine the scale over which bedbugs move to forage. In six of the seven flats that had fewer than 100 bedbugs, all bedbugs were confined to the bed. The only minor infestation in which the bedbugs were not confined to the bed was Case Study 2: Flat D (Figure 2.3, page 39), where the frame of the bed was metal and provided no suitable harbourages. This suggests that where possible bedbugs seek harbourages close to the host. The more peripheral harbourages were only occupied in larger infestations where harbourages close to the host were already occupied. Harbourage availability could potentially therefore be a limiting resource, which may ultimately influence dispersal.

For the four infestations where dispersal is believed to have occurred, the distances from the furthest harbourage to the host were (from case studies 1 to 4 respectively) 220 cm, 245 cm, 175 cm and 225 cm. The distance between the furthest harbourage and the host was notably smaller in Case Study 3 (Figure 2.6, page 45). However, it is possible that dispersal from this flat occurred sooner than it might otherwise have done as a result of the host's 3 month absence. If this is the case, the distance from the host to the furthest harbourage in an infestation may be a good predictor of the onset of dispersal in situations where the host remains present. Based on the distance from the furthest harbourage to the host in the four dispersing infestations,

a 3 metre long arena, with an artificial host at one end, should adequately allow within-infestation movement over a natural scale and distinguish such movement from attempts to disperse.

In all infested flats it was apparent that the bedbugs occupied a patchy distribution of harbourages even when continuous harbourage space was available, thus leaving unoccupied regions in close proximity to the host. This was particularly apparent in Case Study 4: Flat 2 (Figure 2.10, page 50), where good accessibility to the harbourages along the bottom of the bed frame made it possible to isolate and count all bedbugs from each individual harbourage and accurately identify the boundaries of the harbourages (Figure 2.10b, page 50). In Case Study 4 the average number of bedbugs in each harbourage along the bottom of the bed was 30.71±6.56 (n=7) bedbugs, with a distance of 7±0.32 cm (n=6) between each harbourage. The adaptive value of restricting group size could be to avoid detection by the host or reduce disease transmission (Wertheim *et al.* 2001). Females may also choose to move away from large aggregations to reduce sexual harassment from males (Stutt & Siva-Jothy 2001), although there was insufficient data from the case studies to examine this directly.

2.4.3 **Possible causes of dispersal**

In all four case studies active dispersal is believed to have occurred away from the primary infestation to the neighbouring flats. With the possible exception of Case Study 3, active dispersal is believed to have occurred while the host remained present. The reason for this is unknown. Dispersal is a potentially risky strategy as bedbugs are flightless and consequently travel relatively slowly and over limited distances. Since bedbugs take less than 10 µl of blood at each feed (Castaneda & Zinsser 1930) and only feed approximately weekly (Reinhardt & Siva-Jothy 2007), there is little chance of a human becoming a limiting food resource. It is therefore unlikely that food limitation drives active dispersal in situations where the host remains present. It is possible that dispersal could be driven by lack of harbourage availability. If the energetic cost associated with travelling to and from the host limits the maximum distance that the harbourage can be from the host, a lack of available harbourages in the vicinity of the host could potentially drive dispersal. Alternatively, the maximum distance between the harbourage and the host could be limited by the range over which the bedbug can detect the host. If the bedbugs cannot detect the presence of the host from the harbourage they may either never realise that the host is present and thus never get an opportunity to

feed, or have to make multiple potentially dangerous (Usinger 1966) or energetically costly (Mellanby 1938) foraging trips in order to establish if there is food available.

If limited harbourage availability does drive dispersal, this could explain the apparent delay in dispersal in Case Study 1 from Flat D (Figure 2.1, page 35) to the neighbouring flats. In this Case Study the population of bedbugs was estimated to have reached between 50 000 and 100 000 individuals and yet only minor infestations were found in the neighbouring flats. Aside from a slightly higher room temperature, the only apparent difference between the primary infestation in Case Study 1 and the primary infestations in Case Studies 2, 3 and 4, was that the occupant in Case Study 1 had horded newspapers throughout his flat and particularly around the bed area (Figure 2.2, page 36). These newspapers provided ideal harbourages in close proximity to the host and may have allowed the population to get much larger before forcing individuals to occupy more of the peripheral harbourages before ultimately dispersed.

2.4.4 Demography of dispersers

Case Study 4 (Figure 2.10, page 50) provided an unexpected and valuable insight into which individuals within the population actively disperse. Specifically it revealed that adult males as well as nymphs disperse, and not just adult females as has been previously suggested (Stutt & Siva-Jothy 2001, How & Lee 2010b). Wang *et al.* (2010) also used traps in the corridors of an apartment block to catch bedbugs moving between apartments, and found that adults were nine times more likely to disperse than nymphs. However, they did not establish the sex of the adults or the instar of the nymphs that dispersed. How & Lee (2010b) used a laboratory setup comprised of coils of plastic tubing to assess the propensity of different instars of the tropical bedbug *Cimex hemipterus* to disperse. Their findings supported those of Wang *et al.* (2010), showing that adults and large nymphs travelled significantly further within the plastic tubing than the smaller nymphal instars. They also showed that fed adult females travelled furthest (up to 42.3 metres over 120 hrs). This might be predicted, as a mated female can potentially found a new infestation as long as it finds a new host. By contrast, a dispersing male must locate both a new host and a female to copulate with, significantly reducing its chances of dispersal success. However, one problem with How & Lee's (2010b) plastic tubing setup is that it bares little similarity to any natural infestation. Test insects have no access to a host, harbourages or other bedbugs. It is therefore possible that the differences in the distances each instar travelled actually

reflect their physical ability to escape from an unfamiliar, threatening situation, rather than giving a true representation of the propensity of each instar to disperse.

The data from the sticky traps in Case Study 4 (Figure 2.10, page 50) shows that males, females and nymphs all actively disperse between the flats via the corridors. Of 23 bugs caught in the traps in the corridors, eight were adult males, seven were adult females and the rest were nymphs. Of the nymphs caught in the corridors the smallest was in its 3rd instar. All the rest were either 4th or 5th instars. While this could potentially reflect the age structure of the population at the time or the reluctance of small nymphs to enter the sticky traps, the traps close to the bed in Flat 2 had more than 161 1st, 2nd and 3rd instar nymphs, which comprised more than 75% of the total number of bugs caught on the two traps close to the bed. The lack of early instar nymphs captured in the sticky traps in the corridors therefore seems to indicate that early instar nymphs do not tend to actively disperse from the infestation. This finding supports the findings of How and Lee (2010). However, due to the small amount of data available from the case studies, it will be necessary to explore this under controlled conditions.

2.4.5 **Summary**

In this chapter I have:

(1) Measured the range of ambient temperatures and humidities in natural infestations.

(2) Established that harbourages can be found 0-2.5 metres from the host suggesting that a 3 metre long arena with the host located at one end would be sufficient to allow bedbug movement over a natural scale.

(3) Determined that infestations of less than 100 bedbugs were primarily on the bed, while peripheral harbourages were only utilised in the larger infestations.

(4) Shown that few harbourages were found further than 2-2.5 metres from the host, and that the use of these peripheral harbourages seems to tie in with dispersal from the infestation, suggesting that harbourage limitation may be an important factor in the onset of dispersal.

3 Developing a laboratory-based "Infestation Arena"

3.1 Introduction

It is rarely feasible to conduct ecological field studies on natural bedbug infestations and although bedbugs are periodically found infesting poultry breeding houses (Kulash 1947, Axtell & Arends 1990, Lyon & Sprays 1995, Axtell 1999), studies of such infestations are unlikely to provide the insight necessary for informing control strategies for human infestations. Poultry breeding houses are comprised of rows of relatively small adjacent nest boxes, making it easy for bedbugs to move short distances between multiple hosts. If an infested poultry breeding house was considered to be a single infestation with multiple hosts then it is unlikely that active dispersal from the poultry house could be detected at all, as poultry houses tend to be free standing without adjoining buildings into which bedbugs could disperse.

There are practical restrictions on the field data that can be collected from natural infestations of human dwellings, because researchers usually rely on pest controllers to report active infestations. This means that research access to the infestation tends to be limited to the period immediately prior to eradication. In many cases this is restricted to less than half an hour (pers. obs.). Consequently field data tends to be limited to temporal snap-shots in time and information about the origin and duration of the infestations is often vague. For this reason, the factors affecting the distribution and dispersal of bedbugs in infestations of human dwellings have received little attention, despite the obvious importance of such data for informing bedbug control.

Wang *et al.* (2010) conducted one of the only field studies of natural active dispersal in *C. lectularius* (although a similar study was carried out by How & Lee (2010a) on the tropical bedbug *C. hemipterus*). This involved placing pitfall traps in the corridors of a 223 unit high-rise apartment building to catch bedbugs (Wang *et al.* 2010). This study confirmed that bedbugs actively disperse along corridors in apartment buildings and gave insights into the demographic of dispersing individuals (discussed in

Chapter 6). However, the study did not attempt to identify the factors affecting dispersal or provide any explanation as to why certain bedbugs might actively disperse away from an infestation.

The only feasible approach to studying the ecology of the bedbug and the factors affecting its dispersal is to build realistic laboratory arenas to house bedbug infestations under conditions that reflect their natural ecology. Some attempts have recently been made to look at aspects bedbug biology and behaviour in arena setups (Pfiester *et al.* 2009, How & Lee 2010b, Suchy & Lewis 2011). Pfiester *et al.* (2009) used 15 cm diameter glass Petri dishes to assess the tendency of different life stages, sexes and feeding states of bedbugs to aggregate or disperse. Pfiester *et al.* (2009) use the term "active dispersal" to refer to any movement away from the original aggregation, even where the bedbug moves to form a new harbourage within the vicinity of the same host. Consequently their experimental design cannot distinguish movement within and between infestations. They therefore infer that the tendency of females, more than any other life stage, to sit away from the main aggregation may be an indication that this is the primary dispersal stage. However, an alternative explanation for this observation is that females move away from aggregations, while staying within close proximity to the same host, to reduce unwanted male attention (see Stutt & Siva-Jothy 2001) or to find space for egg laying. They may even seek to move closer to the host before laying eggs, to reduce the distance that emerging nymphs travel to feed. With a larger arena it may be possible to distinguish those bedbugs moving around within an infestation from those seeking to disperse from it. However, my field data (Chapter 2) shows that bedbugs are often found residing in harbourages up to 2-2.5 metres from the host. This suggests that an arena capable of distinguishing bedbugs moving within an infestation from those dispersing from it would have to be a minimum of approximately 3 metres long with the host at one end and a system for collecting bedbugs attempting disperse at the other.

Suchy and Lewis (2011) used 90 cm x 90 cm arenas to assess the ability of bedbugs to locate a source of human breath. While this in an improvement in terms of arena size (similar studies have been conducted in 15-20 cm Petri dishes (e.g. Olson *et al.* 2009, Weeks *et al.* 2010)), it still doesn't come close to allowing foraging to occur over the ranges observed in natural infestations (Chapter 2, pers. obs.). As the bedbugs were released in the centre of the arena and the source of the human breath was at one corner, the bedbugs started foraging from a point less than 64 cm from the target. Had it been possible to conduct the same study in much larger arenas it may have been

possible to determine the range over which bedbugs can detect human breath as well as establishing whether bedbugs can determine directionality over the full detection range.

How and Lee (2010) used 20 metre lengths of plastic tubing to look at the propensity of bedbugs of different instars and feeding states to move. Their setup had the benefit of allowing bedbug movement over a scale comparable to that of a natural infestation, and was very easy to observe and measure. However it has little similarity to a natural infestation as it lacks a host, harbourages or conspecifics. Although the investigators postulate that the distance a bedbug travels along the plastic tubing reflects its propensity to disperse, it may alternatively reflect the bedbug's desire or ability to escape from an unfamiliar/hostile situation.

For the bedbugs to behave naturally in laboratory arenas, they are likely to require light and dark phases of the daily cycle; suitable harbourages to hide in during the 'day'; and an artificial host from which they can receive blood feeds during the 'night'. For bedbugs to successfully forage for the artificial host, they are likely to need both CO_2 and temperature gradients (Lehane 2005, Marx 1955 reviewed in Reinhardt and Siva-Jothy 2007). The scale of the arena is important to distinguish within-infestation movement from active dispersal. As harbourages were not found at distances of greater than 2.5 metres from the host (Chapter 2), a 3-metre long arena, with the host situated at one end, should provide sufficient space for unrestricted distributions of harbourages to develop.

3.1.1 Chapter aims

In this chapter I will:

1) Develop an arena in which to house and observe laboratory-based infestations that exhibit near natural foraging, aggregating and dispersal behaviour.
2) Compare four different bedbug stock cultures from different origins to test how stereotyped the observed behaviours are.
3) Assess which of the stock cultures behaves most naturally in the infestation arena and is therefore most suitable to answer questions on the ecology and dispersal behaviour of bedbugs.

3.2 Materials and Methods

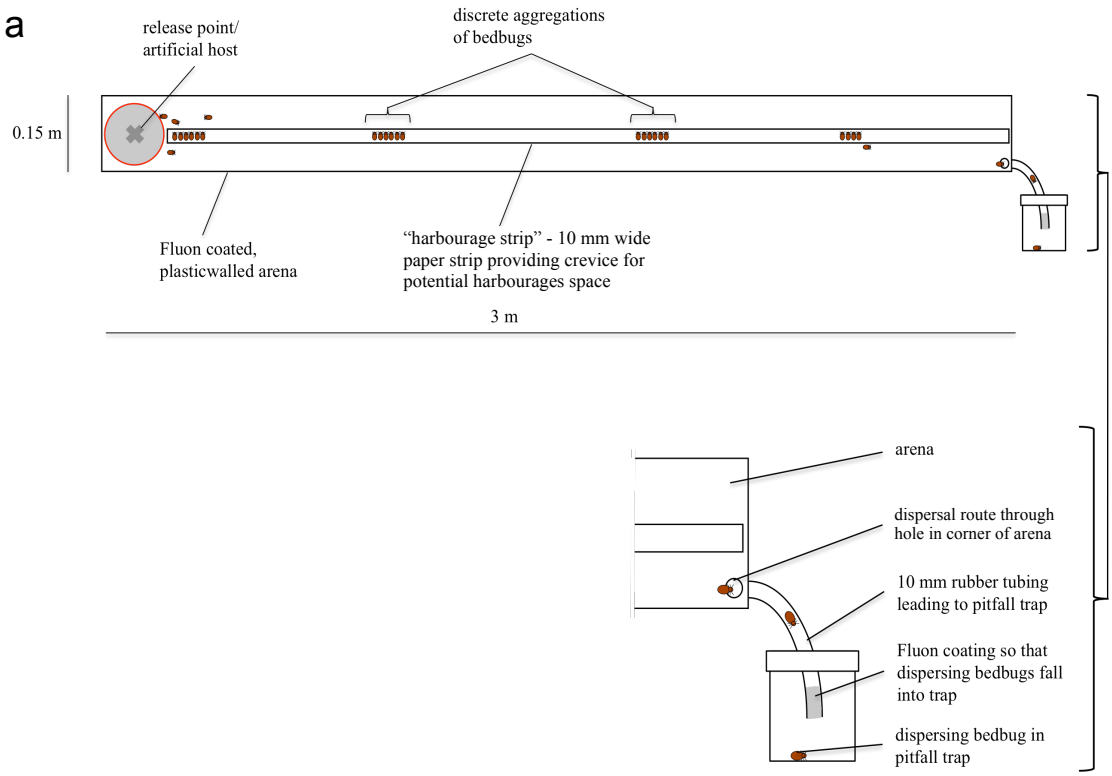
3.2.1 Insect stock cultures

We currently maintain four main stocks (populations) of bedbugs. Two stocks have been housed in the laboratory for more than 40 years (L1 & B1), and two stocks have been collected more recently; one from an infestation in London in 2006 (F4), and one from an infestation in Kenya in 2008 (K1).

Stock cultures are housed in 60 ml plastic containers with gauze lids and fed weekly using the protocol of How & Lee (2010b) to facilitate normal development and egg production. All insects are housed at 26±1^{o}C with 70±5% RH, and a 12:12 light:dark cycle.

3.2.2 Laboratory arenas

Plastic arenas were built to house infestations within the laboratory (Figure 3.1). Since harbourages in natural infestations were never found beyond 2.5 metres from the nighttime location of the host (Chapter 2, page 53), 3 metre long arenas were built. The arenas (and harbourage strips within) were long and narrow. This design allowed the bedbugs to distribute themselves in only one dimension, such that each bug's location could be recorded as its distance from the host (which was placed at one end of the arena (Figure 3.1)). This was not an unnatural situation, as rows of harbourages were often found along the edges of bed frames and skirting boards in the natural infestations (Chapter 2, page 34). Consequently it was only necessary for the arenas to be 0.15 metres wide. A 10 mm wide paper "harbourage strip" was placed down the centre of the arena and secured along the long edge, to form a continuous 10 mm wide paper flap down the full length of the arena, under which bedbugs would be able to form harbourages (Figure 3.1b/c). The paper was slightly bent up to allow bedbugs to crawl underneath. Similar harbourages are often found behind peeling wallpaper in natural infestations (Figure 3.2).



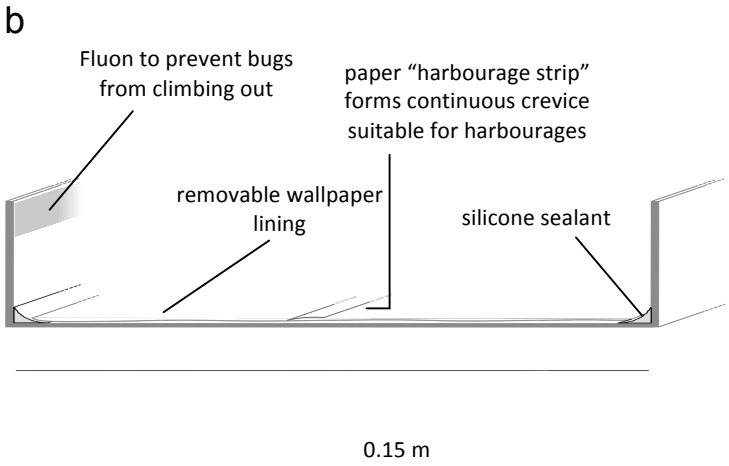


Figure 3.1 shows the arena setup. **a)** shows a plan view of the arena, connected to a side view of the pitfall trap. **b)** shows a cross section of the arena. **c)** shows a photograph of the inside of the arena with the central paper strip folded back to reveal bedbug aggregations.

Figure 3.2 shows a typical bedbug harbourage under a flap of pealing wallpaper.

The tops of the arenas were painted with Fluon^{TM} to prevent the bedbugs from climbing out. The corners were sealed with silicone sealant to prevent the previously sharp right-angled corners from providing potential harbourages for bedbugs. The floor of the arena was covered with plain wall-lining paper to provide a realistic surface for the bedbugs to walk over (Figure 3.1b/c). The lining paper was removable so that it could be discarded and the arena cleaned out after each trial to avoid potential effects of residual aggregation/alarm pheromones from previous trials.

In order to study dispersal, it was necessary to provide the bedbugs with a dispersal route. A 10 mm wide hole was drilled in the corner of the arena at the end furthest from the artificial host (see Figure 3.1a). The hole was fitted with a 30 cm length of tubing that lead into a dispersal trap, so that dispersing bedbugs could be collected. Early observations of prototype arenas revealed that bedbugs in the tubing were easily able to cling to the inside and were reluctant to drop into the trap. Consequently the lower 3 cm of tubing was dipped into Fluon^{TM} and allowed to dry. This prevented the bedbugs from clinging to the inside of the tubing, causing them to lose their footing and fall into the trap.

3.2.3 **Artificial host**

An artificial host (see Appendix 1) containing heparinised sheep blood was used to provide food for the bedbugs, as well as to stimulate the natural foraging and returning behaviour. The artificial host was located at one end of the arena (see Figure 3.1a).

In order to alert the bedbugs to the availability of the artificial hosts, the carbon dioxide (CO_2) concentration in the insectary where the arenas are housed was artificially elevated to 13000 ppm over 8-9 minutes and maintained at the elevated concentration for a further 60 minutes before being allowed to gradually return to ambient. This CO_2 concentration is sufficient to trigger foraging (see Appendix 2).

3.2.4 Do bedbugs show conserved patterns of harbourage distribution?

Bedbug harbourages often showed a patchy distribution relative to the host (Chapter 2). In Case Study 4 (Figure 2.9, page 49) the average group size in each harbourage along the base of the bed was 30.71±6.56 (n=7) with an average distance between harbourages of 5±0.32 cm (n=6). If the bedbugs showed the same patchy distribution of harbourages in the arena as they did in the field, a direct comparison between the harbourage sizes and spacing could be made. If different laboratory stocks show variations in patterns of harbourage usage, then comparison with Case Study 4 could indicate which of the four stocks would be most appropriate for studying aspects of bedbug ecology and dispersal in the later chapters.

In order to simplify the comparison between the distributions of harbourages in the arenas to the distributions observed in Case Study 4, I compared approximately the same number of individuals. For further simplicity, I only compared the distributions of mixed sex cohorts of adults from each of our populations. However, as the bedbugs collected from the harbourages in Case Study 4 were comprised of a proportion of nymphs, some correction had to be made, since the spatial influence of a 1 mm long 1st instar nymph is unlikely to be equivalent to that of an adult.

Of the bedbugs collected from the sample of harbourages in Case Study 4, 67.8% were adult. Fifth instar nymphs are approximately the same sizes as adults and comprised 10.0% of the total number collected. Of the remaining nymphs, 78.8% were in their 1st instar and were thus very small. Therefore, for the purposes of the comparison of spatial distribution, 5th instar nymphs were counted as adults and all other nymphs were disregarded. This resulted in 116 bedbugs being counted across a region of the bed containing 5 harbourages, making an average of 19.83±3.69 bedbugs per harbourage.

For the long-term laboratory stocks (L1 & B1) and the more recently collected field stocks (K1 & F4), 3 replicate cohorts of 116 unfed, mixed sex adult bedbugs were used. All cohorts were released at the release point (see Figure 3.1a) at the beginning of the dark phase of the daily cycle as this is biologically more realistic than releasing in the light when dispersal along the arena and harbourage choice may be influenced by negative phototaxis.

Observations were made at days 1, 2, 7, 14 and 21, however no change was found in the distribution of bedbugs between days 14 and 21, so all except the day 14 observations were abandoned. For each observation the distance of every bedbug from

the release point was recorded so that frequency distributions could be produced for each arena.

3.2.5 Analysis of spatial distribution data

For each laboratory stock the distribution of bedbugs within each arena was plotted as a frequency distribution. The statistical package R was used to calculate the pair-wise distances of every bug to every other bug. To establish if bedbugs distribute themselves randomly in the arenas, 10 000 random distributions were generated to estimate how often the observed distributions could be expected by chance. The statistical probability of obtaining each observed distribution by chance was then calculated.

For each of the bedbug stocks where the distribution of harbourages in the arena was found to be significantly non-random, the mean and standard error of the number of bedbugs per harbourage, the number of harbourages and the distance between the harbourages was calculated for comparison with the data collected from the case studies in Chapter 2.

3.3 Results

3.3.1 Do bedbugs show conserved patterns of harbourage distribution?

For each of the stock cultures tested, Figure 3.3 shows the frequency distributions of the 116 bedbugs in each of the three replicate arenas two weeks after their introduction. None of the replicates of the L1 stock had distributions that differed significantly from random (see Table 3.1).

Stock cultures B1, F4 and K1 were similar in terms of the number of harbourages and number of bedbugs per harbourage, but differed in their mean inter-harbourage distances (Table 3.1). The mean inter-harbourage distance across all replicates was 62.61 cm for the B1 stock, but only 17.66 cm and 10.75 cm for the F4 and K1 stock respectively (Table 3.1).

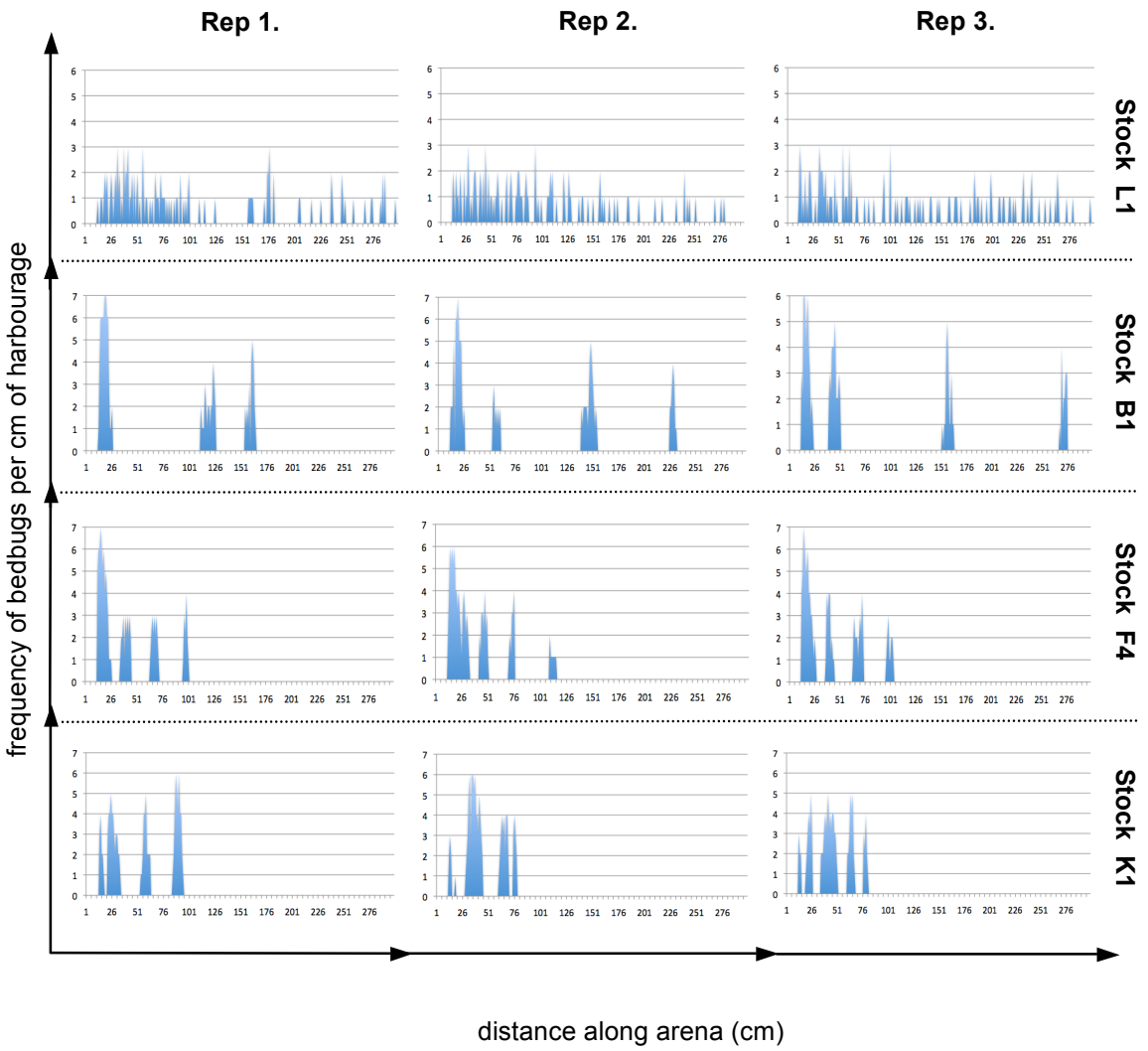


Figure 3.3 shows the frequency distribution of three replicates of 116 mixed sex adult bedbugs from each of the four stocks.

Table 3.1 shows the descriptive statistics of the distributions in Figure 3.2. # denotes significant p-values, indicating non-random bedbug distributions. As the distributions of the bedbugs in the L1 strain did not differ significantly from randomly generated distributions, no further descriptive statistics were calculated.

stock and replicate	probability of distribution occurring by chance	number of bedbugs per harbourage (mean±SE)		distance between harbourages (mean±SE)	
L1, rep. 1	0.2884	-	-	-	-
L1, rep. 2	0.34	-	-	-	-
L1, rep. 3	0.3917	-	-	-	-
L1 means		-	-	-	-
B1, rep. 1	0.00010 #	40	± 11	56.5	± 28.5
B1, rep. 2	0.00000 #	29	± 8.58	58.33	± 15.84
B1, rep. 3	0.00002 #	28.75	± 6.3	73	± 29.02
B1 means		**32.58**	**8.63**	**62.61**	**24.45**
F4, rep. 1	0.00000 #	29	± 10.58	16.33	± 4.41
F4, rep. 2	0.00000 #	29	± 14.91	20.33	± 6.96
F4, rep. 3	0.00050 #	29	± 9.94	16.33	± 3.84
F4 means		**29**	**11.81**	**17.66**	**5.07**
K1, rep. 1	0.00000 #	29	± 6.62	14.33	± 5.70
K1, rep. 2	0.00000 #	28.75	± 13.59	10.67	± 3.38
K1, rep. 3	0.00001 #	23.2	± 7.82	7.25	± 1.11
K1 means		**26.98**	**9.34**	**10.75**	**3.40**

3.4 Discussion

No previous study of bedbug ecology has utilised laboratory arenas of a scale that reflects the distances observed in natural infestations. The arena setup developed in this chapter makes it possible to study the ecology and dispersal behaviour of bedbugs in a controlled laboratory setting.

The first observations of bedbugs in the arena revealed that they establish a patchy distribution of harbourages even where the available harbourage space is continuous. In natural infestations harbourage locations are often influenced by the patchy availability of suitable cracks and crevices. Bedbug-defined spatial patterns of harbourage usage are consequently rarely apparent. However, patchy distributions that can't be explained by harbourage availability can be found where bedbugs utilise long, uninterrupted crevices such as behind the top edge of a skirting board or the junction between the edge of a mattress and a wooden bed frame. The harbourages along the edge of the bed in Case Study 4 (Figure 2.9 & 2.10b, pages 49-50) are a good example of a patchy distribution of bedbug harbourages in a continuous environment.

3.4.1 Comparison of laboratory stocks

Many laboratory studies of bedbug ecology and behaviour have been conducted on bedbug stocks that have been cultured in the laboratory for 25-30 years (e.g. Olsen *et al.* 2009, Pfeister *et al.* 2010, Suchy & Lewis 2011). However, my results show that stocks derived from different populations and cultured for varying durations in the laboratory can differ dramatically in their aggregation behaviour. It is therefore essential that behavioural and ecological studies of bedbugs are carried out on biologically relevant stock cultures that have spent as little time under unnatural laboratory conditions as possible. Similar consideration should be given to behavioural studies of other laboratory model organisms where the subjects are maintained in long-term cultures rather than collected from natural populations (e.g. Bonsall *et al.* 2002, Strevens & Bonsall 2011).

The spatial distribution of the laboratory stock L1 did not differ significantly from a random distribution, suggesting it may have lost its aggregation behaviour, probably as a result of more than 40 years in laboratory culture conditions. Interestingly the laboratory stock B1 appears to have retained its aggregating behaviour despite having been under identical culture conditions for a similar period. Laboratory stock B1 and

field stocks K1 and F4 were all similar in terms of the number of harbourages and number of bedbugs per harbourage, but differed in their inter-harbourage distances. The average distance between harbourages for the B1, K1 and F4 stocks was 62.61 cm, 10.75 cm and 17.66 cm respectively. The average distance between the harbourages along the side of the bed in Case Study 4 was only 7±0.32 cm (n=6). This suggests that the B1 strain would be least suitable, which is perhaps not surprising since this stock has been kept under lab culture conditions for several decades. Although the K1 stock bears closest similarity to those in Case Study 4, in terms of inter-harbourage distance, this strain tends to be reluctant to feed and respond to elevated CO_2. Moreover the K1 strain was originally collected in rural Kenya where ecological conditions of the bedbugs are likely to differ dramatically from those found in the UK. By contrast the F4 strain was recently collected (2010) from a flat in London, making it the most suitable strain for use in the laboratory model system to answer questions about the ecology and dispersal of bedbugs in the UK.

3.4.2 Implications for control

The highly consistent pattern of harbourages between replicates of the same stock, and (to a slightly lesser extent) between stocks suggests that for a given bed design the pattern of harbourages is likely to be highly conserved. This spatial predictability should allow insecticidal treatments and traps to be targeted towards very specific locations. Bedbug monitors designed to mimic harbourages may also be an effective way of establishing if bedbugs are present, as long as they are positioned with reference to the bedbug's natural patterns of harbourage usage.

3.4.3 Summary

In this Chapter I have:

1) Developed an arena to house bedbug infestations under semi-natural, controlled conditions. I have developed an artificial host, which provides the bedbugs in the arenas with a source of nutrition and facilitates the natural foraging and returning behaviour. I have fitted the arena with a dispersal route, which makes it possible to monitor and collect those bedbugs that choose to disperse.
2) Identified variation in the spatial patterns of harbourage usage between bedbug stock populations, which may have implications for the validity of previous studies that have been conducted on long-term laboratory stocks.

3) Selected stock F4 as the most suitable stock culture to explore the ecology and dispersal of bedbugs in the following chapters. The process of evaluating the arena's suitability as a model system for studying bedbug ecology and dispersal will continue throughout the following chapters by reference back to data and observations from the case studies in Chapter 2 wherever possible.

4 The Dynamics of Harbourage Usage

4.1 Introduction

Understanding how new harbourages are formed may make it possible to estimate the age of a population, predict the onset of active dispersal and even explore issues of relatedness. Understanding, how bedbugs use harbourages also has important implications for their control. For example, if bedbugs regularly move between harbourages, then only treating the easily accessible harbourages with a long-lasting residual insecticide may be sufficient to control a population since individuals in untreated harbourages will eventually come into contact with the insecticide.

Chapter 2 revealed that small infestations tend to be spatially associated with the nighttime location of the host, while larger infestations tend to spread out into the peripheries of the room. There may therefore be competition for harbourages in close proximity to the host. In order to examine this, it is necessary to determine if the same patterns can be observed in the experimental arena designed in Chapter 3.

It is presently unknown if bedbugs return to the same harbourage after each feed. It is possible that the bedbugs cycle between different harbourages, utilising the closest ones to the host immediately after feeding and then moving to more peripheral, and potentially safer, harbourages throughout the course of the feeding interval. Alternatively it may be that competition exists between bedbugs for harbourages in close proximity to the host (as suggested in Chapter 2). Thus bedbugs further from the host may have restricted access to food. Harbourage fidelity could also be adaptive; if bedbugs show strong harbourage fidelity, a higher degree of relatedness within, versus between, harbourages could result in the evolution of kin-selected traits such as parental care. Harbourage fidelity may also have implications for male mating behaviour, as it may be necessary for them to visit different harbourages in order to find less related females to mate with.

Aside from the evolutionary implications of harbourage fidelity, there may be important consequences for control. Passive monitors have begun to be used to identify and control bedbug infestations. Passive monitors are designed to provide suitable

harbourages close to the host that can easily be removed and examined. If bedbugs show fidelity to one harbourage then an infestation may go undetected until the population has increased sufficiently for bedbugs to spill out into the passive monitors. However, if bedbugs readily move around between harbourages, one might expect a well placed monitor to be occupied quickly, leading to the early detection of the infestation.

4.1.1 Chapter aims

In this chapter I will explore the factors effecting harbourage usage within an infestation. I will:

1) Establish how the number and distribution of harbourages increases with population size.
2) Determine if bedbugs are faithful to particular harbourages.
3) Determine whether feeding status differs between harbourages
4) Establish if there is a measurable energetic cost associated with commuting from peripheral harbourages to the host.

4.2 Materials and Methods

4.2.1 How does the Pattern of Harbourage Use Change with Population Growth?

Chapter 2 revealed that small infestations tend to be localised to the position of the host, while larger infestations tend to utilise more peripheral harbourages. To see if this pattern of harbourage usage is seen in the laboratory, three replicate arenas were set up as described in 3.2.2. At the beginning of each dark phase of the daily cycle the artificial hosts were replaced and the CO_2 concentration around the arenas was elevated as described in 3.2.3. For every 250 bedbugs in the arena, an additional artificial host was provided (stacked on top) to ensure that food did not become a limiting resource (see Appendix 1 for rationale). Each day, immediately after replacing the artificial host(s), 10 mixed sex adult bedbugs from the F4 strain were introduced at the release point (Figure 3.1a).

All bedbugs introduced into the arenas had eclosed into adults within 1 week of introduction. Thus the newly introduced bedbugs were younger than those already in the arena, thereby simulating natural population growth by reproduction as closely as practicable. Although natural infestations are comprised of a mix of adults and nymphs, nymphs were not included in my experiments for logistical reasons. Furthermore, any nymphs born in the arena, were removed by pooter to limit competition with adults for harbourage space and food.

Each day the number of bedbugs caught in each dispersal trap was recorded. If bedbugs were caught in any of the dispersal traps, the traps were replaced to remove aggregation pheromones that might attract other bedbugs into it. Once per week the location of every bedbug was recorded by its distance from the release point, and plotted as a frequency distribution. The trial was terminated on day 50, at which point each arena contained 500 adults (less those that had dispersed).

4.2.2 Are bedbugs faithful to particular harbourages?

To establish if bedbugs showed any fidelity to particular harbourages three replicate 3 metre long arenas were used (Chapter 3), with a continuous 10 mm wide strip of paper down the centre of the arena to provide suitable harbourage space.

Five males and five females from the F4 stock were introduced daily for 20 days at the beginning of each dark phase, so that harbourages could develop as naturally as

possible. An artificial host, with associated heat and CO_2 (see 2.2.3), was also provided daily at the beginning of each dark phase to facilitate normal foraging behaviour. On day 21 the locations of the harbourages were identified and marked. Harbourages were defined as regions of the paper strip where two or more bedbugs reside with a distance of less than 1 cm between any individual and its nearest neighbour. All bedbugs were then removed from the harbourages and isolated. Any dead individuals, or individuals found not to be in a harbourage, were also removed.

All bedbugs from each harbourage were marked, according to the harbourage they came from, using quick drying enamel paint (Humbrol Enamel, Hornby Hobbies Ltd., UK). As soon as the paint had dried, all bedbugs were returned to their harbourages. A glass barrier, assembled from four glass slides was placed around each harbourage to encourage bugs to resettle in the harbourage from which they had been removed. The glass barrier was removed after 24 hours, and feeding resumed at the start of the following dark phase.

After 21 days, the glass barriers were replaced in the same locations as they had previously been. All individuals in all harbourages were then removed and their sex and colour recorded. Any individuals found not to be in one of the previously defined harbourages were removed separately and their locations recorded.

4.2.3 What is the Energetic Cost of Commuting from Peripheral Harbourages?

Bedbugs consume approximately five times their weight in blood during a single feed (Johnson 1937). It is therefore likely that the return journey, when the bedbug is full of blood, is energetically costly, at least compared to the outward journey (Mellanby 1938). As the distance from the host to the harbourage increases, the net benefit of the foraging trip will decline. This could potentially explain why harbourages appear to be limited to a maximum distance of 2-2.5 metres from the host (see Chapter 2), and potentially explain what drives bedbugs to disperse (i.e. limited harbourage availability within a cost-effective range of the host).

To establish whether there was an energetic cost of travelling over the distances encountered in natural infestations, a cohort of 60 adult female bedbugs from the F4 strain were assigned randomly to one of three treatments: walk then feed; feed then walk; feed only (control). As a metric for energetic cost, the number of eggs produced by each female was recorded over the following week, along with egg length for a random sample of five eggs from each clutch. To control for large between-individual

variations in weekly egg number, individuals were grouped into threes according to their egg production prior to the start of the experiment, and then split randomly across the treatments. This was achieved by establishing the mean weekly egg numbers for each individual, based on a three-week lead-in immediately prior to the start of the experiment. During this lead-in the females were fed but prevented from walking. This was achieved by housing them in isolation in flat-bottomed 96-well tissue culture plates (Scientific Laboratory Supplies Ltd.: MIC9036). Since each well had a diameter of 6.5 mm (only slightly greater than the length of a fed female bedbug), the movement of the bedbugs within the wells was restricted. The same plates were used to house the bedbugs individually throughout the experiment. The mean weekly egg numbers for each individual were ranked and then grouped into threes sequentially. Within each group the individuals were then assigned randomly across the three treatments by dice roll.

So that the females remained fertilised throughout the duration of the experiment they each received a 60 second copulation immediately after the first feed. This has been shown to standardise the amount of ejaculate received and be sufficient to maintain full fertility for more than 5 weeks (Stutt & Siva-Jothy 2001, Reinhardt & Siva-Jothy 2007).

Those bedbugs required to walk were placed into a paper-lined 60 cm x 80 cm arena during the light phase of the daily cycle. Because no harbourage was provided, and because bedbugs are photophobic (Usinger 1966), they actively walked about searching for somewhere to hide. To control the distance the bedbugs travelled they were each followed around with a digital opisometer (map measurer). They were each required to walk 6 metres (slightly further than the maximum distance likely to be encounter in a natural infestation). Once each bedbug had walked the required 6 metres it was removed from the arena and replaced into the 96-well plate.

The treatment was carried out weekly over 5 successive weeks and the eggs were removed immediately prior to each treatment. The eggs from the first two weeks were discarded to give time for any energetic costs to be manifest and for egg numbers to stabilise. Thus a mean egg number and egg size was produced for each individual based on the egg clutches from weeks 3, 4 and 5.

4.2.4 Does the proximity of the harbourage to the host effect the feeding status of the bedbugs within?

Even without measurable energetic costs associated with travelling, travelling is likely to increase exposure to predators (Reinhardt & Siva Jothy 2007), sexual harassment (Stutt & Siva-Jothy 2001), or even detection by the host (Reinhardt & Siva Jothy 2007). Bedbugs in the peripheries of the infestation may therefore have a lower optimum feeding frequency compared to bedbugs living adjacent to the host, resulting in a lower fecundity. The mean feeding frequency was established from the current feeding status of a sample of the individuals within the infestation using the protocol of Reinhardt *et al*. (2010).

Following on from the experiment in 4.2.2, a photograph was taken of every individual on day 21 after marking (the same day that they were removed from the arenas for assessment of harbourage fidelity), using an image analysis setup (camera: Micropublisher 3.3 RTV, Q Imaging, USA, with software: Image-Pro Plus 5.1.2, Mediacybernetics, USA) for analysis of feeding status.

4.2.4.1 Analysis of feeding status

The abdomen length of each individual was measured from the images using image analysis software (ImagePro Plus 6.2.1). Pronotum width was also measured to correct for body size as this does not change size when the bedbug feeds and subsequently digests it food. The ratio of abdomen length to pronotum width changes predictably over time as the bedbug digests its blood meal. Using Figure 4.1 (modified from Reinhardt *et al.* 2010) the abdomen length : pronotum width ratio of each individual was converted into days since feeding. As most of the change in body size occurs in the first six days after feeding, it becomes more difficult to assess the time since feeding after the sixth day (pers. obs.). For this reason feeding status was recorded as days since feeding to the nearest day for the first 5 days and then as '6 days or more'.

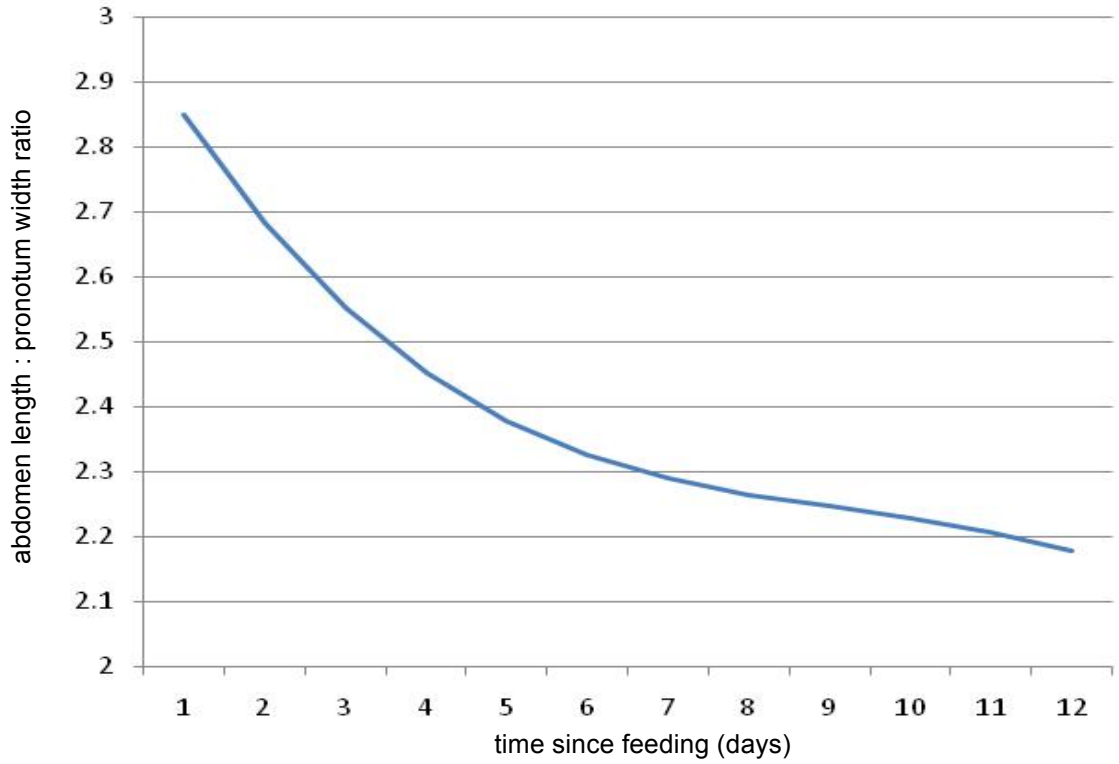


Figure 4.1 shows the decrease in abdomen length : pronotum width ratio over time at 26˚C. The formula of the polynomial cubic fit was: Y = 3.0576 + (−0.0096 * t) + (4.0E-05 * t2) + (−6.E-08 * t3), where t is time (number of days since last blood meal)(adapted from Reinhardt *et al.* 2010).

4.2.4.2 Propensity of unfed bedbugs to feed

To establish whether unfed bedbugs would feed if given the opportunity, all bedbugs from the "6 days or more" feeding category were removed from the arena at the end of the experiment and offered food. This was achieved by placing groups of up to 100 individuals into small paper-lined arenas (L:W:H, 25:15:10 cm) with an artificial host on a heat mat. The number of fed individuals was scored after 1 hour.

4.2.5 **Statistical Analysis**

The effects of harbourage space availability (number of harbourage strips) on (1) the rate of new harbourage acquisition and (2) the rate of increase of the maximum host-harbourage distance were analysed using generalised linear mixed models (GLMM) using the lmer function contained within the lme4 and matrix packages in R. Models were fitted using Poisson and Gaussian error structure respectively. Replicates of each arena setup were included as a random effect. Minimum adequate models were derived using backwards, stepwise procedures to remove non-significant effects. The effects of harbourage availability on dispersion time were analysed using two-sample weighted log-rank test contained in a R package "surv2sample". Both models were checked for normality using plots in R (Residuals vs Fits and Normal Q-Q).

Fidelity was assessed at two levels; 1) harbourage fidelity - the number of bedbugs found in exactly the same harbourage after 21 days, presented as a percentage of the total; and 2) local fidelity – the tendency of bedbugs to return to the same area after feeding, but not the same harbourage presented as a linear regression between the initial location (x-axis) and the final location (y-axis) of any individuals *not* found in the same harbourage after 1 day. A significant positive linear regression between the initial and final locations of individuals that left their original harbourages would be evidence for fidelity to the locality of their original harbourage.

Mixed-model nested ANOVAs were used to test the significance of the null hypotheses that the energetic cost of walking has no effect on either the number or size of eggs produced. To prevent the large inter-specific variation in weekly egg output from masking any potential treatment effects, individuals were grouped into threes according to their mean weekly fecundity based on a three week lead-in. Each group was then split randomly across the treatments and group was used as a factor, nested

within treatment, in the model. Both models were checked for normality using plots in r (Residuals vs Fits and Normal Q-Q).

Chi-square contingency table was used to test the null hypothesis that the feeding status of bedbugs is unaffected by their location in the arena. Because expected values were too low, feeding statuses 1 to 5 were combined. This resulted in less than 80% of expected values falling below 5 and no expected values falling below 2.6 in any of the three replicates.

4.3 Results

4.3.1 How Does Harbourage Use Change with Population Growth?

Figure 4.1 shows the spatial distribution (recorded weekly) of all bedbugs in the three replicate arenas. There was a significant positive correlation between harbourage number and population size (GLMM with Poisson error, $F_{6,14}$=20.27, $p<0.001$, Figure 4.2). There was also a significant positive correlation between the maximum host-harbourage distance and population size (GLMM with Gaussian error, $F_{6,14}$=118.39, $p<0.001$, Figure 4.3). This supports the field observations that small bedbug populations are localised in the immediate vicinity of the host, while peripheral harbourages are only utilised as the bedbug population increases.

4.3.2 Are Bedbugs Faithful to Particular Harbourages?

Of the 200 bedbugs initially present in each of the three arenas 194, 186 and 192 (for arenas 1 to 3 respectively) were still alive and occupying harbourages after 21 days. Of these individuals 188, 183 and 187 (for arenas 1 to 3 respectively) were occupying the previously defined harbourages after a further 21 days.

Figure 4.4 shows the frequency distribution of the bedbugs within each arena 21 days after the bugs had been marked with paint. The pie charts represent the proportion of bedbugs of each colour occupying each harbourage. The "harbourage fidelity" for each replicate (i.e. the proportion of bedbugs found in exactly the same harbourage after 21 days) is presented in Figure 4.5. The mean "harbourage fidelity" across the replicates was 40.67±1.56% (n=3).

Figure 4.5 shows the initial location plotted against the final location of every bedbug that left the harbourage, for each of the three replicates. There was only a significant positive correlation between the initial and final location of the non-harbourage faithful bedbugs in Replicate 1. This suggests that some fidelity to the locality of the original harbourage exists. However the low R^2 value of 11.9% suggests that the proportion of individuals that moved to nearby harbourages was relatively small.

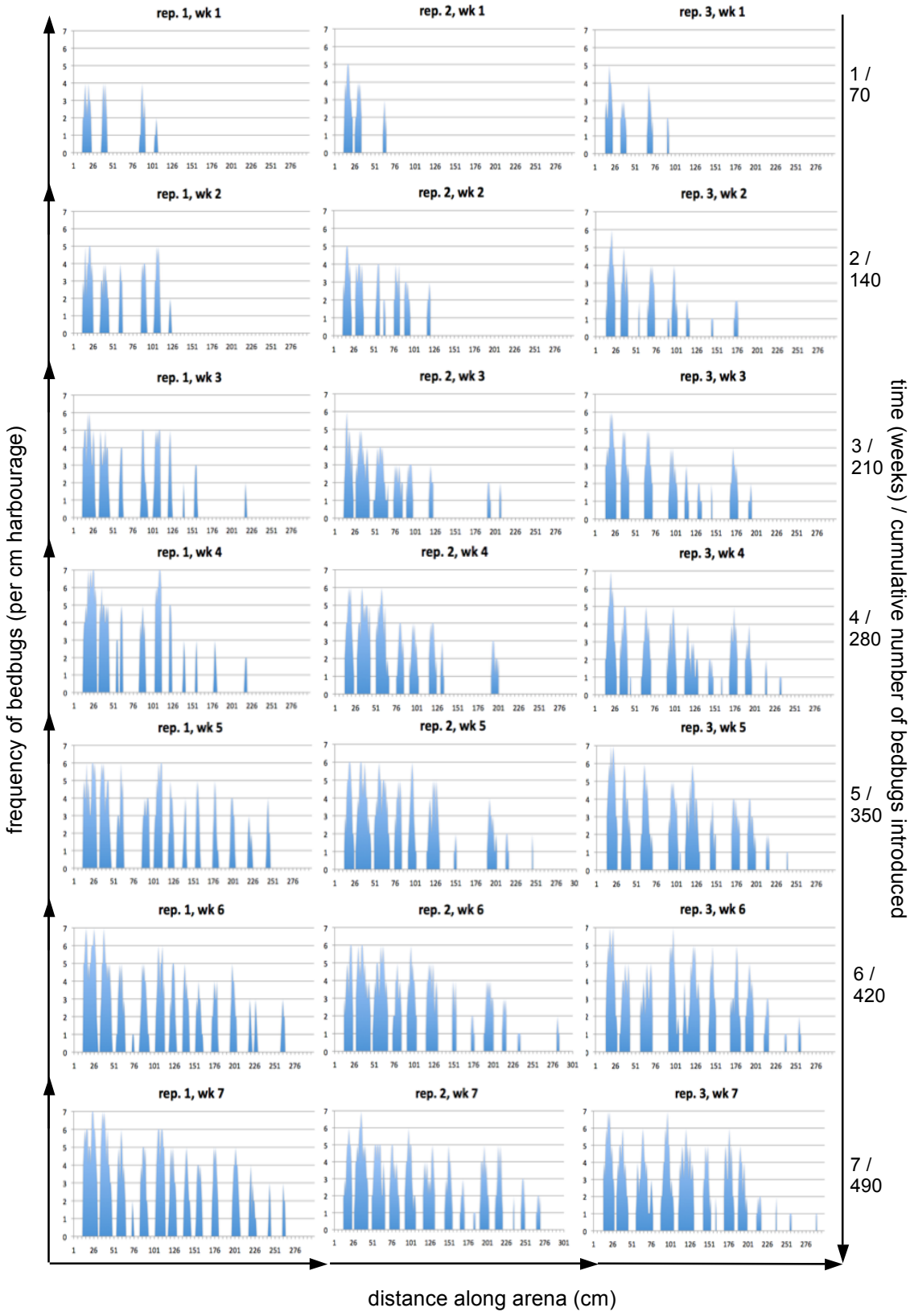


Figure 4.1 shows the change in distribution of bedbugs with population growth for three replicate arenas.

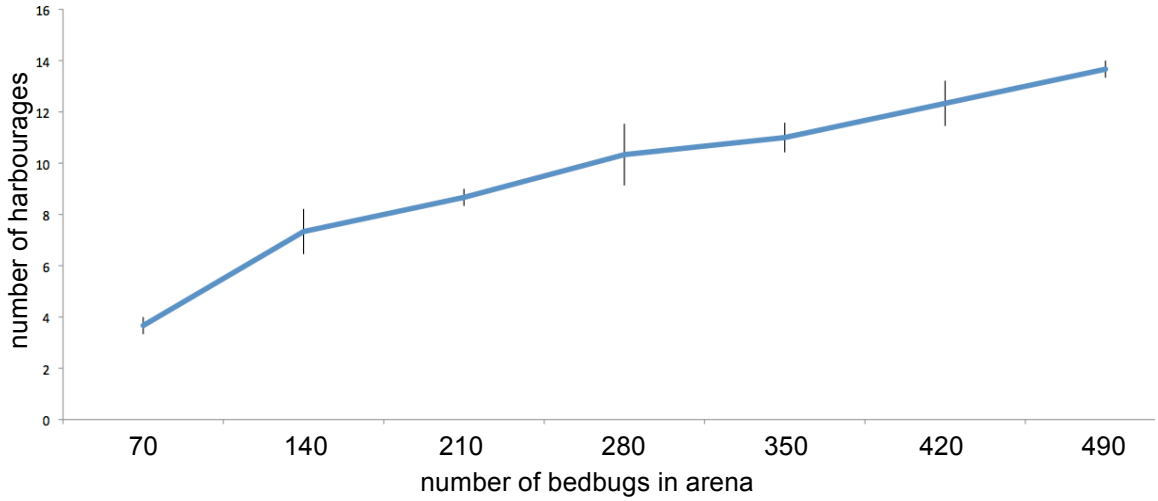


Figure 4.2 shows a significant increase in number of harbourages with population size for each of the three replicate arenas (GLMM with Poisson error, $F_{6,14}$=20.27, p<0.001). Error bars represent 1 standard error, n=3 at all data points.

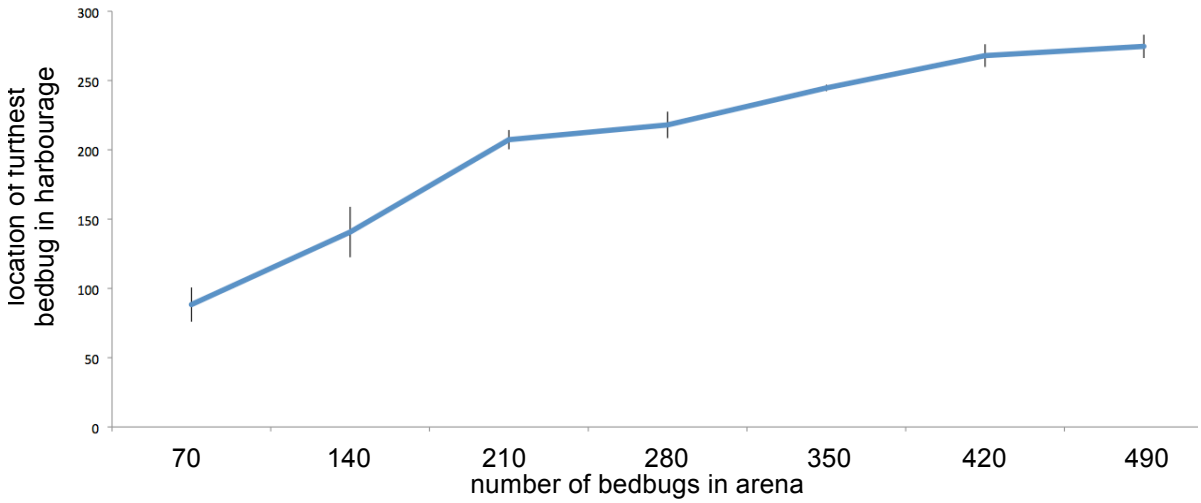


Figure 4.3 shows how the distance between the artificial host and the furthest bedbug in a harbourage increases with population size (GLMM with Gaussian error, $F_{6,14}$=118.39, p<0.001). Error bars represent 1 standard error, n=3 at all data points.

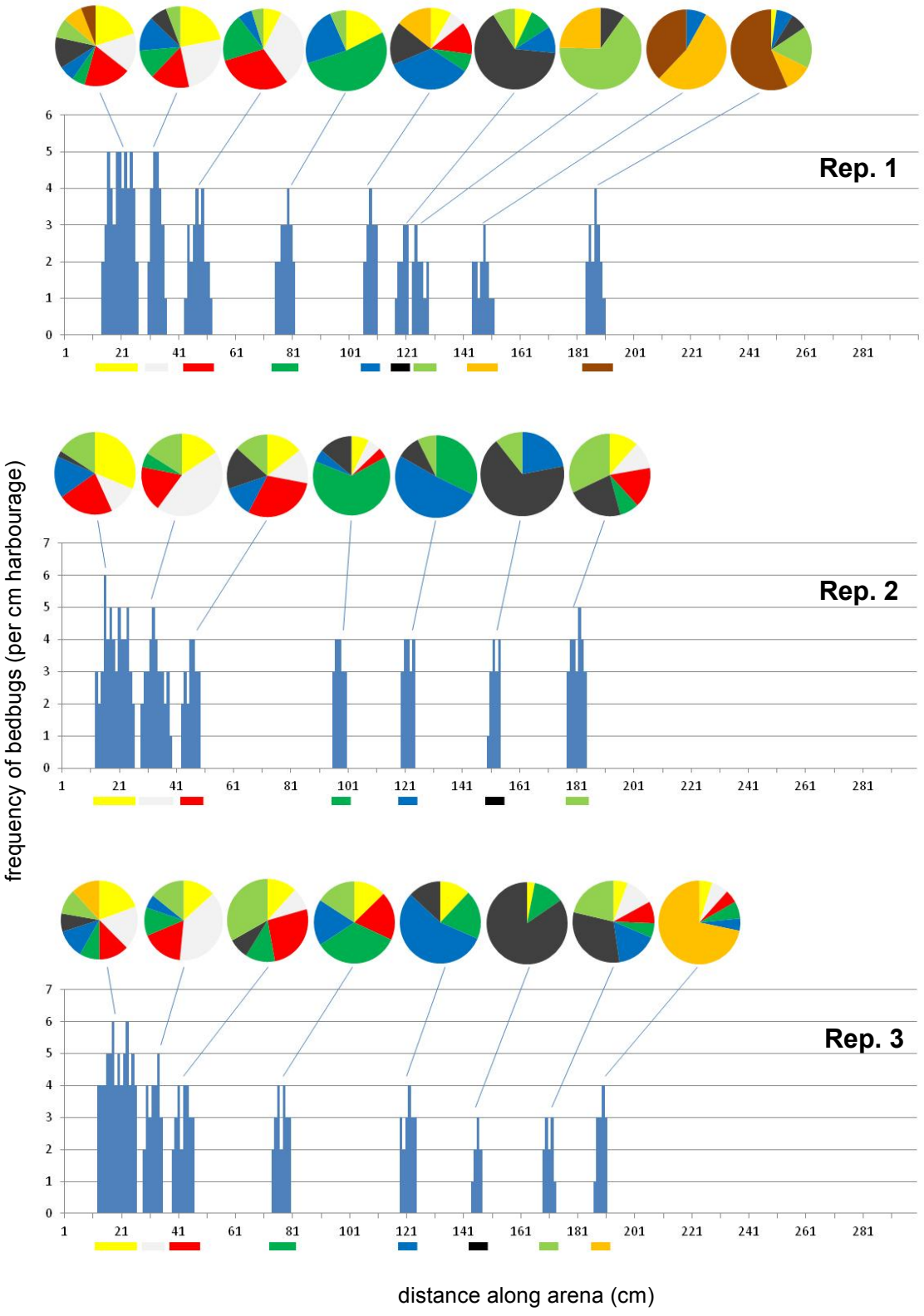


Figure 4.4 shows the frequency distribution of bedbugs in the three replicate arenas. Pie charts indicate the proportion of bedbugs of each colour in each harbourage. Colour marks along the x-axes indicate the colours assigned to all bugs in each corresponding harbourage at t_0.

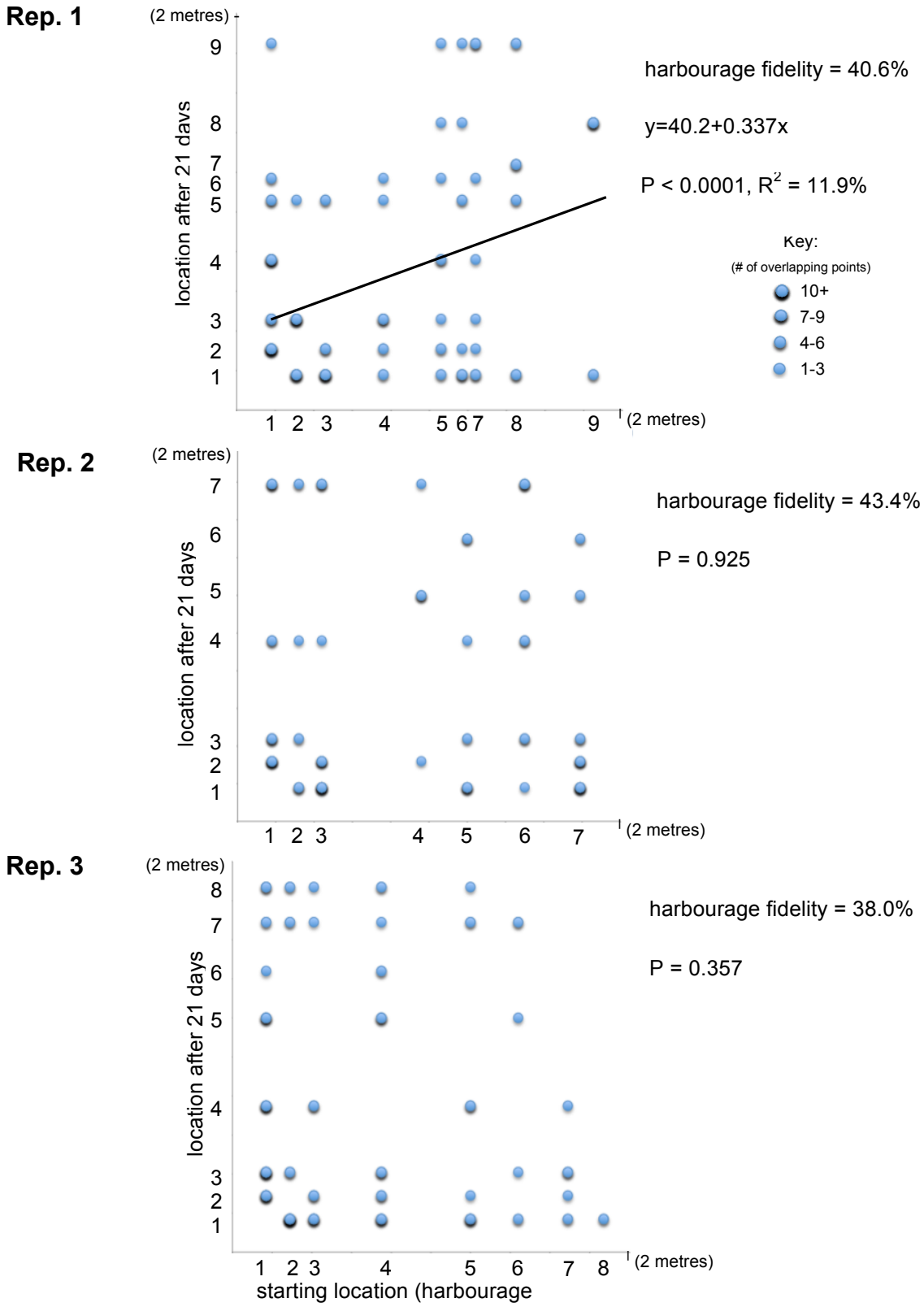


Figure 4.5 shows the initial distribution of non-harbourage faithful individuals (x-axis) against the final distribution of non-harbourage faithful individuals (y-axis) for the three replicates. There was a significant linear correlation between the initial and final locations in Replicate 1, but not in Replicates 2 or 3. Harbourage-faithful individuals were not included in the analysis, but the percentage of harbourage faithful individuals is presented against each replicate for reference.

4.3.3 Does the proximity of the harbourage to the host affect the feeding status of the bedbugs within?

Figure 4.6 shows the variation in feeding status across the harbourages for the three replicate arenas. Bedbugs nearest the host were significantly more likely to have fed in the past 5 days (χ^2 (Contingency Tables)=84.79, 92.42, 55.34 respectively; df=8, 6, 7 respectively; all p values < 0.00001).

Propensity of unfed bedbugs to feed

All bedbugs in the "6 days or more" feeding category (115.67±6.94, n=3) were moved to a small arena and given access to an artificial host. Within 1 hour 96.4% had fed to repletion, suggests that the low feeding status of the bedbugs in the peripheral harbourages did not reflect a negative appetitive state.

4.3.4 What is the Energetic Cost of Commuting from Peripheral Harbourages?

There was no effect of walking 6 metres on the number of eggs produced (Mixed-model nested ANOVA: $F_{2, 59}$=0.036, p=0.965) or on the size of the eggs produced (Mixed-model nested ANOVA: $F_{2, 59}$=0.157, p=0.855) for either recently fed or unfed bedbugs, suggesting that the energetic cost of commuting between peripheral harbourages and the host is unlikely to constrain the maximum host-harbourage distance or explain why bedbugs in peripheral harbourages tend to be unfed.

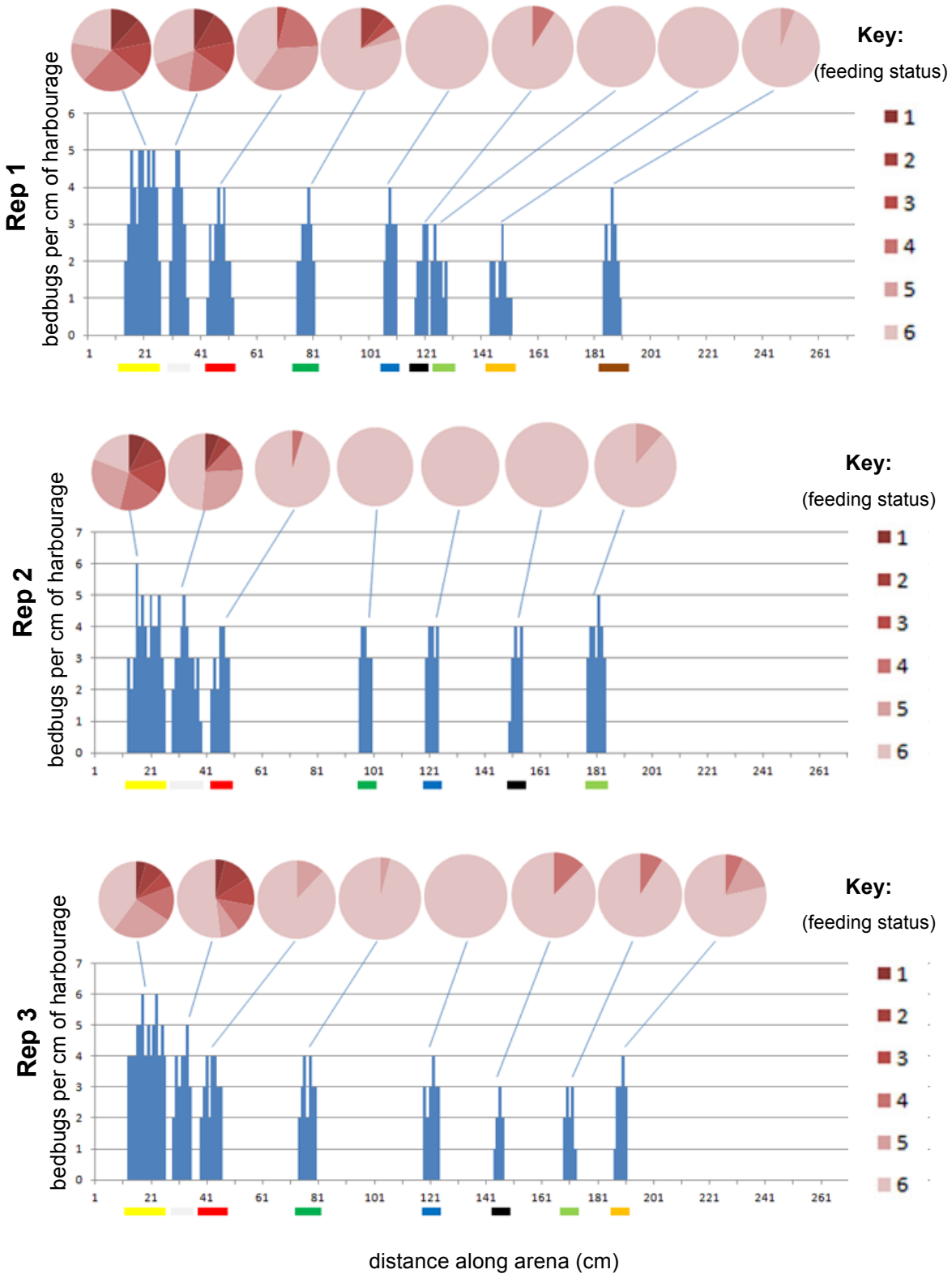


Figure 4.6 shows the proportion of bedbugs of each feeding status in each harbourage. Feeding statuses 1 to 5 denote individuals that fed 1 to 5 days ago respectively. Feeding status 6 denotes individuals that fed 6 or more days ago.

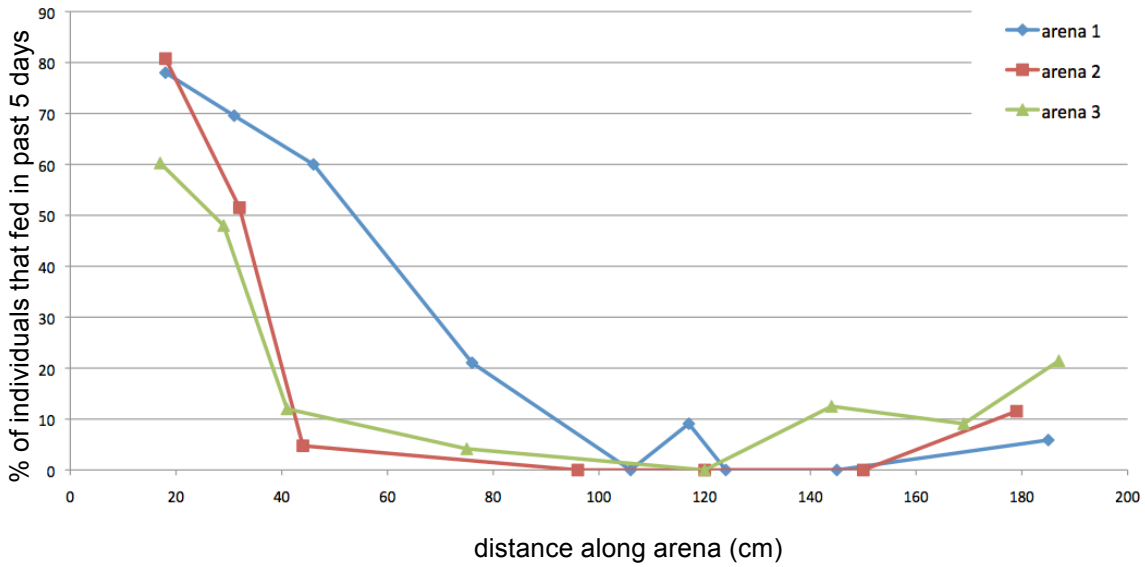


Figure 4.7 shows the percentage of bedbugs in each harbourage that had fed in the past 5 days. There was a highly significant effect of the location of the harbourage on the proportion of occupants that had fed in the previous 5 days (χ^2 (Contingency Tables)=84.79, 92.42, 55.34 respectively; df=8, 6, 7 respectively; all p values<0.00001).

4.4 Discussion

4.4.1 Effect of population size on number and distribution of harbourages

Figures 4.1, 4.2 and 4.3 demonstrate that bedbugs occupy harbourages close to the host first, and spread out into the peripheral harbourages as the infestation develops. It is not clear why bedbugs have a preference for harbourages close to the host but still retain a patchy distribution, leaving regions of unoccupied harbourage space in relatively close proximity to the host. This suggests that there is some benefit of aggregation that is limited by a density-dependent effect that eventually causes the costs of aggregation to outweigh the benefits (Pulliam & Caraco 1984, Wertheim *et al.* 2005).

Siljander *et al.* (2008) observed that when aggregation pheromones were supplied in higher concentrations, aggregated bedbugs quickly dispersed, leading the authors to conclude that the same chemicals could act as an alarm pheromone above a threshold concentration. If aggregation pheromones are continually produced while the bedbugs are present in the harbourage, a high density of bedbugs may eventually raise the concentration of aggregation pheromone, making it unattractive or even repellent to approaching bedbugs. This density dependent feedback on harbourage size could potentially provide a proximate mechanism capable of controlling harbourage density, however, it does not provide an ultimate explanation.

Aggregating bedbugs have been shown to benefit from enhanced water conservation giving them greater resistance to dehydration compared to solitary individuals (Benoit *et al.* 2007). It is possible that the same mechanism could be responsible for restricting the maximum group size as high humidity can promote bacterial and fungal growth (Kemper 1936). If this were the case one might expect harbourage size, number and spacing to be influenced by microclimatic humidity. Alternatively limiting the size of aggregations may help reduce the likelihood of being discovered by the host, or even reduce the spread of bedbug pathogens.

4.4.2 Fidelity of bedbugs to particular harbourages

It was important to see if bedbugs show fidelity to particular harbourages or localities as this has implications for relatedness and a range of associated issues. More than 40% of bedbugs were found in the same harbourages after 21 days.

Fidelity of bedbugs to the locality of their original harbourage was assessed by correlating the initial and final locations of all individuals that moved away from their

initial harbourage. A significant positive correlation between the initial and final locations was only found in one of the replicates suggesting that bedbugs that move away from their initial harbourage tend not to move to the nearby harbourages more than would be expected by chance. Even in the replicate where a significant positive correlation between the initial and final locations was identified, the low R^2 value (11.9%) suggests that only a small proportion of individuals actually showed fidelity to the locality of their original harbourage.

One limitation of the experimental design was that it was only possible to assess harbourage fidelity at a single time point, because identifying the colour code of each bedbug involved removing them from the harbourages. It is likely that harbourage fidelity, or lack thereof, is a function of time, but without additional time points, we cannot infer anything about this dynamic.

These results suggest that bedbugs are, to some extent, faithful to specific harbourages. However, true harbourage fidelity implies that the bedbugs leave their harbourages to forage and then return to their original locations after feeding. It was therefore necessary to examine the feeding status of the bedbugs within each harbourage for evidence that they had left and then returned.

4.4.3 Variation in feeding status between harbourages

There was a clear negative relationship between feeding status and distance from the host (Figure 4.7). Approximately 60-80% of the bedbugs in the harbourages adjacent to the host had fed in the past five days, while the majority of bedbugs in harbourages further than 50 cm from the host had not fed for at least 6 days. One limitation of using body size as an estimate of the duration since the last feed is that after the first 6 days (at 26ºC) there is little additional shrinkage. It is therefore impossible to determine if the bedbugs in the harbourages that hadn't fed in the past 5 days had in fact fed during the first 2 weeks after being marked. However it seems highly unlikely that successful foraging of bedbugs in the peripheral harbourages was occurring in the first two weeks of the trial but then ceased for the week immediately prior to recapture and analysis of feeding status.

A limitation of the laboratory arena is that the artificial host is considerably smaller than a natural host. It is therefore possible that the range over which the bedbugs are able to detect the artificial host is shorter than the range over which they are able to detect a host in a natural infestation. Host detection range could influence the feeding status of bedbugs within harbourage if, for example a bedbug is unable to detect

the presence of the host from within its harbourage, it may never receive the cues necessary to trigger foraging. Further fieldwork is needed to look specifically at the feeding status of bedbugs in harbourages at different distances from the host.

Even if the feeding status of bedbugs in harbourages is influenced by the range over which the bedbugs can detect the host and the detection range is reduced in the arena setup, this should not influence the qualitative result that feeding status is higher nearer the host. Assuming the observed variation in feeding status is not an artefact of the arena setup, there are two possible biological explanations for the observed distribution. Firstly, it may be that within a single feeding cycle, bedbugs move between harbourages. There are several reasons why this could occur. It may be that a bedbug in the peripheries of the infestation locates and feeds on the host, and then hides in the nearest established harbourage. Freshly fed bedbugs move relatively slowly and freshly fed females in particular are vulnerable to male harassment (Reinhardt *et al.* 2009), so it would be in the interests of the individual to find a harbourage as quickly as possible. Once the blood meal has been partially digested, the bedbug might then benefit by moving further away, for example, to avoid aggression from conspecifics, detection by the host, or to find a suitable space for egg laying. An alternative explanation for the skewed distribution of feeding status may be that only the bedbugs in those harbourages closest to the host have regular opportunities to feed. This could therefore generate competition for access to the high quality harbourage resources (i.e. those close to the host). However, I have never observed aggression between bedbugs (over >10 years) and it has never been reported, although it is possible that the "resident always wins" (as suggested by Maynard Smith & Parker 1976 and demonstrated by Davies 1978). If it is difficult to displace a resident bedbug from a harbourage and harbourages tend to be abundant, then aggression may never evolve.

The variation in feeding status, and specifically the lack of recently fed individuals over most of the length of the arena, combined with the data on harbourage fidelity (4.3.2), suggests that the majority of bedbugs in harbourages beyond 50 cm from the host do not return to the same harbourages after feeding. Fidelity to harbourages appears to be a consequence of bedbugs failing to leave the harbourages over the 21 day period, rather than returning to the same place after foraging.

4.4.4 Energetic cost of travelling

It is not known why bedbugs in peripheral harbourages do not feed regularly. One explanation is that the cost of travelling greater distances to and from the host

outweighs the nutritional benefit. There are several potential costs of long foraging trips, which are not mutually exclusive. Firstly, females outside harbourages are at greater risk of being traumatically inseminated by males, especially when returning from the host, when their distended abdomens restrict their ability to escape (Reinhardt *et al.* 2009), and high male-imposed mating rates have already been shown to be costly to females (Stutt & Siva-Jothy 2001). Any time spent out of the harbourage also increases exposure to predators such as spiders and discovery by the host (Reinhart & Siva-Jothy 2007). Lastly, there must be some energetic cost of travelling, particularly on the return trip when they are carrying a large blood meal. However, I found no measureable energetic travelling cost (in terms of egg number or size) over the distances normally encountered in natural infestations, suggesting that this is unlikely to be a factor in the decision to not forage.

An observation from the case studies (Chapter 2) was that harbourages tend not to be found further than 2-2.5 m from the nighttime location of the host. The argument for the energetic constraint of long foraging trips could also be applied here, but since no measurable cost was detected over simulated foraging trips of 6 metres, it seems unlikely that energetic constraints could be responsible for restricting the maximum host-harbourage distance either.

4.4.5 Implications for control

No explanation was found for the apparent maximum host-harbourage distance of 2-2.5 metres found in Chapter 2 (although the energetic cost of travelling can now be ruled out). In all infestations where harbourages were found beyond 2 metres, dispersal to neighbouring flats was already occurring. Severe infestations tend to develop as a result of underlying social issues such as a mentally or physically impaired host, which delays the reporting and treatment of the infestation (pers. obs. from 4 severe infestations). However, the probability of an infestation going undetected/unreported declines dramatically once dispersal to neighbouring flats begins. All the infestations in Chapter 2 where dispersal is believed to have occurred were identified and reported, not by the tenant, but by neighbours as a result of active dispersal to the neighbouring properties. Assuming the probability of dispersal increases as bedbugs are forced to occupy harbourages further from the bed then infestations where harbourages have formed beyond 2.5 metres may be scarce. The maximum host-harbourage distance would therefore tend to be limited by the increasing likelihood of the infestation being detected by people in the neighbouring flats.

The discovery that feeding status is much higher in harbourages adjacent to the host has implications for the use of passive monitors that mimic harbourages. Many of these monitors do not trap the bedbugs, but work by providing them with an ideal harbourage in close proximity to the host (e.g. BB Alert Passive, MIDMOS Solutions Ltd., UK), which can be removed and checked for signs of bedbugs. If the monitors are checked regularly they have the potential to prevent an infestation from becoming established, particularly if alternative harbourages are limited. However, if the monitors are setup but not checked regularly, the bedbugs will benefit from ideal harbourages in the vicinity of the host, which could facilitate a higher feeding rates than in the monitor's absence and thus speed up population growth.

4.4.6 **Summary**

In this chapter I have shown that :

1) The number of harbourages increased with population size, retaining a patchy distribution of harbourages in continuous space. This process pushes harbourages out into the peripheries of the infestation.
2) Approximately 40% of bedbugs were found in the same harbourages after three weeks, suggesting some level of harbourage fidelity exists. However…
3) Feeding status declined dramatically with distance from the host, suggesting that the apparent harbourage fidelity was due to many individuals failing to leave the harbourage over the duration of the experiment. This finding also raises questions over competition for harbourage resources, although no overt conflict has ever been observed.
4) There was no measurable energetic cost commuting to the host (in terms of female fecundity), suggesting that energetic constraints are unlikely to be responsible for either the apparent 2.5 metre limit on host-harbourage distance, or on the low feeding status of individuals in peripheral harbourages.

5 Factors Affecting Active Dispersal

5.1 Introduction

Passive dispersal in bedbugs occurs when individuals are carried accidentally on clothes, furniture or other belongings. It has been studied and described by several authors (Usinger 1966, Boase 2001, Reinhardt & Siva-Jothy 2007, Kilpinen *et al.* 2008). However, active dispersal - where the bedbugs actively move between nearby rooms and buildings - has received relatively little attention (but see Wang *et al.* 2010, How & Lee 2010b – tropical bedbug). Consequently the factors that initiate active dispersal from an established infestation are poorly understood, and perhaps as a result of this, active dispersal is a major problem in gaining control of bedbug infestations (Pinto *et al.* 2007, Doggett & Russell 2008).

Chapter 2 demonstrated how the population size at which infestations began to disperse varied greatly. One explanation for this could be that with more available harbourage space infestations can become larger before bedbugs begin to disperse. In Flat D of Case Study 1 (Figures 2.1/2.2, page 35-36), horded newspapers provided considerably more suitable harbourage space than was available in any of the other infestations. Flat D also had at least 25 times more bedbugs than any of the other infestations visited, and yet dispersal to the neighbouring flats had only been apparent for a short time. Using the arena setup it is possible to test if harbourage availability influences the onset of dispersal by varying harbourage availability and increasing the population size until dispersal occurs.

There are important practical implications of understanding how harbourage availability affects bedbug ecology and dispersal. Beds with few available harbourages may cause infestations to occupy peripheral harbourages more rapidly and consequently be harder to treat. Passive monitors, designed to provide suitable harbourages for bedbugs, that can be easily removed and checked, may have the added benefit of delaying dispersal to neighbouring rooms or flats.

Female bedbugs pay a 25% longevity cost, as a result of natural mating rates (Stutt & Siva-Jothy 2001), and are most vulnerable to male mating attempts

immediately after feeding, as the large blood meal reduces their mobility and exposes the region of the abdomen where traumatic insemination usually occurs (Reinhardt *et al.* 2009). It has been proposed that female bedbugs might attempt to disperse from infestations to avoid unwanted male attention (Stutt & Siva-Jothy 2001, Pfiester *et al.* 2009). However, field studies have yet to identify any differences in the natural sex ratios that would be expected if females were dispersing in significant numbers. Furthermore, Case Study 4 (Chapter 2) showed that both sexes were caught in roughly equal numbers on sticky traps located in the corridors of a multiple occupancy dwelling, which suggests that both sexes disperse, although the sample size was too small to draw firm conclusions.

As well as dispersing from an infestation, female bedbugs may be able to avoid males simply by moving to new harbourages. In this case one might expect to find variation in the sex ratios between harbourages. Using the laboratory arena setup I have developed, it is possible to test whether female bedbugs disperse in response to the presence of males as well as assess if there is any variation in sex ratio between harbourages.

Active dispersal may be an accidental consequence of having to utilise peripheral harbourages beyond the range that the host can be directly detected. Direct detection of the host by heat, host kairomone(s) and/or CO_2 has only been shown over distances of up to 1.5 metres (reviewed in Reinhardt & Siva-Jothy 2007). However, elevated CO_2 has been observed to trigger foraging over several metres in a semi-enclosed environment where the CO_2 concentration was able to build up (see Appendix 2). Although CO_2 and/or other chemical components of breath have been shown to provide directional cues to bedbugs, this has only been shown over a distance of less than 65 cm (Suchy & Lewis 2011). It is unlikely that CO_2 and other chemical cues provides directional information at the peripheries of the infestation, since the concentration gradient of these chemicals will decline exponentially with distance from the host. Consequently at the peripheries of the infestation, the local gradient in CO_2 concentration is unlikely to be sufficient to indicate the direction of the host to the foraging bedbug. It is therefore possible that bedbugs in a harbourage at the peripheries of an infestation can detect elevated CO_2 due to the presence of the host, begin foraging, but in the absence of any detectable directional cues, walk in the wrong direction, resulting in an 'accidental' departure from the established infestation. In this situation one would expect the dispersing individuals to be a random sample of bedbugs from the peripheral harbourages rather than individuals in a particular phase of their life cycle.

5.1.1 Chapter aims

In this chapter I will explore factors affecting active dispersal in bedbugs. I will:

1) Determine whether dispersal is influenced by the availability of space for harbourages.
2) Establish if females disperse to avoid sexual harassment from males.
3) Look for variation in the sex ratios within each harbourage as an indication of within-infestation male avoidance.

5.2 Methods

5.2.1 Does Harbourage Space Availability Influence the Onset of Dispersal?

To test the effect of harbourage availability on dispersal, three replicate arenas were set up as described in 3.2.3, but instead of a single 10 mm wide harbourage strip down the centre of the arena, each arena had two parallel harbourage strips, spaced such that the width of the arenas were divided equally into thirds. A second set of three replicate arenas was set up as above but with three 10 mm wide paper harbourage strips running parallel up the length of each arena, and spaced such that the width of the arenas were divided equally into quarters. In all the arenas the artificial host was replaced at the beginning of each dark phase of the daily cycle, in conjunction with a period of elevated CO_2 (see 3.2.3). An additional artificial host was added for every 250 bedbugs. Immediately after replacing the artificial hosts, 10 mixed sex adult bedbugs from a newly eclosed cohort were introduced into each arena at the release point. In order that the distributions of bedbugs in the arenas with two and three harbourage strips could be compared to the distributions in Chapter 3 (where only a single harbourage strip was provided) all the arenas in this Chapter were set up on the same day as those in Chapter 3 and populated with bedbugs from the same cohorts.

The number of dispersed bedbugs was checked daily. However, it was not feasible to establish the distribution of all bedbugs for all arenas every week. Consequently the weekly distribution of all bedbugs was only established for Replicate 1 of the double harbourage arenas and Replicate 1 of the triple harbourage arenas. For Replicates 2 and 3 of the double and triple harbourage arenas, the number of harbourages and distance from the host to the furthest bedbug in a harbourage was recorded weekly (in addition to the daily check for dispersers). Dispersal traps containing bedbugs were replaced daily to avoid lingering aggregation or alarm pheromones from influencing dispersal.

The experiment was terminated on day 77 when the total number of bedbugs introduced into the arena was 770.

5.2.2 Do Males Influence the Onset of Female Dispersal?

Typically, freshly fed female bedbugs must pass a series of occupied harbourages on their return journey following a foraging trip (pers. obs.). Male sexual

interest, and the resulting copulations, are therefore likely to increase proportionally with the length of the occupied harbourage that the females must pass before reaching a suitable, unoccupied space to reside. Male reproductive 'attention' will therefore increase with population size, despite the sex ratio remaining constant.

Sexual harassment is unlikely to be the only trigger for dispersal as the field data from Case Study 4 (Chapter 2) revealed that males and females, as well as nymphs, disperse. However, if sexual harassment is an important factor in driving dispersal, it would be predicted that populations without males would disperse later than populations where males and females ware both present. To examine whether male bedbugs influence the onset of female dispersal, six identical 3 metre long arenas were set up as described in 3.2.2 (page 60), with a continuous 10 mm wide strip of paper down the centre of the arena to provide potential harbourage space. Five males and five females from the F4 stock were introduced into three of the arenas daily at the beginning of each dark phase. Into the remaining three arenas, ten females were released at the beginning of each dark phase.

An artificial host, with associated heat and CO_2 (see 3.2.3, page 63), was provided daily at the beginning of each dark phase (immediately prior to introducing the bedbugs) to facilitate normal foraging and returning behaviour. For every 250 bedbugs released into the arena, an additional artificial host was added to prevent food limitation from influencing dispersal. The number of dispersed bedbugs was recorded daily. Any dispersing bedbugs were removed from the dispersal traps and sexed. The dispersal traps were replaced daily to remove any lingering aggregation or alarm pheromones. The experiment continued until day 55, at which point 550 bedbugs had been introduced

At the termination of the experiment, the number of males and females in each harbourage of the mixed sex arenas was recorded to see if sex influenced the patterns of harbourage usage. This could indicate within-infestation male harassment avoidance by females.

5.2.3 Statistical Analysis

A Linear Mixed-Effects Models (LME) were used in the statistical package R to assess the effect of increased harbourage space availability on the rate of harbourage acquisition, and the rate of increase of distance between the host and furthest bedbug, with increasing population size.

Survival Analysis (Prentice-Wilcoxon's weighted log-rank test) in the statistical package R was used to test the effect of increased harbourage space on time to dispersal.

Survival Analysis (Prentice-Wilcoxon's weighted log-rank test) was also used to test the effect of male presence/absence on time to dispersal, and a T-test was used to compare the final number of dispersers of each sex in the mixed sex arenas.

A Chi-Square contingency table was used to test the null hypothesis that the distribution of bedbugs across the harbourages in the mixed sex arenas is not influenced by sex.

5.3 Results

5.3.1 Does Harbourage Space Availability Influence the Onset of Dispersal?

Figure 5.1 shows the distributions of all bedbugs in the first replicate of the double harbourage strip and triple harbourage strip arenas. As expected, the number of harbourages increased with population size (LME, $LRT_1 = 174.82$, $p < 0.001$), however there was no difference in the rate of harbourage acquisition with increasing population size between the single, double and triple harbourage strip setups (LME, $LRT_2 = 1.15$, $p = 0.56$) suggesting that harbourage availability does not affect total harbourage number or mean group size.

The distance between the artificial host and the furthest bedbug was used as a measure of peripheral harbourage usage. The number of harbourage strips had a significant effect on the rate at which the distance increased between the host and the furthest bedbug within a harbourage (LME, $LRT_1 = 43.89$, $p < 0.001$, Figure 5.2), suggesting that peripheral harbourage use is driven by lack of harbourage space near the host.

Figure 5.3 shows the cumulative number of bedbugs that dispersed from the double harbourage strip arenas over time on the same axis as the cumulative number of bedbugs, which dispersed from the single harbourage strip arena. Doubling the available harbourage space significantly delayed bedbug dispersal by on average 1.67 times (Prentice-Wilcoxon's weighted log-rank test; $p<0.001$, Figure 5.3). A third harbourage strip delayed dispersal still further, as no bedbugs had dispersed from the triple harbourage arena by the time the experiment was terminated (week 11). These results support the field observation that greater harbourage availability delays the onset of dispersal.

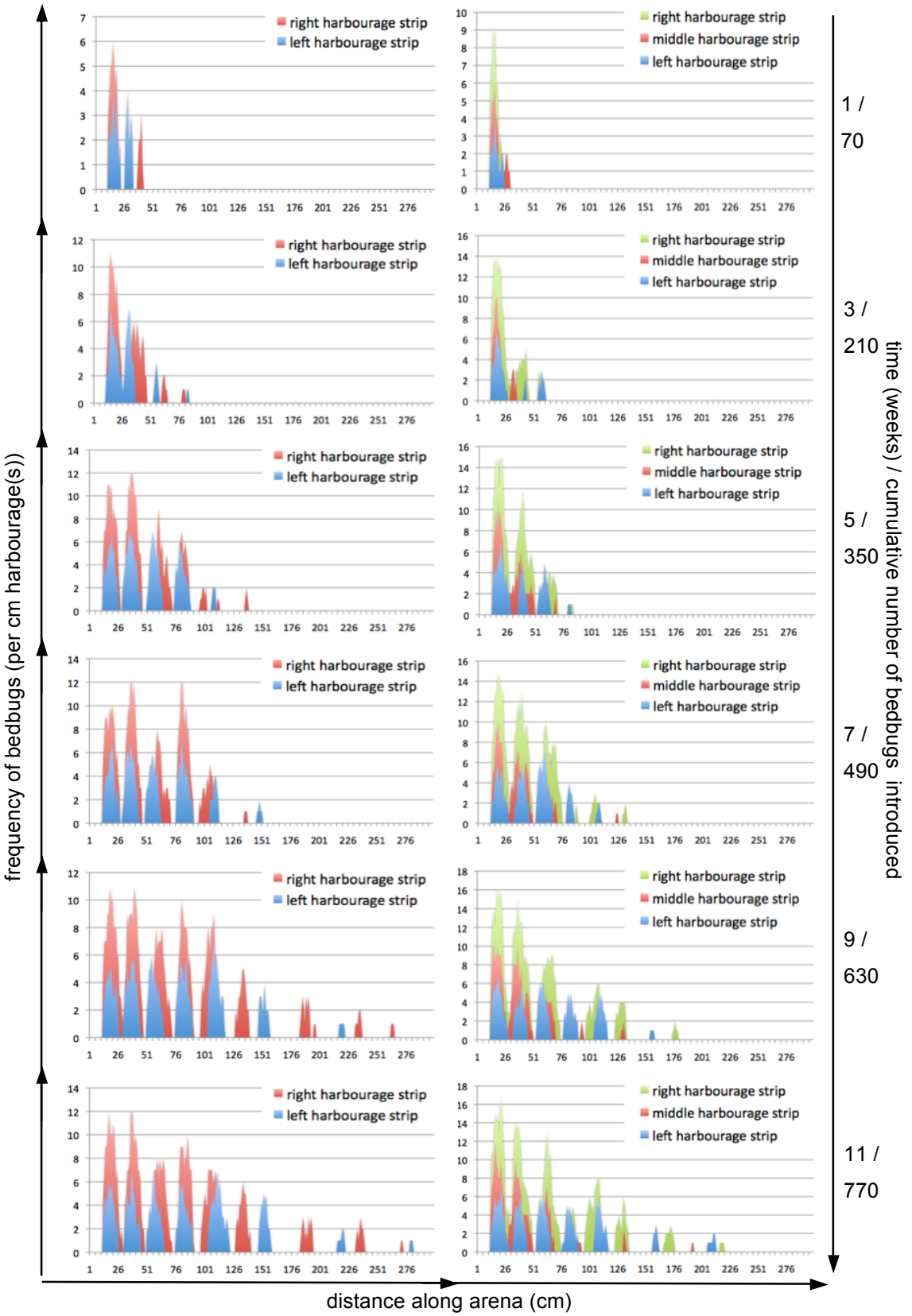


Figure 5.1 shows the change in distribution of bedbugs with increasing population size for rep. 1 of 3 of the double harbourage arenas (left column) and rep. 1 of 3 of the triple harbourage arenas (right column). For each time point the frequency distributions of bedbugs under each of the parallel harbourage strips are combined onto a single set of axes but remain distinguishable by colour. The distributions at weeks 2, 4, 6, 8 and 10 are not shown.

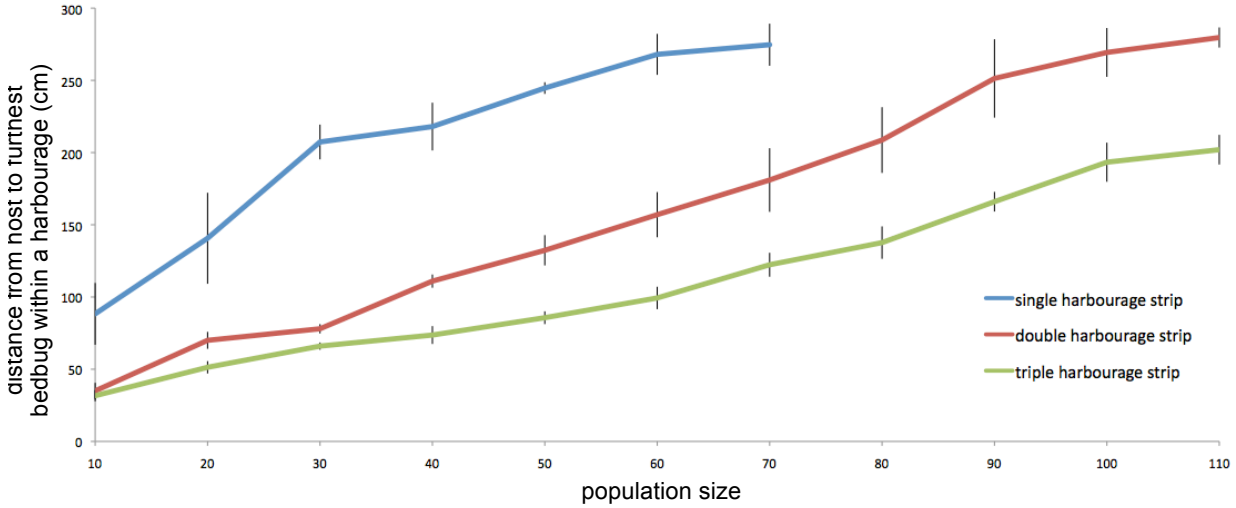


Figure 5.2 shows how the distance between the host and the most peripheral bedbug within a harbourage increases with population size, and how the effect of doubling and tripling the available harbourage space reduces the rate and which the distance between the host and most peripheral bedbug increases with population size. There was a significant effect of population size (GLMM, $t = 4.30$, $p < 0.001$), number of harbourage strips ($t = 17.29$, $p < 0.001$), and the interaction between the two ($t = 2.51$, $p = 0.014$) on the distance from the host to the furthest bedbug. Error bars represent 1 standard error, n=3 at all data points.

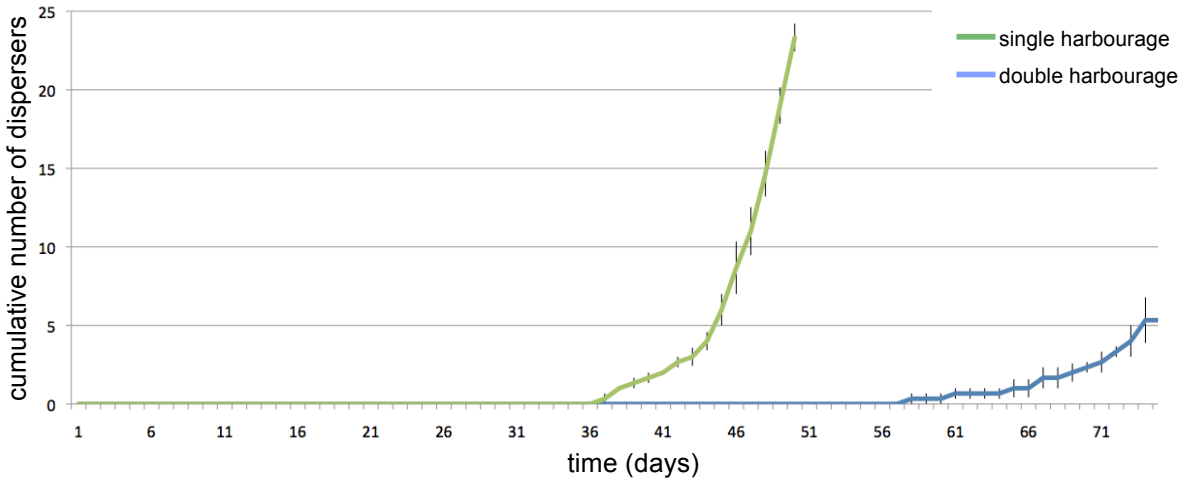


Figure 5.3 shows the cumulative dispersal of bedbugs from the single harbourage strip arena and the double harbourage strip arena. At the 11 week time point, when the experiment was terminated, no bedbugs had dispersed from the triple harbourage strip arena. There was a significant effect of the number of harbourage strips on the time to dispersal (Prentice-Wilcoxon's weighted log-rank test; $P < 0.001$). Error bars represent 1 standard error, n=3 at all data points.

5.3.2 Does the Presence of Males Influence the Onset of Female Dispersal?

There was no difference in time to dispersal of bedbugs from the female only arenas compared to the mixed-sex arenas ($P > 0.05$; Prentice-Wilcoxon's weighted log-rank test, Figure 5.4). Furthermore, there was no significant difference in the numbers of males and females that dispersed from the mixed sex arenas (T-test, $p>0.05$), supporting the finding from Case Study 4 and suggesting that dispersal is not driven by female avoidance of sexual harassment.

In two of the three replicates, the distribution of bedbugs across the harbourages in the mixed sex arenas was influenced by sex (χ^2 (Contingency Tables)=25.45, 20.45 respectively, df=13,12 respectively, 0.025<p values<0.01, Figure 5.5), while in the third arena the difference from the null hypothesis was marginally non-significant (χ^2 (Contingency Tables)=24.27, df=13, 0.1<p value<0.05, Figure 5.5) indicating that at least in some cases bedbugs select harbourages on the basis of the sex of the resident bugs within. This supports the hypothesis that females may select harbourages to avoid males.

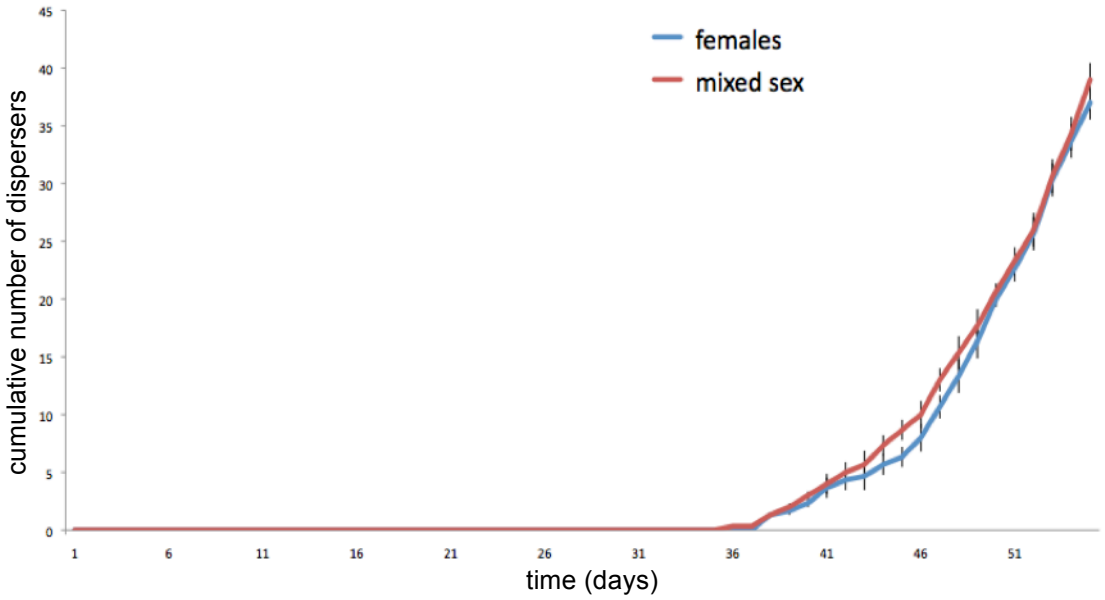


Figure 5.4 shows cumulative dispersal over time for the female only and mixed sex arenas. Error bars represent 1 standard error. There was no significant difference in the time to the onset of dispersal between female-only and mixed sex arenas (Prentice-Wilcoxon's weighted log-rank test, $P > 0.05$). Error bars represent 1 standard error, n=3 at all data points.

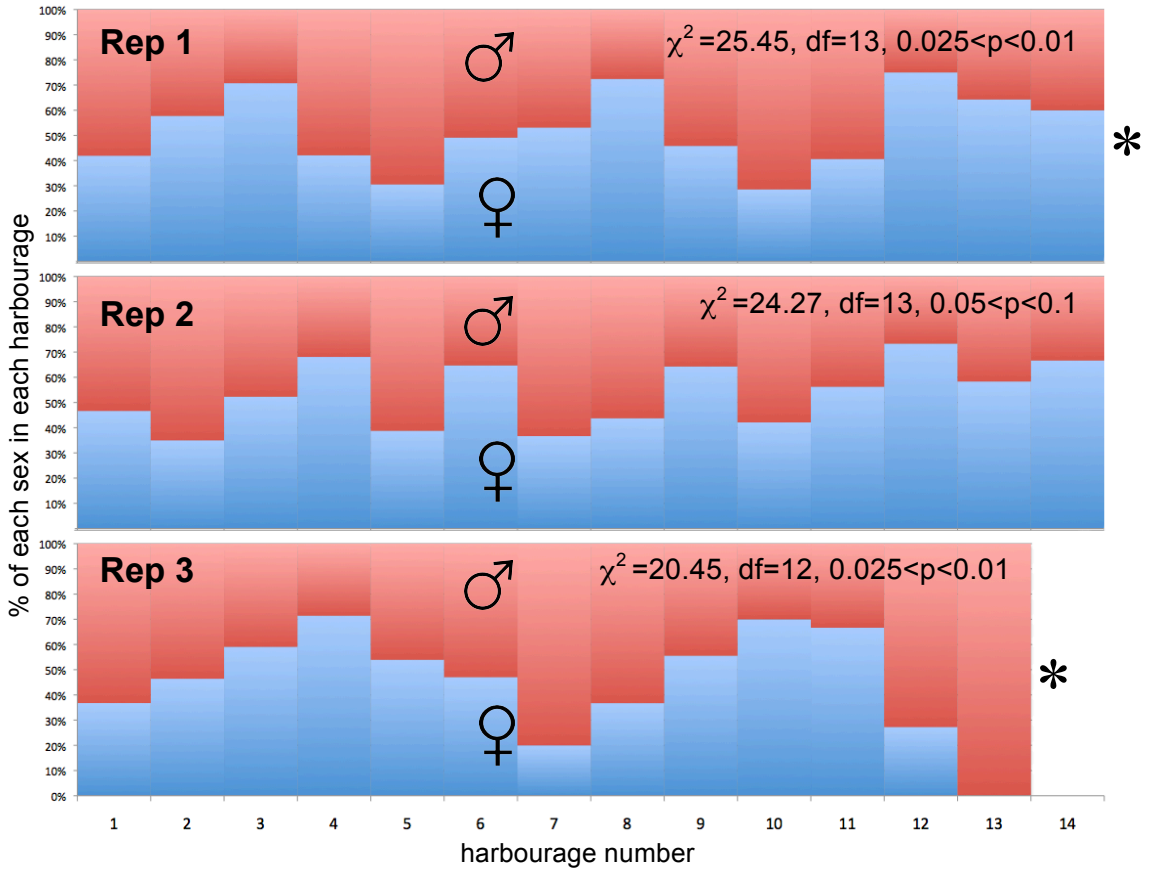


Figure 5.5 shows the proportion of males and females in each harbourage for each of the three replicates. The Chi-square statistic is presented for each replicate. * denotes replicates where the distribution of bedbugs across the harbourages was significantly influenced by sex.

5.4 Discussion

5.4.1 Effect of harbourage space availability on dispersal

The case studies in Chapter 2 suggested that the population size of infestations at the onset of dispersal varied considerably. This was attributed to variation in the abundance of harbourage space. The data in Figure 5.3 supports this hypothesis, showing that increased harbourage space availability in the arena setup did significantly delayed dispersal.

Doubling the available space for harbourages corresponded to a 1.67 fold increase in the population size at the onset of dispersal, suggesting that the two harbourage strips were not used equally. Data on the relative usage of the different harbourage strips within each arena was only available from the first replicate, in which 53% of bedbugs occupied the right harbourage strip compared to 47% on the left (viewed from above with the artificial host at the top). This is likely to be due to a tendency of bedbugs to follow the edges of the arena before turning and crawling under the harbourage strip. Since all harbourage strips were attached down the left side and were therefore open down the right side, bedbugs walking down the right side of the arena may have found it easier to discover/access the harbourages than bedbugs walking down the left side.

5.4.2 Effect of male presence on female harbourage selection and dispersal

Despite the measurable costs associated with natural mating rates (Stutt & Siva-Jothy 2001), there was no evidence to suggest that female bedbugs made any attempt to avoid males through active dispersal. This result supports the findings of Stutt & Siva-Jothy (2001) and Johnson (1942), who found no difference in natural sex ratios, indicating that females were not dispersing in significant numbers to avoid males.

Pfeister *et al.* (2009) looked at the propensity of bedbugs to aggregate in a 15 cm diameter Petri dish arena. They found that females tended to be found away from aggregations significantly more often than males or nymphs. They also found that females aggregate more with increased population density, and suggest that females were likely to be the founders of new harbourages and that they were likely to aggregate together to avoid male harassment. In two of the three replicate arenas I found evidence to support these suggestions as the distributions of males and females within the same

arena differed significantly, although it is also possible that the difference in distribution could be caused by males avoiding females.

There were some striking differences in the aggregation behaviour of the bedbugs in my study and that of the Pfeister *et al.* (2009) study. Firstly, the mean number of bedbugs per harbourage was 3.5±0.20 (n=9) in the equivalent 50:50 sex ratio treatment. By contrast the mean number of bedbugs per harbourage for the F4 strain in the arena setup was 29±10.81 (n=3)(see Chapter 3, Table 3.1). Pfeister *et al.* (2009) found that in the 50:50 sex ratio treatment 64.4±2.16% (n=9) of individuals were in aggregations, while the remaining individuals sat on their own. However, I found that in the arena solitary bedbugs were rarely if ever found.

There are a number of factors that could explain the observed differences between the results of Pfeister *et al.* (2009) and this study. Firstly, the number of bedbugs per arena was dramatically higher in my study (10-40 versus ~500). Pfeister *et al.* (2009) show that the number of individuals per aggregation and the proportion of individuals that aggregate both increase with population size in the arena (although they attribute the effect to population density rather than number of individuals).

Another factor that might explain some of the differences is that Pfeister *et al.* (2009) use the 'Harlan' bedbug strain, which has been in culture since 1973 (e.g. Polanco *et al.* 2011). Chapter 3 showed that bedbug populations can differ dramatically in their aggregation behaviour. This was particularly evident in our L1 stock, which has been in culture for approximately the same duration as the Harlan strain (see Chapter 1).

The arena design that Pfeister *et al.* (2009) used did not provide the bedbugs with any kind of harbourage structure for them to hide under or squeeze into. Instead they were forced to aggregate on the floor of an upturned Petri dish, which is quite an unnatural situation. For example, aggregations may have been more transient as the bedbugs may have moved frequently to find a suitable harbourage. Furthermore, in my arenas, and presumably in natural infestations, the confines of the crevice seem to prevent males from being able to copulate with females (pers. obs.), so once the female is in the harbourage, sexual harassment may be minimal. However, because the arenas Pfeister *et al.* (2009) use lack any confined crevices, females may have been exposed to unnaturally high levels of sexual harassment from males. This could potentially explain why Pfeister *et al.* found that >35% of their bedbugs were found away from aggregations.

Lastly, all my observations of bedbug distribution and harbourage usage were collected during the light phase of the daily cycle. Pfeister *et al.* (2009) covered their

arenas in a coloured filter gel so that the bedbugs behaved as if they were in darkness for the duration of the experiment. Bedbugs are photophobic (Usinger 1966) and therefore mainly move around at night. It is likely that if I had conducted my observations at night I would also have found a greater proportion of individuals away from the main aggregations.

The lack of evidence for females dispersing to avoid males suggests that the costs associated with dispersal are likely to be higher than the costs associated with unwanted male attention. This would not be surprising as dispersing bedbugs move slowly compared to other haematophagous insects, they are also at high risk of predation and have no guarantees of finding a new host. The cost of dispersal is likely to be even higher for males, since they require both a host and females to mate with, while a mated female would have the potential to found a new infestation as long as a new host was located. It is therefore surprising that males do actively disperse and even more surprising that they appear to disperse at approximately the same rate as females.

In order to better understand the nature of active dispersal, it will be necessary to examine the dispersing individuals to see how they compare in terms of age, feeding status and mating status. If active dispersal is an adaptive decision one would predict that freshly mated females should be more likely to disperse since they would be best able to establish a new infestation than a virgin.

5.4.3 Implications for control

The findings in this chapter have several important implications for control. Firstly, dispersal only occurs as a consequence of increasing population size and lack of space for harbourages in the vicinity of the host. This highlights the importance of early detection, particularly in environments where harbourage space is sparse. Furthermore, it challenges the use of mattress and bed frame encasements (Pinto *et al.* 2007) and cavity fillers (Cain & Strand 2009), which are designed to eliminate potential bedbug harbourages, as these could potentially accelerate dispersal.

Both the laboratory model and the field data suggest that the onset of dispersal ties in with the use of harbourages in the region of 2.5 metres from the host (268.7±8.41 cm (n=3) in the arena setup). It would therefore be prudent to consider any infestation with harbourages beyond 2 metres from the host a dispersal risk and take steps to screen neighbouring rooms/flats for signs of an infestation.

5.4.4 **Summary**

In this chapter I have:

1) Shown that increased availability of space for harbourages delays the onset of dispersal from the infestation.
2) Found no evidence that females disperse to avoid males.
3) Found some evidence to suggest that females may choose harbourages with reduced numbers of males, although the possibility that males may be avoiding females could not be ruled out.

6 Characteristics of Dispersers

6.1 Introduction

It is not known if a dispersal phase exists at some point in the lifecycle of the bedbug. This *is* know to occur in swallow bugs (*Oeciacus vicarius*), which annually transit into a dispersal phase, waiting around the entrance of the swallow nest for an opportunity to climb onto a swallow and disperse with it (Foster & Oikowski 1968, Loye 1985, Brown & Brown 2005). For bedbugs however, little is known even about which life stages actively disperse from established infestations. Chapter 5 revealed that both sexes disperse in roughly equal numbers. This, along with the case studies from Chapter 2, contest the commonly held belief that dispersal is primarily a female strategy to avoid sexual harassment, and copulatory wounding.

If a dispersal phase exists in bedbugs, analysis of the dispersed individuals could reveal traits that make it possible to predict those individuals within the population that are likely to disperse. If certain individuals are predisposed to dispersal, it does not necessarily mean that these represent a dispersal phase or that they are best suited to the task. Dispersal may be the only option for the older and/or less competitive individuals that, for example, are no longer able to compete for harbourages in close proximity to the host.

If no traits distinguish dispersing from the non-dispersing individuals, it is likely that no specific dispersal phase exists. If bedbugs do not have a dispersal phase, dispersal could instead be a consequence of having to occupy harbourages situated beyond the range that they are able to directionally detect the host. This hypothesis is supported by the observation from the field work (Chapter 2) and the arena dispersal studies (Chapter 5), i.e. that dispersal only occurs once bedbugs are occupying peripheral harbourages.

The “accidental dispersal” hypothesis predicts that dispersing individuals will be unfed and hungry. It also predicts that there will be a positive relationship between distance from the host and likelihood of dispersal, as the likelihood of being able to discern directionality from host cues will decline with distance from the host. Since

there is believed to be no difference in host detection ability between the two sexes (Suchy & Lewis 2011), the accidental dispersal hypothesis also predicts that males and females are equally likely to disperse (a prediction that is supported by the results of Chapter 5, which showed that equal proportions of males and females dispersed from the mixed sex arenas).

6.1.1 Chapter aims

In this chapter I will look for evidence for a dispersal phenotype in the bedbug by attempting to identify common characteristics that distinguish them from non-dispersers. I will compare dispersers and non-dispersers for variation in:

1) Feeding status.

2) Mating status (using the number of eggs laid without re-mating as a proxy).

3) Sexual harassment (females only - using copulatory wounding scars as a proxy).

4) Body size.

6.2 Methods

6.2.1 General experimental design

The data collection followed on from the experiment described in 5.2.2, in which ten mixed sex bedbugs were released into three replicate arenas (described in Chapter 3) daily for 55 days.

All dispersing bedbugs were removed from the dispersal traps daily, photographed dorsally and ventrally with a digital camera (Canon 1D Mk II N with MP-E 65 macro lens, 68 mm extension tubes and 2x teleconverter), and isolated in flat-bottomed 24-well tissue culture plates (SIGMA: Z707791). Dispersal traps containing bedbugs were replaced daily to prevent lingering aggregation pheromones from influencing dispersal.

At the termination of the experiment in 5.2.2, all harbourages were identified and the number of males and females in each harbourage was recorded. In order to be able to draw comparisons between the bugs that had dispersed and those that had remained in the arena, 10 males and 10 females were selected at random from three locations within each arena; the proximal harbourage (nearest the host), the midrange harbourage (half way along the distribution of harbourages), and the peripheral harbourage (furthest from the host). All selected individuals were photographed dorsally and ventrally, and then isolated in flat-bottomed 24-well tissue culture plates.

6.2.2 Feeding status of dispersers

Given that feeding status varies dramatically between individuals in harbourages adjacent to the host and individuals in the peripheries of the infestation, the feeding status of the dispersers gives an indication of where they have come from. For example, if most individuals have recently fed, this could indicate that dispersal in primarily occurring from the harbourages adjacent to the host. However, if dispersal is an a consequence of residing in harbourages beyond the range that the host can be detected one would expect all dispersing bedbugs to be unfed and from the periphery of the infestation.

The time since feeding was calculated for each disperser from the photograph taken on the day of dispersal. This was done using the abdomen length : pronotum width ratio as described in 5.2.2, which was modified from Reinhardt *et al.* (2010). Time since feeding was categorised into days from 1 to 5, and then 6 days or more.

6.2.3 Mating status of dispersers

The chances of successfully founding a new infestation are likely to be much greater for a recently mated female than a virgin one. This is because a mated female only needs to find a suitable host, while a virgin female must also find a male to mate with. Consequently, if females are selected to disperse, they would be expected to do so after mating.

As a proxy for the current mating status of the individual, female bedbugs can be isolated and fed (a necessity for egg laying, Usinger 1966). The total number of eggs the female lays without re-mating and the number of weeks over which the female is able to lay fertile eggs is a good indicator of her mating status and therefore ability to found a new infestation.

All dispersed females and all females selected from the arena were fed weekly to facilitate egg laying. The eggs were removed at weekly intervals and counted. Females were discarded when they did not produce any eggs for two consecutive weeks.

6.2.4 Sexual harassment status of dispersers

Traumatic insemination produces visible melanised mating scars between the sternites in the region of the ectospermalege (Usinger 1966). Since mating scars remain visible indefinitely, this was used as a proxy for sexual harassment. If the decision to disperse is driven by sexual harassment, one might predict a difference in the number of mating scars compared to non-dispersers.

The number of mating scars might also be expected to correlate with mating status, however Stutt & Siva-Jothy (2001) have shown that natural mating rates are far above what is required for a female to remain fully fertile. Therefore, if males show a preference for particular female phenotypes, there could be large variation in copulatory wounding scars with little variation in the number of fertile eggs the female is able to lay before re-mating. Wounding scars might therefore be a better predictor of dispersal than mating status if dispersal was driven by sexual harassment.

Once all isolated females had stopped laying fertile eggs for two consecutive weeks (see 6.2.3), they were starved to death. This process causes the gut and fat stores to shrink out of the way so that light can be shone through the cuticle from underneath to visualise the dark, melanised copulatory wounding scars. The area of the abdomen containing the spermalege and associated scarring was visualised on a compound microscope (Leitz Diaplan, Wild Leitz GmbH, Germany).

It is not usually possible to discern individual scars in multiply-mated females, as the scarring quickly developed into a large melanised mass. Therefore, the amount of scarring was categorised subjectively into three classes: 1) little or no scarring; 2) moderate scarring; 3) considerable scarring.

6.2.5 Body size of dispersers

Chapter 5 revealed that many individuals within the infestation do not feed, despite being hungry. One explanation for this is competitive exclusion. Body size is a potential factor influencing competitive ability. If larger individuals are able to displace smaller ones, a negative relationship between distance from the host and body size could be expected.

Pronotum width was already used as a proxy for body size in 6.2.2 as the pronotum width does not change in size when the bedbug feeds (Reinhardt *et al.* 2010). The pronotum widths of males and females that had and had not attempted to disperse was therefore compared to see if body size influences dispersal.

6.2.6 Statistical analysis

The Normal Probability Plot in the statistical package Minitab (version 16.0) was used to check for normality in both measures of mating status (egg number and laying duration). Since neither data set was normally distributed, the nonparametric Kruskal-Wallis test used to see if significant differences exist in the median number of eggs laid or the duration of egg laying for females collected from different areas of the arenas including those that had dispersed.

Where the Kruskal-Wallis test revealed significant effects of location on mating status, a Kruskal-Wallis Multiple Comparison test (using macro: KrusMC.MAC for Minitab 16.0 by Steve Orlich, Minitab Inc.) was used to determine where significant differences lay.

A Chi-square contingency table was used to test the null hypothesis that the proportion of females with each class of copulatory wounding was the same across all harbourages as well as for the dispersers. Chi-square values were calculated in Microsoft Excel 2008 for Mac.

The Normal Probability Plot in the statistical package Minitab (version 16.0) was used to check for normality in male and female body sizes. Bartlett's test was used to test for equal variances in the body sizes of the males and females isolated from different areas of the arena. ANOVA was used to test for significant differences in the

mean body size of male and female bedbugs isolated from different areas of the arenas including those that had dispersed.

6.3 Results

6.3.1 Feeding status of dispersers

A total of 146 bedbugs dispersed from the three replicate arenas (44, 47 and 55 respectively), none of which had fed in at least 6 days. Given that 60-80% of individuals adjacent to the host had fed in the past 5 days (see Figure 4.7), this result suggests that the dispersers are not dispersing directly from the harbourages adjacent to the host, and supports the hypothesis that dispersal occurs from the peripheral end of the arena.

6.3.2 Mating status of dispersers

Non-dispersing females were isolated from the proximal, midrange and peripheral harbourages (relative to the artificial host), for comparison of their mating status with that of the dispersers. There was a significant overall effect of location on the mating status of females, both in terms of total egg production without re-mating (Kruskal-Wallis, H_3=66.21, $p<0.001$, Figure 6.1a) and egg laying duration (Kruskal-Wallis, H_3=59.44, $p<0.001$, Figure 6.1b).

As expected (from the feeding status data in Chapter 4) mating status was highest in the harbourages closest to the host (Figure 6.1). The mating status of the dispersers was lowest and did not differ significantly from that of individuals collected from the midrange harbourages, but was significantly lower than that of individuals collected from the peripheral harbourages (Kruskal-Wallis Multiple Comparison test, see Figure 6.1a & b). Therefore, the dispersing females are among the least suited to the task, both in terms of the number of offspring they can produce, and the duration over which they can lay fertile eggs without re-mating. These results do not support the dispersal phase hypothesis, but do support the accidental dispersal hypothesis.

Figure 6.2 shows the proportion of females from the harbourages adjacent to the host in each mating status class (measured as weeks of fertile egg production), overlaid onto a series expected distributions based on fixed weekly mating probabilities. This figure suggests that the weekly probability of being mated is around 20-40%.

6.3.3 Sexual harassment status of dispersers

There was no significant effect of the location from which females were isolated on the level of copulatory wound scarring (χ^2 (Contingency Tables)=10.2, df=6,

p=0.115, Table 6.1). Since mating status is highest in the harbourages nearest the host (6.3.2), this result suggests that females may move away from the proximal harbourages, towards the peripheries of the infestation. Otherwise, a lower harassment status would be expected in the harbourages that midrange and peripheral harbourages where mating status was lowest.

6.3.4 **Body size of dispersers**

There was no significant effect of harbourage location on body size, for either males (ANOVA, $F_{3,154}$=0.79, p=0.499, Figure 6.3) or females (ANOVA, $F_{3,164}$=1.84, p=0.142, Figure 6.3), suggesting that body size is neither related to harbourage occupancy or dispersal.

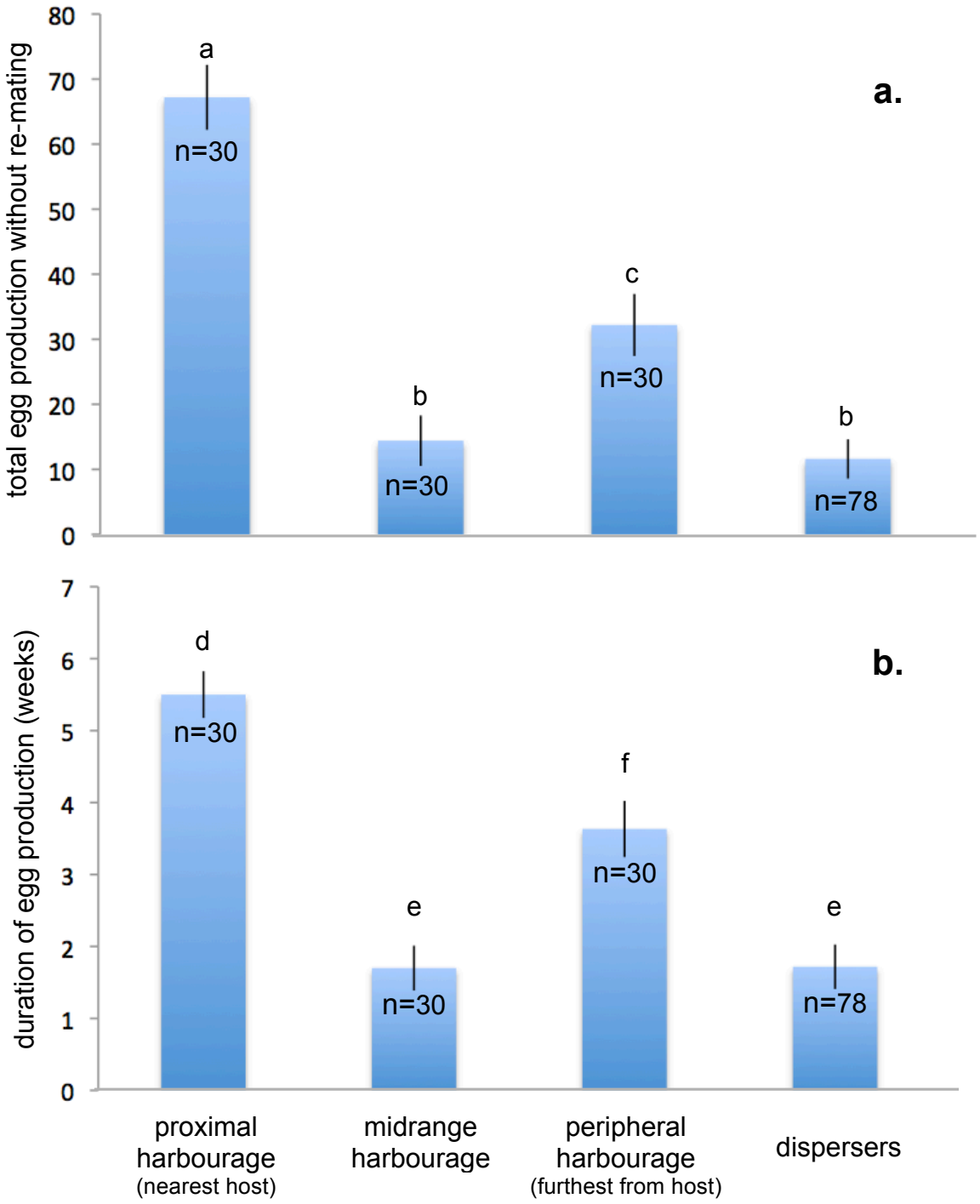


Figure 6.1 shows; a) the significant difference in total egg production from weekly-fed females isolated from different areas of the arena (Kruskal-Wallis, H_3=59.44, $p<0.001$), and b) the significant difference in the number of weeks over which those isolated females laid fertile eggs (Kruskal-Wallis, H_3=66.21, $p<0.001$). Bars with the same letter do not differ at $p<0.05$ (Kruskal-Wallis Multiple Comparison test). Error bars represent 1 standard error.

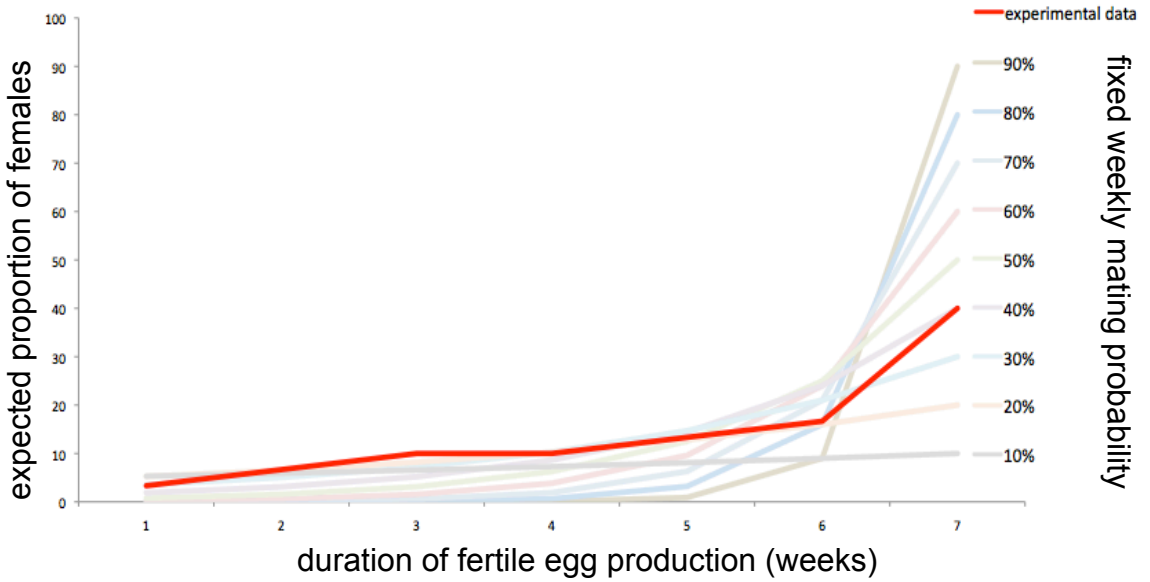


Figure 6.2 shows the expected proportion of females of each mating status class (measured as duration of fertile egg production) based on a series of fixed weekly mating probabilities. The red line shows the actual experimental data for females in harbourages adjacent to the host, suggesting that the actual weekly mating probability is approximately 20-40%.

	copulatory wound scarring level					
location	little or no scarring		moderate scarring		considerable scarring	
proximal harbourage	6	20%	9	30%	15	50%
midrange harbourage	13	43.3%	8	26.7%	9	30%
peripheral harbourage	8	26.7%	13	43.3%	9	30%
dispersed	33	42.4%	26	33.3%	19	24.4%

Table 6.1 is a contingency table showing the frequency and severity of copulatory scarring for each location in the arena. Percentages of location (row) totals are presented for each frequency. There was no significant effect of location on the level of copulatory wounding of female bedbugs (χ^2=10.2, df=6, p=0.115).

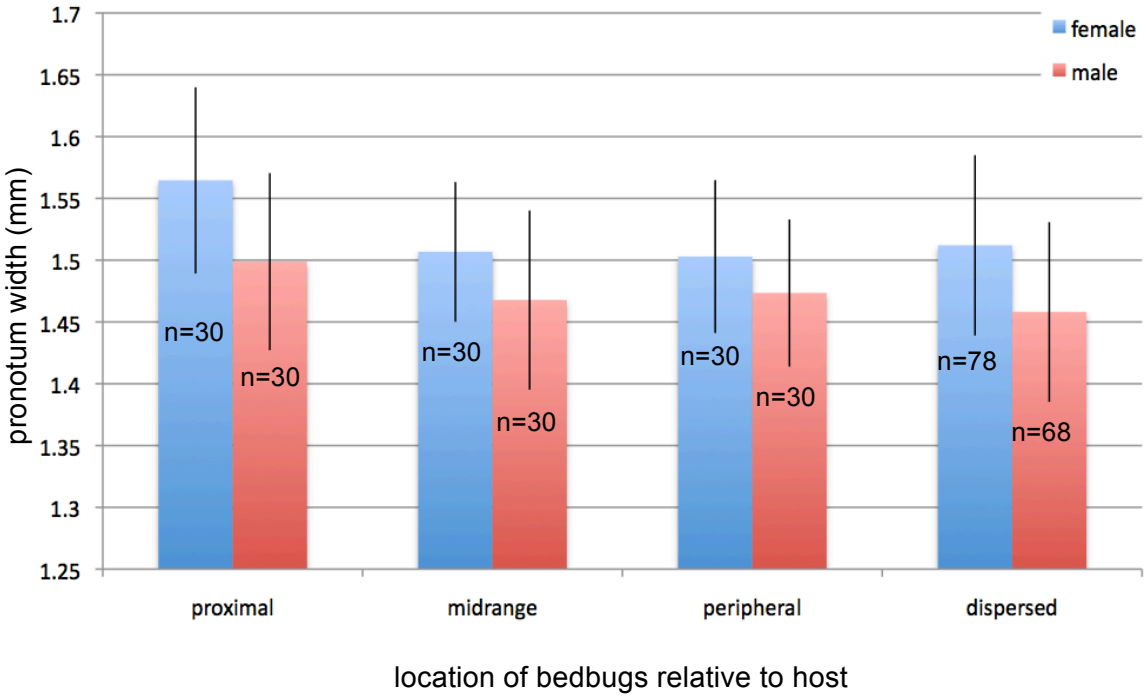


Figure 6.3 shows the body size (pronotum width) of bedbugs isolated from three locations in the arena including those that dispersed. There was no effect of location on the body size of the bedbugs within (sexes analysed separately, males: ANOVA, $F_{3,154}$=0.79, p=0.499, females: ANOVA, $F_{3,164}$=1.84, p=0.142). Error bars represent 1 standard error.

6.4 Discussion

None of the four characteristics I examined (feeding status, mating status, harassment status and body size) varied between dispersers and non-dispersers. Dispersers *were* characterised by their low feeding and mating status, but this is likely to indicate where in the arena they dispersed from, rather than indicating a phenotype adapted for dispersal *per se*.

6.4.1 Variation in mating status

As expected, mating status was highest in the harbourages nearest the host (Figure 6.1). However, female bedbugs isolated from the midrange harbourages had a significantly lower mating status than those in the peripheries, suggesting that bedbugs displaced from the harbourages adjacent to the host tend to travel out to the peripheries of the infestation, before finding somewhere to settle.

The mating status of the dispersers is similar to that of the bedbugs in the midrange harbourages. This could either indicate that dispersal is primarily occurring from the midrange harbourages, or that the dispersers are a subset of the bedbugs from the peripheral harbourages that have a lower than average mating status. A colour marking experiment similar to 4.2.2 should be carried out to establish where in the infestation bedbugs are dispersing from.

Stutt & Siva-Jothy (2001) observed that under an equal sex ratio, female bedbugs are mated 5±3.16 times (n=20) after each blood meal. Since this is approximately twenty times higher than is required to remain fully fertile (Stutt & Siva-Jothy 2001), one would expect that virtually all of the bedbugs collected from the harbourages adjacent to the host should be able to lay fertile eggs for approximately seven weeks. However in this experiment only 12 of the 30 isolated females laid fertile eggs for 7 weeks. Figure 6.2 shows that the observed proportion of females of each mating status is in line with that which would be expected by a fixed weekly mating probability of 20-40%. This is very much lower than Stutt & Siva-Jothy's (2001) estimate.

Stutt & Siva-Jothy (2001) established their estimate of weekly mating rate by placing five satiated bedbugs of each sex in a 5 cm diameter Petri-dish and observing it for 3 days with a digital video camera. In this setup it was neither possible for females to move away or squeeze into a harbourage (as no harbourage was provided), which may have resulted in an unnaturally high copulation rate. Stutt & Siva-Jothy's (2001)

estimate of five copulations per blood meal could therefore be a better indication of male mating propensity than natural mating rates, as their setup did not take into account the bedbug's ecology.

Reinhardt *et al.* (2011) looked at the constraints of seminal fluid availability on male mating rates and noted that Stutt & Siva-Jothy's estimate of mating rate may be overly high as well, as it does not take into account male recovery time. Reinhardt *et al.* (2011) suggest that 6.5 matings per 17 days may be a more realistic estimate of male mating rate, which equates to approximately 2.7 matings per week; only slightly more than half the previous estimate, but still much higher than the apparent mating frequency calculated from the mating status data in 6.2.

6.4.2 Variation in harassment status

There was no difference in the number of copulatory wounding scars between females isolated from different regions of the arena (including the dispersers). This finding was surprising, given that only bedbugs in close proximity to the host feed regularly (Chapter 4) and that copulation tends to be associated with feeding (Reinhardt *et al.* 2009). One explanation is that sexual harassment, linked to feeding, in the harbourages closest to the host, drives females towards the peripheries.

6.4.3 Variation in body size

Body size had no effect on the distribution of males or females (6.3.4) suggesting that if competitive exclusion is responsible for pushing certain individuals out into the peripheries of the infestation, then body size is probably not related to competitive ability. An alternative explanation is that the resident always wins regardless of competitive ability (see Maynard-Smith & Parker 1976, Davies 1977 and Krebs 1982). This situation is normally associated with territory disputes where the cost of elevated fighting is high and the benefit of winning is low, or where the resident has more to gain than the intruder through better knowledge of the territory. However, bedbugs may simply be unable to force a resident out of an occupied harbourage. The harbourage residents would therefore be defined solely by the order in which they arrived, and would only be subject to change when a resident left the harbourage to forage or, potentially in the case of males, look for mates. In this situation one would predict that new harbourages would primarily be founded by those individuals that had most recently arrived in the arena/infestation, which is supported by the observed pattern of mating status (Figure 6.1).

6.4.4 **Summary**

In this chapter I have shown that:

1) None of the dispersers were recently fed, suggesting that they are unlikely to have dispersed from the harbourages nearest the host.
2) The mating status of the dispersers was very low compared to the proximal and peripheral harbourages, indicating that they are not the individuals within the population that would be best able to disperse and found new infestations.
3) Neither body size or sexual harassment status had any effect on dispersal or location in the arena.

7 General Discussion

7.1 Introduction

Bedbug research over the past decade has focussed primarily on control, with relatively little attention given to understanding the basic ecology and behaviour of this ubiquitous pest. However, without an understanding of the fundamental aspects of this insect's biology, control strategies will be poorly informed and thus more likely to fail. The aim of this thesis was to develop a laboratory model system and use to study bedbug ecology in a controlled environment, so as to unravel questions associated with bedbug ecology, focusing particularly on their dispersal.

7.2 Thesis Overview

In Chapter 2 I presented a number of case studies and used them to characterise key parameters in a typical bedbug infestation.

In Chapter 3 I used my observations and field data from Chapter 2 as the basis for developing the laboratory arena. This also necessitated the development of an artificial host. Trials with the laboratory arena using a variety of different stock cultures revealed that bedbugs from different stocks vary in the way they utilise harbourage space. Most notably, my long term laboratory culture, stock L1, appeared to have lost its tendency to aggregate. Based on these findings, field stock F4 was selected as the most appropriate for use in the subsequent research.

In Chapter 4 I conducted experiments to determine how bedbugs utilised harbourage space. These results of these experiments revealed that: i) bedbugs occupied harbourages closest to the host first, and spread into more peripheral harbourages as the population increased; ii) bedbugs produced a patchy distribution of harbourages in continuous space; iii) there appeared to be some level of harbourage fidelity although it is likely that this was driven by bedbugs in the peripheries not leaving the harbourages,

rather than individuals feeding and then returning to the same harbourage; and iv) average feeding status declined dramatically with distance from the host.

In Chapter 5 I conducted experiments the established which factors influence dispersal. The results of these experiments revealed that: i) increasing the availability of harbourage space in the vicinity of the host delayed dispersal; ii) contrary to popular belief females did not disperse to avoid males; iii) there was some variation in the distribution of males and females between harbourages suggesting that some females avoided harbourages with too many males or vice-versa.

In Chapter 6 I compared bedbugs that had dispersed from the arenas with those that had not in order to characterise dispersers and potentially identify a dispersal phase. I found no evidence for a dispersal phase but dispersers were characterised by their low feeding and mating status. Copulatory wounding scars were used as a metric for the level of sexual harassment females had received. There was no measurable difference in the scarring between dispersed and un-dispersed individuals, providing further evidence that females do not appear to disperse as a result of sexual harassment. Body size was not a predictor of dispersal. Recording the number of eggs a female is able to produce with regular feeding in isolation revealed that the mating rate is highest adjacent to the host and lowest in the middle of the distribution of harbourages, with mating rates in the peripheral harbourages falling between the two. This could suggest that bugs pushed out of the harbourages adjacent to the host tend to move out to the peripheral harbourages.

7.3 Results in the Context of Dispersal Theory

Chapter 5 revealed that harbourage availability (in the vicinity of the host) was by far the most important factor influencing the onset of dispersal. Population density in the harbourages therefore seems to be the main driving force for dispersal. Density-dependent dispersal is predicted by many theoretical models (see Poethke & Hovestadt 2002, Amarasekare 2004a/b, Poethke *et al.* 2007, Strevens & Bonsall 2011, Nowicki & Vrabec 2011 and references therein). But empirical evidence is sparse and inconsistent (see review in Lambian *et al.* 2001, Matthysen 2005, Nowicki & Vrabec 2011 and references therein).

The research presented herein develops a new laboratory system for studying dispersal, which allows careful manipulation of population size and structure, as well as environmental factors such as food and harbourage resource availability. In natural

infestations it is likely that kin show a high degree of relatedness as a result of multiple bottle-necks in the population of just one or a few individuals at each colonising event of the population's history. However, in the arena setup it is also possible to manipulate the genetic diversity of the population and examine its effects on dispersal.

Few studies have attempted to document dispersal in species where competition is localised and among kin (Lambin *et al.* 2001), although some examples have been found between birds, where one sibling forces the dispersal of the other (see Lambin *et al.* 2001 for review). The empirical data collected from the bedbug laboratory model system is therefore valuable for the validation of theoretical models of dispersal.

Hamilton and May (1977) present simple mathematical models that demonstrate that even in temporally stable, patchy environments, avoidance of kin competition can favour parents who enforce dispersal of a large proportion of their offspring, even if the potential cost of dispersing is high. The assumptions of the models are fairly specific, requiring spatially structured populations in stable environments, however these assumptions are met perfectly by the bedbug system. Although aggression between bedbugs has never been observed (pers. obs. over >10 years), evidence from the mating status data collected in Chapter 6, suggests that new arrivals to the infestation (potentially including new offspring) tend to be displaced to the peripheries of the infestation. While this needs to be examined further, it supports the idea that the parents are responsible for the dispersal of their nymphs.

Gandon (1999) developed a model incorporating: i) the cost of dispersal ($0 \leq c \leq 1$); ii) the coefficient of relatedness ($0 \leq R \leq 1$); and iii) the cost of inbreeding ($0 \leq \delta \leq 1$). Gandon (1999) used the model to clarify the importance of these three factors in the evolution of dispersal, generating predictions about the evolutionarily stable (ES) dispersal rate (d^*) in different situations.

For the special case where $\delta = 0$, the ES dispersal rate is described by the simplified equation:

$$d^* = (R - c)/(R - c^2) \text{ when } R > c$$

$$\text{and } d^* = 0 \text{ when } R \leq c.$$

(Gandon 1999)

Unpublished data by Otti & Fountain, suggests that the cost of inbreeding between siblings of inbred lines of bedbugs is minimal, and only detectable under severe starvation stress, making Gandon's simplified equation (above) appropriate to this system.

Fountain (unpublished data) also reveals that within an infestation the coefficient of relatedness is extremely high (approaching 1), as could be expected given that infestations are probably founded by one or few individuals, resulting in extreme bottle-necks every time a new population is founded.

No study has attempted to quantify the cost of dispersal in bedbugs. Given that dispersal occurs is the absence of alternative host cues (see previous chapters), it is likely that in some situations a dispersing bedbug may take considerable time to find an alternative host. Dispersing bedbugs are also likely to be exposed to increased risk of predation (Reinhardt & Siva-Jothy 2007) and desiccation (Benoit *et al.* 2007). In the case of nymphs and males, dispersers also need to find conspecifics to mate with for there to be any benefit of the dispersal decision. It is therefore likely that the cost of dispersal is high, although given the extremely high levels of relatedness, it is likely that R is still greater than c, and therefore Gandon's model predicts that dispersal will always occur.

Gandon's (1999) model makes no attempt to incorporate ecological factors, which are now known to be important in driving bedbug dispersal (Chapter 5). However it still manages to provide a compelling explanation for how dispersal can be adaptive in the bedbug system: even in a situation where food is apparently unlimited and the cost of dispersing is potentially very high, dispersal will remain adaptive as long as the coefficient of relatedness is sufficiently high. Dispersal in highly related populations can therefore be viewed as a form of altruism to avoid competition between relatives (Gandon 1999) and the cost of dispersing is therefore offset by kin selection. Kin selection also provides an alternative explanation (to "resident always wins", see Chapter 4) for the lack of aggression and overt competitive behaviour between individuals for harbourages adjacent to the host.

7.4 Results in the Context of Control Strategies

The bedbug resurgence (Boase 2001, Doggett et al. 2004, Kilpinen 2008 and others) combined with (and probably resulting from (Romero *et al.* 2007)) almost universal resistance to the most common classes of insecticides (Doggett *et al.* 2004, Potter 2005, Romero *et al.* 2007) has necessitated the development of non-chemical control products and strategies. These include passive monitoring devices, which provide bedbugs with a suitable harbourage in the vicinity of the host that can easily be checked and removed if bedbugs are found (Pinto et al. 2007, Cain & Strand 2009), and active monitors based on a similar principal but utilising attractants designed to mimic either the host or the harbourage (Pinto et al. 2007, Cain & Strand 2009). Other non-chemical control strategies include mattress encasements designed to eliminate many of the cracks and crevices, simplifying the treatment process, and interception/isolation devices designed to prevent bedbugs from being able to climb onto the bed (Pinto et al. 2007). All these products attempt to exploit the bedbug's natural behaviour and ecology, with varying success. Robust empirical studies of bedbug ecology and behaviour are therefore critical to the development of successful control products, as well as for the evaluation of existing products on the market.

7.4.1 Active and passive monitors

The fundamental principal of any pest monitoring device is to facilitate early detection and thus simplify the treatment process. The success of monitors that mimic harbourages is therefore down to their ability to provide a more suitable and attractive harbourage than any of the other potentially numerous harbourages present in the room, such that the monitor is occupied while the infestation is still extremely small. Monitoring devices that only start to catch bedbugs once the infestation has developed and begun to disperse are of little value. The results from Chapter 3 show that in small infestations harbourages are closely associated with the host. It is therefore essential that harbourage mimicking monitors are placed as close to the host as is feasible. Harbourage-mimicking monitors placed under the bed (as is often the case) are only likely to catch bedbugs once the harbourages on and around the bed frame and mattress are saturated and bedbugs are pushed out towards the peripheries of the infestation.

Chapter 4 revealed that bedbugs in harbourages closest to the host feed with a much higher frequency than those in the peripheries. Since a well placed harbourage-

mimicking monitor would be situated close to the host, it is essential that it is checked regularly as the monitor could contribute to an increased population growth rate.

7.4.2 Bed isolation / interception devices

A variety of products exist to try and prevent bedbugs from gaining access to the bed. These tend to either be pitfall traps around the legs of the bed or sticky tapes that bedbugs can't cross and/or get stuck to. As long as no alternative route exists by which bedbugs can gain access to the bed, then these devices have the potential to be successful as a preventative measure. If the devices are put in place during an infestation, they also have the potential to reduce the spread of bedbugs into harbourages away from the bed and potentially therefore reduce active dispersal. The success of these devices is dependent on their ability to prevent bedbugs crossing as well as the elimination of any alternative route (for example, up the wall).

7.4.3 Mattress and bed frame encasements

Eliminating harbourages around the mattress and bed frame with encasements is often recommended by pest control operators to simplify the treatment of an infestation (Pinto *et al.* 2007). However, the results from Chapter 5 show that with fewer harbourages available bedbugs spread out into the peripheries of the infestation and ultimately disperse more rapidly. This problem may be appeased by the use of harbourage-mimicking monitors in conjunction with the encasements. The monitor should provide a suitable harbourage, allowing the infestation to remain in the vicinity of the host, as well as delaying dispersal until the room can be treated. The encasements are also likely to amplify the success of the monitor by reducing alternative options available to the bedbugs.

7.5 Conclusions and Future Direction

The work described herein is of fundamental importance to the design of effective bedbug control strategies. However, the results generated from the laboratory model have an application that extends well beyond understanding bedbug ecology. Numerous mathematical models attempt to explain dispersal under various situations, but empirical studies to validate these models are scarce and controlled laboratory model systems of dispersal are scarcer still. There is consequently considerable potential for further work to be done on this system, from both applied and theoretical perspectives.

7.5.1 Nymphal dispersal

No attempt was made to quantify nymphal dispersal, although from Case Study 4 (Chapter 2) as well as the study by Wang *et al.* (2011), nymphs are known to disperse in natural infestations. Availability of harbourage space is now known to be an important factor in bedbug dispersal (Chapter 5), and because nymphs are smaller than adults, it is likely that a harbourage can accommodate more nymphs than adults. It is therefore likely that nymphal dispersal would be delayed compared to adult dispersal simply due to the relative difference in body size and comparatively larger amount of harbourage space available. This is supported by the observations from Case Study 4 (see Figure 2.11, page 52), which showed that of the nine nymphs that dispersed only one was in its third instar and the remainder were all either in their fourth or fifth instars. Wang *et al.* (2010) also found that nymphs were nine times less likely to disperse than adults when taking population structure into account, although they did not attempt to distinguish instars. It would be relatively straightforward to use the arena setup to ascertain the population size at the onset of dispersal for each instar. This might also be predictable from the body size measurements, which would provide further evidence of the importance of harbourage availability on dispersal.

7.5.2 Assessing variation in competitive ability

Size was shown not to be important in individual distribution between harbourages or dispersal, suggesting that competitive ability may not be a factor in bedbug ecology. However, variation in their ability to acquire and hold harbourages close to the host can not be ruled out without further investigation. Age, for example, may be a factor in competitive ability. This was not tested due to the time restrictions of

producing cohorts of "old" bedbugs, which would take six to seven months. One way to test directly for the presence of variation in competitive ability would be to move the host to the opposite end of the arena and see if the bedbugs "re-sort" themselves accordingly. Alternatively one could transplant harbourages from near the host to the peripheries and vice-versa and see if the bedbugs within those harbourages return to their original positions. As the arenas are lined with a removable wall-paper lining paper, it would be relatively straight forward to carefully cut around a harbourage with a scalpel and relocate it with minimal disturbance to the occupants.

As discussed in Chapter 5, one alternative to the competitive exclusion hypothesis is that the resident always wins. Assuming bedbugs always seek to occupy harbourages closest to the host first, as shown in Chapter 3, and newly arriving bedbugs never displace harbourage residents, then the distribution of bedbugs within the arena, should precisely reflect the order in which they were introduced. This can easily be tested within the arena setup by introducing cohorts of colour-marked bedbugs and determining where in the arena they settle.

7.5.3 Origin of dispersers

As discussed in Chapter 6, it is not known from where in the infestation the dispersers are dispersing from. This could be explored relatively easily with a colour marking experiment similar to that used in Chapter 4. If dispersal is primarily occurring from the harbourages furthest from the host it would support the hypothesis that dispersal is a consequence of foraging beyond the range that the host can be detected.

7.5.4 Influence of ambient humidity on harbourage size

Aggregations are predicted to increase in size until the costs associated with aggregating outweigh the benefits (Wertheim *et al.* 2005, Pfiester *et al.* 2009). This is apparent in the way bedbug harbourages are formed, ultimately producing a patchy distribution of harbourages in a continuous environment (Figure 3.2). However, the underlying mechanism responsible for producing this patchy distribution has not yet been determined. Benoit *et al.* (2007) showed that bedbugs in aggregations benefit from resistance to dehydration, presumably due to elevated humidity within harbourages containing multiple individuals. Elevated humidity could also provide the mechanism for limiting the maximum size of a harbourage, since high humidity is often associated with bacterial and fungal growth (Kemper 1936). It would be possible to test the effect of humidity on harbourage size and distribution simply by varying the ambient humidity

of the insectary in which the arenas are housed and monitoring harbourage formation with increasing population size. If high humidity limits harbourage size then a negative correlation between humidity and harbourage size could be predicted.

7.5.5 Future direction for control

One of the most striking findings of Chapters 2 and 3 was the spatial predictability of bedbug harbourages. Novel approaches to exploiting this aspect of their ecology should be explored further. Beds could be designed so that the only available harbourages are easily accessible or even removable. In the hotel industry, where the time allocated to processing rooms between guests is very limited, a bed design that facilitates rapid screening for bedbugs could be a considerable advantage in limiting their spread.

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Appendix 1: Developing the artificial host

Introduction

Artificial hosts for haematophagous insects are comprised of a membrane (through which the insect can feed) and a source of vertebrate blood (which must usually be treated with an anticoagulant to prevent clotting). Many insects detect temperature as a host cue, so it is necessary for the artificial host to incorporate a system for keeping the blood at a constant temperature in the region of 30-40˚C.

Although blood feeding systems for haematophagous insects are commercially available, standard designs were found to be unsuitable for feeding foraging bedbugs (i.e. bedbugs freely moving around in an arena setup). This is because bedbugs are often reluctant to climb onto the artificial host to feed and they are unable to reach the feeding membrane from the floor of the arena. It was therefore necessary to design an artificial host specifically suited to foraging bedbugs.

Artificial host design

For bedbugs to be able to feed through the membrane without climbing onto the artificial host, the membrane had to be mounted vertically (unlike commercially available designs) and rest on or close to the floor of the arena, so that the bedbugs could reach it. Since competition for space to feed on a host is likely to be minimal in natural infestation, a relatively large surface area was required to minimise competition around the artificial host. However, since the arena is only 0.15 m wide the maximum dimensions of the artificial host are limited by the arena with. For these reasons a circular structure with a vertical membrane around the outside and an overall diameter of 0.12 m was devised. This allowed sufficient room for the bedbugs to walk around the feeder and feed from any point.

The artificial hosts were constructed out of three CDs, as these were found to be a good source of plastic discs of the required dimensions. The radius of one of the CDs

was reduced by 2 mm using a bench sander and jig. The CDs were then assembled in a sandwich, with the smaller disc in the middle, and held together with silicone sealant (Figure A1.1b). The membrane was then wrapped around the circumference of the discs, and the void left by reducing the radius of the middle disc, was filled with blood by injecting through the membrane with a hypodermic needle. This produced a very low profile artificial host design with a feeding membrane easily accessible to bedbugs standing on the floor or the arena (See Figure A1.1c). To keep the artificial host at a constant, elevated temperature, the whole structure was placed on a 7 watt aquarium heat-mat (HabiStat^{TM}: HHM006), which was found to maintain the temperature at approximately 36˚C.

Bedbugs are approximately 3 mm wide, and the artificial host has a total circumference of almost 0.38 m, so there should be sufficient room for approximately 125 adult bedbugs to feed simultaneously. One case study by Reinhardt *et al.* (2010), showed that bedbugs kept at 26˚C fed approximately once every 2.5 days. It is therefore reasonable to assume that an artificial host of this design should be sufficient to support a laboratory-based infestation of bedbugs in the region of 312 individuals without feeding rate being restricted by access to the host. The volume of blood in one artificial host is approximately 1.5 ml. Adult bedbugs consume an average of 11.02 ul of blood (based on collective weight gain of 20 mixed sex adult bedbugs from the F4 strain, and an average human blood density of 1060 mg/m^3 (Cutnell & Johnson 1998)). Therefore one artificial host should contain enough blood for approximately 136 bedbugs. Based on a mean feeding interval of 2.5 days (Reinhardt *et al.* 2010), this should sustain a population of 340 individuals. To ensure that there is never competition for access to the host or availability of blood, an additional artificial host will be added (stacked on top) for every 250 bedbugs in the arena.

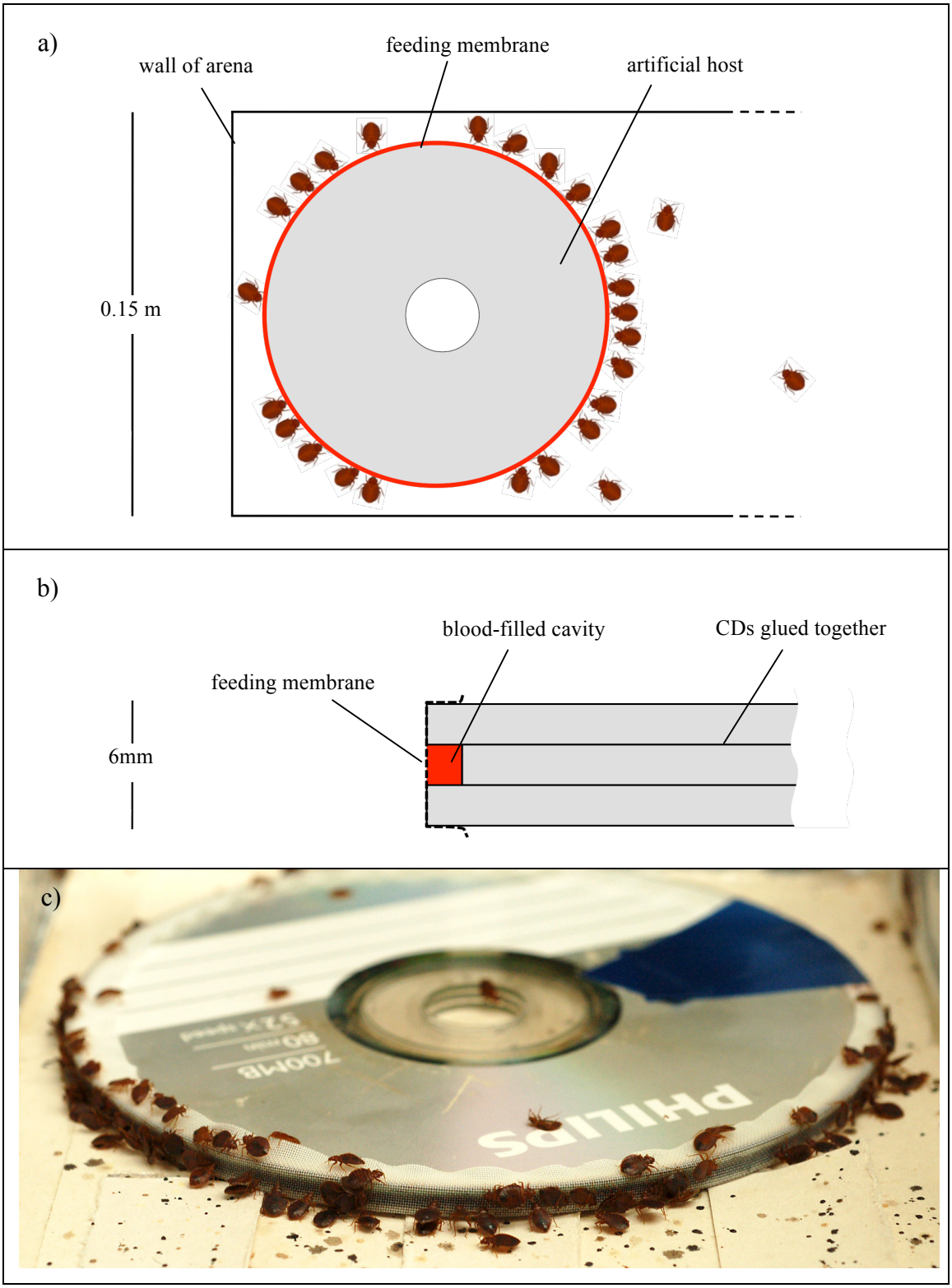

Figure A1.1 shows the design and function of the artificial host. a) shows the size and location of the artificial host relative to the end of the arena; b) shows a cross-section of part of the artificial host revealing the blood-filled cavity; c) shows a photograph of the artificial host in use, surrounded by feeding bedbugs.

Blood

Previous studies have found that the most suitable blood for the artificial membrane feeding of bedbugs to be heparinised sheep blood (Montes *et al.* 2002). This was obtained from TCS Biosciences (TCS: SB 075). It was found that this blood separates into distinct layers of plasma (on top) and cellular material (below) within a few hours if left to stand. This is not usually a problem for most blood feeding setups, where the insects are generally given limited time to feed. However, the artificial hosts in the arenas need to be available to foraging bedbugs for approximately 8 h per night in order to replicate natural conditions as closely as possible. Blood separation is therefore a potential problem, as bugs would be unlikely to receive all blood components in a single feed.

By freezing the blood at -80^{o}C until completely solid, it was found that the cellular material could be lysed, which prevented it from separating out even after several weeks without being agitated (pers. obs.). Some early trials with adults from the F4 strain showed good feeding success when offered the frozen/thawed blood (34/40 fed to repletion within 2 hours). However, more than half of the individuals that fed had died by the following day, possibly as a result of bacterial infection of the blood.

Plating out the blood on blood-agar (SIGMA: 70133) revealed that the blood did contain bacterial contaminants. It was consequently necessary to sterilise it before use. This was achieved by first centrifuging the blood at 20000 rcf for 40 mins at 4˚C in a refrigerated centrifuge (Eppendorf: S417R), to remove the lysed cellular material. After discarding the cellular material in the pellet, the blood was pasteurised for 1 hour at 60^{o}C. The resulting product had the same consistency as the original untreated blood, although it was slightly brown in colour. Plating out the heat-treated blood confirmed that the contaminant had been removed.

Membrane

Parafilm® 'M' laboratory film is often used as a membrane for artificial host setups (Montes *et al.* 2002, Romero *et al.* 2007, 2009, Benoit *et al.* 2009). However, in most cases the membrane can only be used for up to an hour and must then replaced. If Parafilm® is used for prolonged periods it tends to lose its integrity and begins to let blood seep through. In preliminary arena trials using Parafilm® it was found that the blood had drained out into the arena over the course of the night in 4 out of 9 replicates. It was therefore necessary to explore alternative feeding membranes.

Table A1.1 summarises the membranes tested and the proportions of adults and 5th instar nymphs that successfully fed to repletion in an arena setup within 8 hrs. Adults and nymphs were starved for 2 weeks prior to feeding to encourage foraging. The blood offered, and artificial hosts design used, were as described above.

By far the most successful membrane tested was the Sylgard® silicone elastomer (Dow Corning: Kit 184) spread over a fine nylon mesh (1089 holes per cm^2). Although the trial was run for 8 hours, more than half of the bugs offered blood through this membrane had fed to repletion within the first 20 minutes. Furthermore, Sylgard® is resistant to high temperatures as well as many chemicals including ethanol, making it very easy to sterilise between uses. Daily use of the same Sylgard®/mesh membranes over > 6 months reveals that these membranes have a considerable lifespan compared to any of currently used alternatives (see Hunt & Kinnon 1990).

Table A1.1 shows the proportion of bedbugs that fed to repletion within 8 hours for each membrane type tested. * indicates membranes that allowed blood to leak out during the trial.

membrane	manufacture	proportion that fed to repletion	
		5th instar nymphs	adults
TCP™ Spray-On Skin/nylon mesh	fine nylon mesh laid onto Parafilm, Spray-On Skin applied, Parafilm removed when dry	0/40 *	0/40 *
silicone sealant	silicone sealant spread thinly over Parafilm, Parafilm removed when dry (24 hrs)	3/40	4/40
silicone sealant/fine nylon mesh	fine nylon mesh laid onto Parafilm, silicone sealant applied thinly with plastic spreader, Parafilm removed when dry (24 hrs)	2/40	1/40
Sylgard® silicone elastomer	Sylgard® silicone elastomer poured onto Parafilm and spread out with plastic spreader, Parafilm removed when dry (24 hrs)	0/40 *	0/40
Sylgard® silicone elastomer/fine nylon mesh	fine nylon mesh laid onto Parafilm, Sylgard® silicone elastomer poured on and spread out with plastic spreader, Parafilm removed when dry (24 hrs)	32/40	29/40

Appendix 2: Elevating CO_2 to Facilitate Foraging

Introduction

Carbon dioxide (CO_2) is known to be an important foraging trigger for bedbugs and potentially also aids in host location (Reinhardt & Siva-Jothy 2007, Suchy & Lewis 2011). It was therefore necessary to artificially elevate the CO_2 concentration in the artificial infestation setup each time the artificial host was replaced, in order to alert the bedbugs to the hosts presence. A tank of compressed CO_2 with a regulator was used to control the CO_2 concentration of the insectary where the artificial infestation setups were housed.

Natural fluctuations in CO_2 concentrations

To establish the CO_2 concentrations likely to be present in a natural infestation, a CO_2 sensor (Telaire™: 7001i) with a data logger (Telaire™: HO8-007-02) was placed in the bedrooms of ten volunteers for two nights per room. For the first night the sensor was placed in the bad, adjacent to the volunteer. For the second night the sensor was placed under the mattress at the head of the bed (a common location of bedbug harbourages in infested rooms, see Chapter 2). The sensor was placed in the bed 10 minutes before the volunteer got into the bed and was left in position until the following evening, so that the ambient CO_2 concentration during the day could also be established. The volunteer was asked not to be present in the room during the day, so as not to elevate the CO_2 concentration of the ambient reading. Figure A2.1 shows a typical readout from the CO_2 monitor. As expected, the CO2 concentration was highest adjacent to the (potential) host (1214.78±97.69 ppm). Although there was a slight increase in the CO_2 concentration at the site of the harbourage, as a result of the "host's" presence, the difference was not significant (Figure A2.2).

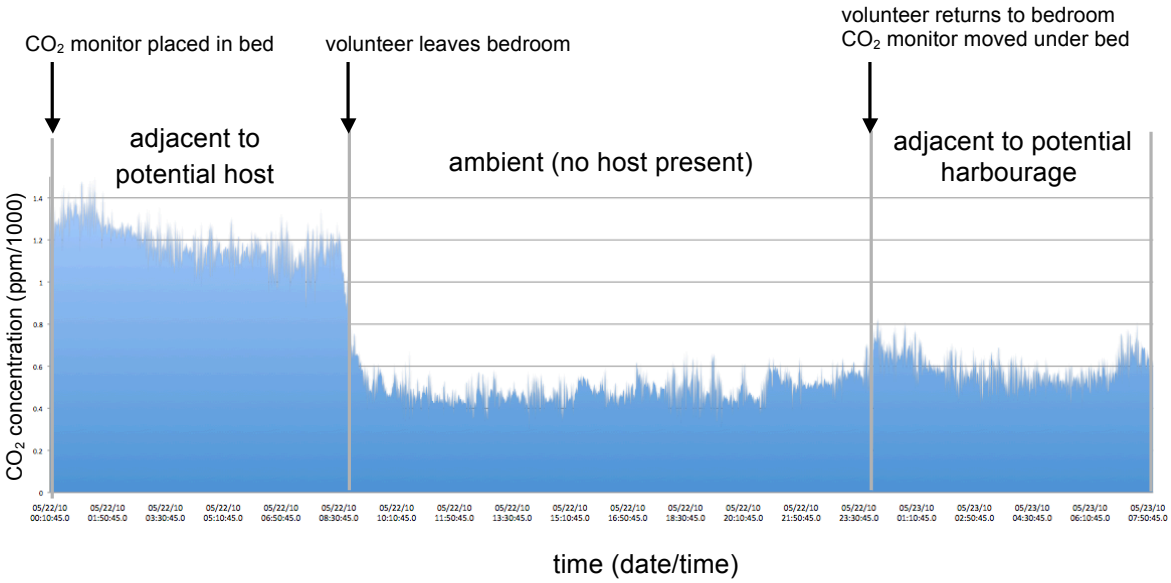

Figure A2.1 shows a typical CO_2 monitor readout from the bedroom of one of the un-infested volunteers, labelled to indicate the location of the monitor and presence of the host.

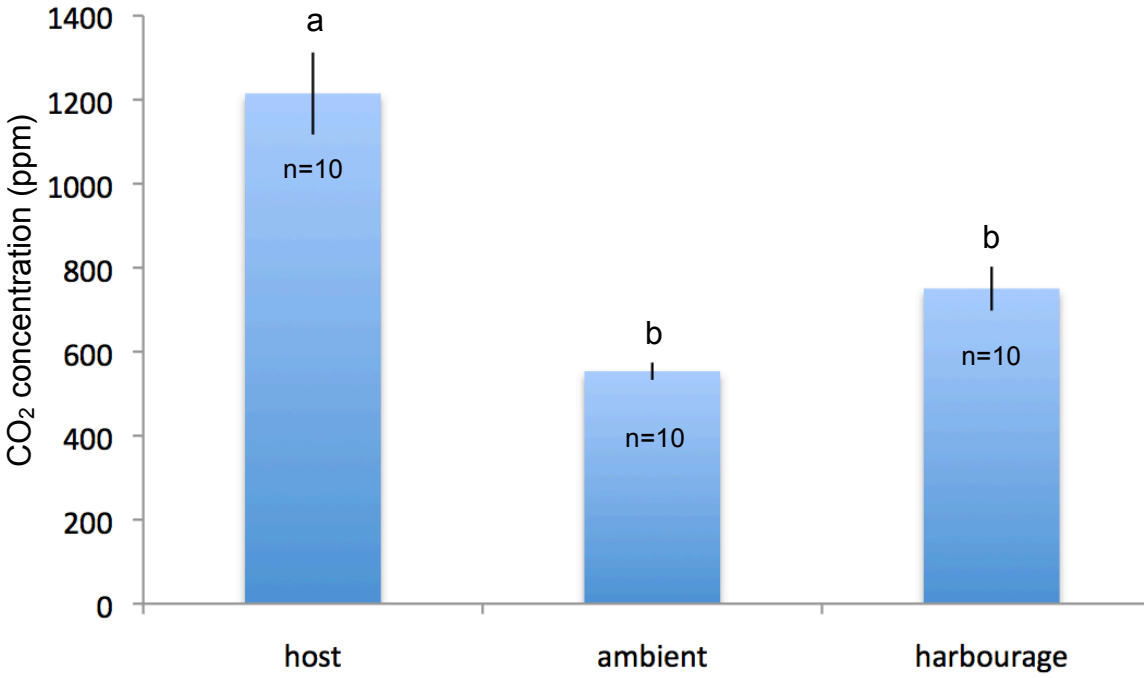

Figure A2.2 shows the variation in mean CO_2 concentration (parts per million) in the bedrooms of ten un-infested volunteers. The CO_2 concentration was recorded on the bed, adjacent to the volunteer (host), underneath the head-end of the mattress (harbourage), and on the bed, during the day, when the volunteer was absent (ambient). Error bars represent 1 standard error. Bars with the same letter do not differ significantly at $p<0.05$ (ANOVA, $F_{2,27}=27.24$, $p<0.001$; followed by Tukey Multiple Comparison test).

Elevating CO_2 to trigger foraging

To establish if the ambient of CO_2 concentration in the insectary (where the infestation arenas are housed) is realistic, a CO_2 monitor (Telaire^{TM}: 7001i) with a data logger (Telaire^{TM}: HO8-007-02) was placed adjacent to the infestation setups for 48 hrs. The ambient CO_2 concentration in the insectary was 484.1±2.6 ppm (n=2880 reads). The mean ambient CO_2 concentration from the bedrooms of the volunteers was 553.51±20.60 ppm (n=10) (Figure A2.3). The CO_2 concentration of the insectary is therefore slightly lower than the average of the volunteers' bedrooms, but it is still within the same range (slightly higher than the three lowest bedroom readings).

As the CO_2 source will be located at the end of the arena nearest the artificial host, and the concentration will be monitored from approximately the same location, the target concentration should be approximately the same as the levels encountered in the beds of the volunteers when the "hosts" were present (ca. 1200 ppm). To establish if this concentration is sufficient to trigger foraging in the arena setup, 300 adult female bedbugs were taken from the F4 population stock cultures, fed and split evenly between three arenas (see Chapter 3). The bedbugs were allowed 3 weeks to settle, establish harbourages and then become sufficiently hungry that all individuals should have the desire to forage if a host (or host-like cue) was detected. Under red light the CO_2 concentration in the room was steadily elevated by approximately 100 ppm/min for 15 minutes and the number of bedbugs out of the harbourages was recorded at 1 min intervals, along with the current CO_2 concentration.

A2.3 shows the increase in the number of foragers with elevating CO_2 concentration. Approximately 88% of bedbugs had left their harbourages by the time the CO_2 concentration had reached 12000 ppm. This climbed slightly to approximately 94% by the time the CO_2 concentration had reached 13000 ppm, and thereafter relatively few additional bedbugs began foraging.

Triggering foraging with CO_2 is likely to be an effect of time as well as CO_2 concentration. For example a lower CO_2 concentration may have been adequate to trigger equal levels of foraging if more time had elapsed. The relationship between CO_2 concentration and time, and their effects on the onset of foraging could be explored further. However, for the purposes of the laboratory infestation setup it is only necessary that the bedbugs are presented with host-like cues at biologically realistic levels, so that they are able to forage naturally. I will therefore elevate the CO_2

concentration of the insectary to 13000 ppm (at a rate of 100 ppm/min) each time the artificial hosts are introduced. I will maintain the elevated CO_2 concentration for a further 60 minutes to allow ample time for the foraging bedbugs to locate the artificial host.

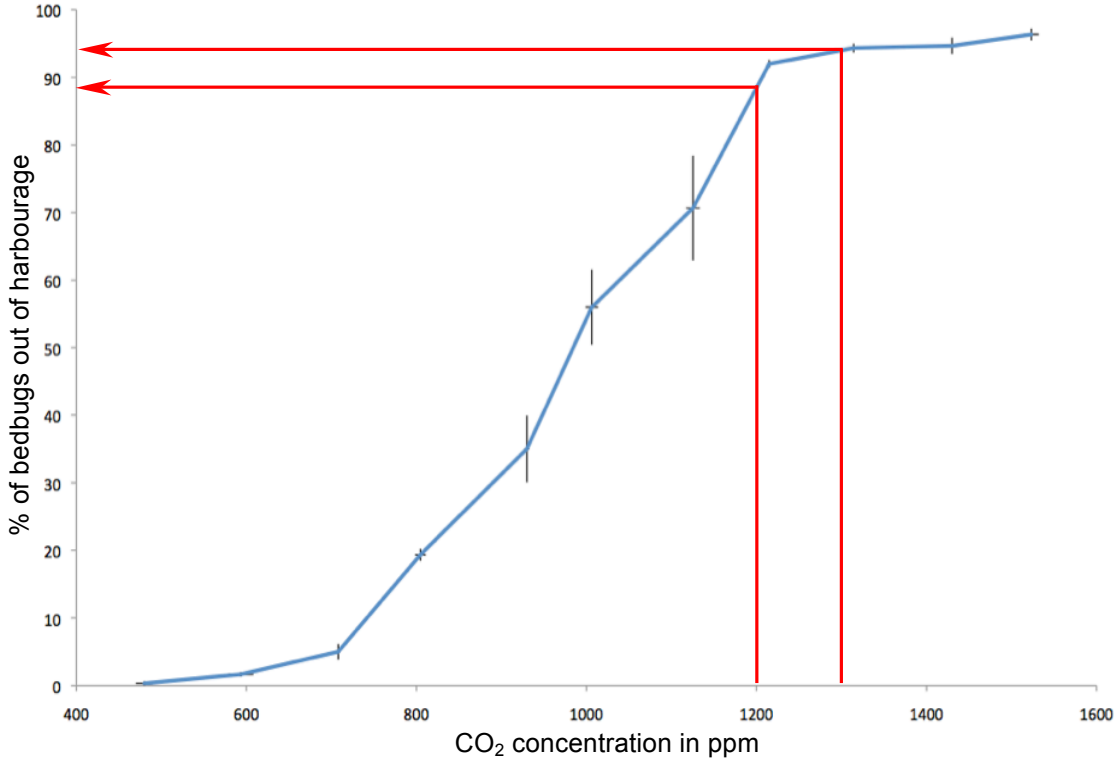

Figure A2.3 shows the percentage of bedbugs out of their harbourages at increasing concentrations of CO_2 for three replicate cohorts of 100 females from the F4 stock. Red arrows indicate the effect of increasing the CO_2 concentration from 1200 ppm to 1300 ppm. Error bars (X & Y) represent 1 standard error, n=3 at all data points.