Norwegian University of Life Sciences
Faculty of Environmental Science and Technology
Department of Ecology and Natural Resource Management

Master Thesis 2015
60 credits

Sub Lethal Heat Stress - Can it be Applied in Bed Bug Control?

Ranjeni Sivasubramaniam

Table of Contents

Abstract

This study examined the short and long term effects of long exposure to sub lethal temperatures on *Cimex lectularius*. Adult bed bugs and fifth instar nymphs were exposed to sub lethal temperatures, 34°C, 36°C and 38°C for 14 and 21 days with both constant and disrupted exposure. The treatments with constant exposures had lowest survival compared to the disrupted treatments. The two treatments with highest temperature had the lowest survival rate within the constant exposure treatments. Some mortality did occur in the 34°C as well. The treatments with disrupted exposure did not have as high mortality like the constant exposure treatments. Feeding abilities was reduced in the constant exposure treatments, but not in the disrupted treatments. Egg production and hatching success was reduced in both constant and disrupted exposures. The highest treatment temperatures had lowest egg production and reduced hatching success. After the eight week recovery period, egg production and hatching success had increased in both the constant and in the disrupted exposure treatments. Moulting abilities of the offspring's produced after thermal stress was reduced in both constant and disrupted treatments. The higher the temperature exposure was, the more it effected development. Although sub lethal heat treatment did not induce high mortality immediately, the long term effects that the long exposure caused can be detrimental for the development and growth of the surviving population in the future. Sub lethal heat treatment does affect bed bug if exposure is long enough. This method is not a stand-alone solution against bed bugs, but could be an efficient method if used in combination with IPM.

Sammendrag

Dette studiet undersøkte korttids og langtids effekter av forlenget eksponering av subletale temperaturer med både konstant og avbrutte eksponeringer på *Cimec lectularius*. Voksne veggdyr og femte stadium nymfer ble eksponert for subletale temperaturer, 34 °C, 36 °C og 38 °C, i 14 og 21 dager med både konstant og avbrutte eksponeringer. Behandlingene med konstant eksponering hadde lavest overlevelse i forhold til behandlingene med avbrutte eksponeringer. Behandlingene utsatt for de to høyeste temperaturene med konstant eksponering hadde lavest overlevelse. Noe dødelighet forekom også i 34 °C behandlingene. Behandlingene med avbrutte eksponeringer hadde ikke like store effekter med dødelighet som i de med konstant eksponering. Fôrings evner ble svært redusert etter konstant eksponering, men ikke i behandlingene med avbrutt eksponering. Egg produksjon og klekkesuksess var redusert i behandlinger med både konstant og avbrutte eksponeringer. Behandlingene med høyest temperatur eksponering hadde lavest eggproduksjon og klekke suksess. Etter den åtte

uker lange restitusjons perioden, hadde eggproduksjon og klekkesuksess økt betraktelig i både konstant og avbrutte eksponerings behandlingene. Avkommene som ble produsert etter behandlings perioden, hadde reduserte evner til å skifte hud og utvikle seg til neste stadium i både konstant og i avbrutte behandlings typene. Jo høyere temperatur eksponeringen var, jo mer var evnene til å utvikle seg redusert. Selv om subletal varme behandling ikke gir umiddelbare effekter, kan effektene som vedvarer påvirke utviklingen og populasjonsveksten til de overlevede individene negativt. Dette kan svekke populasjonen og minske sjansene for ny spredning og vil hindre fremtidig vekst. Subletal varmebehandling har en effekt på veggdyr, så lenge eksponerings tid er lang nok. Denne metoden er ikke en løsning som burde brukes aleine, men kan være effektiv hvis den brukes i kombinasjon med IPM.

Introduction

The bed bug, *Cimex lectularius* (Hemiptera: Cimicidae) is a flightless blood-sucking parasite that has been one of the most common and annoying pests in human history (Potter 2011; Usinger 1966). Bed bugs have co-existed with humans for more than 4 millennia and the earliest record of bed bugs was made by archaeologist, who found preserved bed bug remains in a 3550 year old workmen's village in Egypt (Davies et al. 2012). Bed bugs are believed to have evolved in caves within the Mediterranean and Middle Eastern regions where they were parasites on bats and birds. They probably started to exploit man when they moved into caves during the Pleistocene, Palaeolithic and Neolithic periods and they have remained nearby our bed sides ever since (Koganemaru 2013; Potter 2011; Usinger 1966). There are several species of cimicids, but only three are known to be ectoparasites on humans; *Leptocimex boueti*, primarily found in West Africa, *Cimex hemipterus* found in tropical and sub-tropical regions and *Cimex lectularius* also known as the common bed bug which is most common in the temperate regions (Lehane 2005). The common bed bug is the species that this study is going to focus on.

Before 1940's bed bugs were common worldwide, but with the development and use of highly effective organochloride insecticides such as DDT and other modern pesticides, bed bugs became less common in the latter half of the 20th century,- at least in developed countries (Busvine 1957). By the end of the 1960's bed bugs became resistant to DDT and other pesticides, and as a consequence, the last 15 years bed bugs have made a global re-emergence (Benoit 2011; Brown 1971). In Norway the number of reported infestations has risen from 500 infestations a year in 2007, to 2000 in 2014 (Folkehelseinstituttet 2015). Their resurgence is most likely caused by increased travel and pesticide resistance (Davies et al. 2012; Koganemaru 2013). The lack of awareness, knowledge and experience about the bed bugs among the general public and inadequate bed bug control measures are also important contributing factors to their resurgence (Cooper 2011).

Bed bug biology

Bed bugs are obligatory hematophagous ectoparasites, feeding exclusively on blood, and all nymph stages and both sexes require blood to survive, develop and reproduce (Davies et al. 2012). The adult female depends on the acquisition of a blood meal to produce eggs and it is

believed that a blood meal is also necessary for the adult male to produce sperm (Reinhardt & Siva-Jothy 2007). After hatching from the egg, the nymphs progress through 5 instars before they moult into adults. Bed bugs are hemimetabolous insects and require a blood meal to progress from one instar to the next. The insects prefer human blood, but in the absence of humans they will feed on mice, rats, chickens and other warm-blooded animals (Rozendaal 1997). With regular access to a food source bed bugs will feed weekly at room temperature. Blood is a great source of proteins and other resources, but it also lacks important key nutrients. For a bed bug to have normal growth and reproduction, a provision of B vitamins is vital, and this is solved by harbouring symbiotic bacteria within the mycetomes of the bed bug. Some studies have identified one of these symbionts as *Wolbachia* which major biological role is the provisioning of B vitamins (Hosokawa et al. 2010; Nikoh et al. 2014).

Bed bugs are predated upon by spiders, mites, pseudoscorpions and can also be infected by various fungus and other diseases, but these factors are not known to reduce populations noticeably. With no genuine threats from predators,- pathogens and other limiting factors except host availability, the bed bugs have the ability to grow exponentially once they have been introduced to a new environment. A female bed bug can lay 15-25 eggs per week and it takes about 4-21 days under normal conditions (20 ˚C -22 ˚C) for the eggs to hatch. A female bed bug is able to produce 200-500 eggs throughout its life (Harlan 2006; The Bedbug 2013). Under favourable conditions (25˚C-32˚C) the bed bugs can complete their entire development within just above a month but with lower temperatures it can take up to a whole year (Benoit et al. 2009a). If a bed bug population starts off with a few individuals, these few have the potential to grow to be several thousand within a couple of months. Bed bugs are in fact one of few species of insects that can withstand high levels of inbreeding (Fountain et al. 2015). Even after several generations of inbreeding they can produce healthy and viable offspring's with no signs of inbreeding depression. All that is needed to establish infestation in a room is one fertilized female with access to blood meals and the disaster has begun. Bed bugs are therefore considered to be excellent founders of new populations (Fountain et al. 2015). They are also able to survive without any blood meals for a year or more which enables the bed bug infestation to persist even in vacated rooms without any hosts (Bacot 1915).

Bed bugs tend to live close to their host, and to avoid being detected, they remain hidden in cracks or crevices in furniture or beds during daytime (Potter 2006). These harbouring sites are maintained by the presence of chemical ques like aggregation pheromones and contact stimuli with surfaces or bodies of other bed bugs (Johnson, 1941). Increased clustering of bed bugs reduces water loss and allows mate access (Benoit 2011). Bed bugs are nocturnal animals; active when host activity is minimal (Romero et al. 2010; Usinger 1966). The insects react on host cues such as temperature, CO_2 and other kairomones, and if interrupted during feeding, the bed bug will terminate feeding and resume when interruption ceases (Weeks et al. 2011). This is probably the main reason for multiple bites in one area (Benoit 2011). If bed bugs are disturbed, it can lead to the release of alarm pheromones warning other bed bugs to disperse from the area (Mellanby 1939).

Importance as pests

Bed bugs can severely reduce quality of life as they cause discomfort by biting which causes itching and blisters that can eventually result in secondary infections. If biting occurs frequently and over a long period with population size reaching high numbers, it can also lead to anaemic condition for the host. Anxiety, sleep deprivation, ostracism and possibly even depression are other consequences of having a bed bug infestation (Hwang et al. 2005; Susser et al. 2012). The discomforts that follows with a bed bug infestation can cause, are not acceptable, and a complete eradication of an infestation is the only acceptable outcome for most people. Although bed bugs are previously not known to spread diseases, recent studies indicate that bed bug are potential vectors of *Trypanosoma cruzi*, the etiologic agent of Chagas disease (Salazar et al. 2015), and *Bartonella quintana*, the agent of Trench fever in laboratory environments (Leulmi et al. 2015).

Bed bug control

Increasing front-page news featuring bed bugs in mainstream press and an increase in public awareness have given a renewed interest in management of these flightless parasites. Today there is no cheap management method that can guarantee success. Bed bugs can hide almost anywhere and escape detection by even the most experienced inspector as their small size and their nocturnal and elusive behaviour make them hard to eradicate (Davies et al. 2012). Often a great proportion of the bed bug population is exterminated during control, but a few

individuals manage to survive and can cause reoccurrence within a few months. Because of the high levels of insecticide resistance among the bed bugs, non-chemical control and integrated pest management are essential components in current bed bug management practices (Doggett et al. 2012; Koganemaru 2013). Bed bug control efforts often fail if only chemical treatments are used (Cooper 2011).

Insecticides in the form of liquids, dusts or aerosols are often used in bed bug control (Potter 2011), but leaving behind residues is a great concern as it can lead to development of resistance of the insecticide and future use of the same chemical on the same population of bed bugs could have reduced effects. High rates of resistance to insecticides has given a high demand to develop new and more efficient insecticides to use against bed bugs, but applying pesticides can create a high risk of human/pesticide exposure (Romero et al. 2007; Wang et al. 2009). Human exposure to pesticides can cause disease, including various forms of cancer (Sharpe & Irvine 2004). The increased awareness of health risks linked to pesticide use reflects on the higher demand of environmentally biodegradable compounds with less mammalian toxicity (Potter 2011). Chemical treatments should be kept to a minimum if possible to avoid potential adverse health effects on humans. Combined with high chances of resistance this method is just a temporary solution as we are dependent on creating new compounds as resistance develops, but this process is both expensive and time consuming (Doggett et al. 2012). However, few other options that is just as cheap and applicable like chemical treatments has not yet been developed (Potter 2011).

Other methods to treat bed bug infestation are with the use of cold temperatures. Exposure to -20°C for more than 48 hour will give 100% mortality for bed bugs in all stages (Benoit et al. 2009a; Olson et al. 2013). Cold treatment can be applied by using freezer containers to treat beds and other infested furniture that can be removed from an infested room. Cold temperature treatment has the same limitations as heat treatments; not reaching lethal core temperature of the infested item and it is therefore recommended that extreme temperature treatments are applied in combination with chemical treatments or other treatment methods (Koganemaru 2013).

The use of heat has long been a non-chemical bed bug control method. Within other species there is more variation of resistance to low temperature than there is to high temperatures (Chown & Nicolson 2004). Current studies has presented that 100% bed bug mortality can be reached with a couple of minutes exposure to 60°C, with 1 hour exposure to 48°C, 48 hours of exposure to 40°C and over a month of exposure to 37°C (Benoit et al. 2009a; Kells & Goblirsch 2011; Rukke et al. 2015). The goal for all bed bug treatments is to kill bed bugs instantly or within a few hours by exposing them to high temperatures. There has been little study on lower temperatures with longer exposure period and if it could be used as an option against bed bugs. The key points of thermal treatment is to ensure that the bed bugs are exposed to the lethal temperature for a specified time and that the critical temperatures are reached in the core of the infested item (Kells 2006). Heat treatment can be applied to both infested items and rooms. Exposure of bed bugs to extreme temperatures up to 50°C for 1 hour is currently being used as a standard by most pest management professionals (Austin 2013). When utilizing heat treatment in whole rooms there could occur some cold spots where the heat is below lethal temperature, and some constructions can provide harbourages for the bed bugs (Koganemaru 2013). It also comes with the risk of spreading the infestation, as bed bugs will seek cooler areas when exposed to temperatures above 30°C to 35°C (Doggett et al. 2012). Structural heat treatment therefore require special equipment and trained personnel to ensure thorough and safe applications of lethal temperatures (Olson et al. 2013). Exposing bed bugs to extreme temperatures through hot temperature laundering and drying, steaming and using portable heat boxes are methods you can use to treat a bed bug infestation with.

Intense thermal stress can perturb the structure of an organisms proteins as it may result in unfolding of proteins and then reduce the cellular pool of functional proteins and it may also be cytotoxic (Chown & Nicolson 2004). When the insects are exposed to heat, they produce heat shock proteins that can protect proteins from unfolding in the cells and improve survival. However, continuous expression of heat shock proteins reduces survival and fecundity, inhibits growth and thus affects development time. This has been identified in several insect species such as moths, ants and parasitic wasps (Chown & Nicolson 2004). Bed bugs that have survived exposure to sub lethal temperatures below 40°C have been shown to have reduced fitness with lowered numbers of laid and hatched eggs (Rukke et al. 2015) as thermal wounding by sub lethal temperatures may be as detrimental without obvious effects by reducing reproductive abilities, ability to develop into adults, feeding abilities and by making

individuals more prone to chemical treatments (Pereira 2009). Thermal treatment has direct effects on bed bugs, but elevated temperatures can also affect bed bug symbionts due to changes in microbes within the mycetomes of the bed bug (Chang 1974; Hosokawa et al. 2010; Nikoh et al. 2014), and consequently prevent bed bug reproduction (Pereira 2009; Rukke et al. 2015). Earlier studies done by Chang (1974) have shown that temperature exposure to 36°C can lower the fecundity of bed bugs due to loss of symbionts, however it also notes that the lowered fecundity could be caused directly from the high temperature (Chang 1974).

The efficiency of a temperature treatments is dependent on the temperature and the exposure time, and non-lethal temperatures could be lethal if exposure time is prolonged (Chown & Nicolson 2004; Rukke et al. 2015). I want to explore the use of sub-lethal heat treatment on bed bugs and discus if this method could be applied as a part of an integrated pest management in the future by investigating the effects of sub lethal heat at the temperatures 34°C, 36°C and 38°C on bed bugs in a laboratory environment. The three treatment temperatures were selected based on information from previous studies(Rukke et al. 2015; Usinger 1966). In Rukke et al 2015, the bed bugs reached 100% adult mortality after 2 days of exposure to 40°C and after 9 days with exposure to 38.5°C. The study also looked at sub lethal effects on reproduction and the long term effects on the second generation. The temperatures used in this study seem to be below the critical temperature range for bed bugs. I also want to see if there is any difference in effects whether the temperature treatment is continuous or disrupted during the treatment period. Can it for instance be sufficient to have elevated temperature treatment during the weekends and no treatment during the workdays for an extended period of two or three weeks? Most bed bug studies only measure the immediate effects of the treatment on the tested generation. My study will also investigate the long term effects on the surviving bed bugs and their offspring's and thus observe how the treatments will affect the surviving population in the long run. How the different temperature treatment affect the overall survival, feeding abilities, the number and quality of the eggs and overall development of offspring's are investigated. Based on previous studies I expect that the effects will increase with extended time and with the increase of temperatures between the ranges of 34-38°C.

Materials and method

Study animal

In appearance and size the nymphs are relatively different from the adults (Figure 1). The first instar nymph is no larger than one millimetre, is almost translucent and has only 1 row of spines on each abdominal tergite. The second and third instar nymphs are larger but still have a translucent cuticle, and you can still see all the internal organelles through. The second instar has 2 rows of spines on the abdominal tergite and the third instar has at least 3 rows of spines and in addition the last 2 antenna segments are sub equal. The fourth instar has antennas where the fourth segment is distinctly shorter than the third segment and the hind margin of the mesonotum is barely broadly concave at the middle. The fifth instar is more similar to the adults, but they are slightly smaller in size and they have two light stripes on their back. They can also be recognized by the hind margin of mesonotum which is noticeably broadly concave at the middle. The adults are larger in size and have a darker and a more hardened cuticle. The females are a bit larger than the males, and they have a more rounded and symmetrical abdomen than the males which have a narrower and pointier abdomen. The females also have a paragenital sinus located at hind margin of fifth abdominal sternite. (Usinger 1966).

Bed bug culturing and experimental preparations

The study animals were collected from an existing stock culture that was sampled from two hotels in Oslo, Norway, 2009. The stock cultures were maintained in a 16:8 hour cycle (00:00 – 16:00 daytime and 16:00 – 00:00 night) at 22˚C and 65% relative humidity. The bed bugs were fed with artificially heated human blood through a Parafilm membrane once every two weeks according to the feeding regimen described in Aak&Rukke (2014).

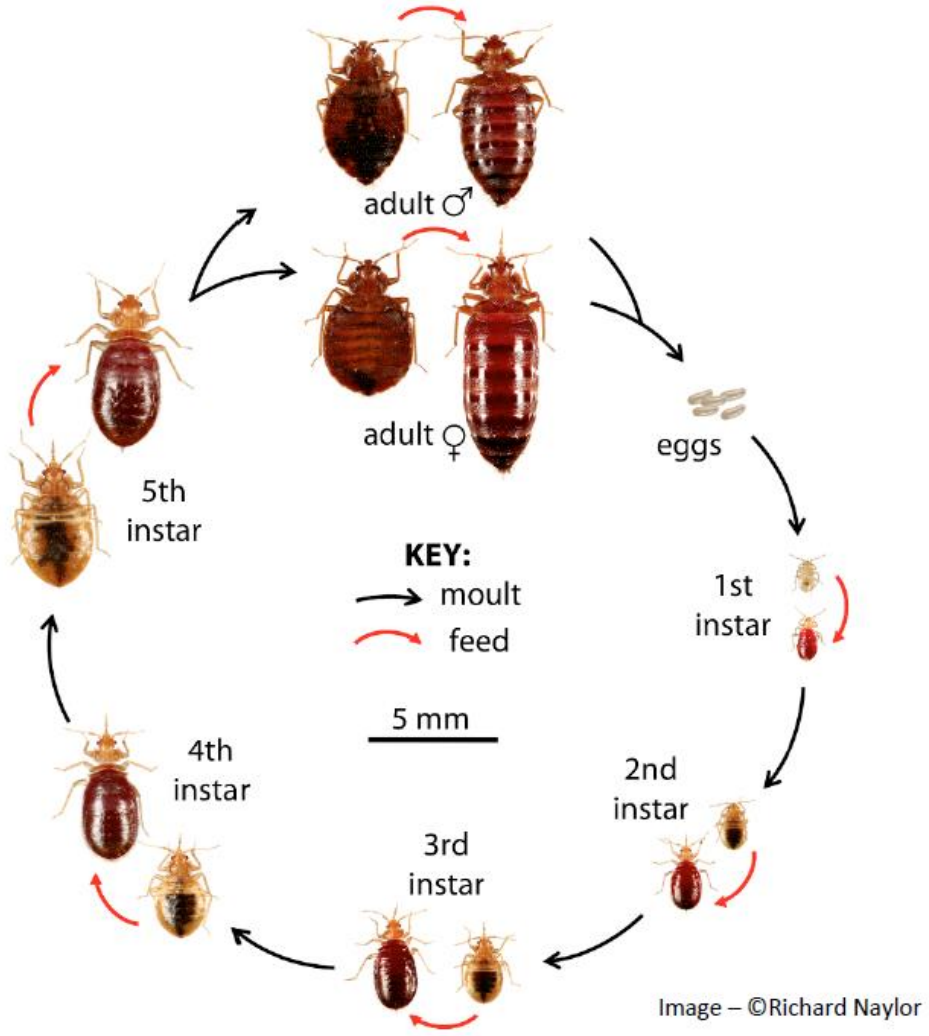


Figure 1. The various life stages of the common bed bug, *Cimex lectularius.* Depicted are the eggs, the five nymph instars, and both sexes, all before and after being fed(The Bedbug 2013).

Climate chambers

All the experiments were conducted in climate chambers (Sanyo – MLR-351H, Medinor ASA, Oslo, Norway) with a 16:8 hour light:dark cycle and 65% relative humidity (RH). The four climate chambers were set at 22˚C (21.7˚C ± 0.3˚C (SE)), 34˚C (33.7˚C± 0.7˚C), 36˚C (36.0˚C ± 0.5˚C) and 38˚C (37.9˚C ± 0.6˚C). The 22˚C chamber is hereafter denoted as room temperature.

Experimental units

Bed bugs were kept in polyethylene boxes (VWR straight sample container – VWR, Oslo, Norway) during the experiment. The boxes measured 140 ml with a diameter of 47 and

contained a filter paper, 47 mm in diameter. The lid of the boxes had a circular hole of 40 mm in diameter drilled into it. The hole was then covered and sealed completely with a metal mesh screen of 0.25mm (Burnmeister AS, Oslo, Norway) by using a welder. The experiment is based on units represented by boxes with a total of 12 bed bugs in each box. Each box included 3 male adults, 3 female adults and 6 fifth instar nymphs. A total of 130 boxes were used and gave a total number of 1560 bed bugs for the entire experiment on the initial start of the treatment period.

Thermal Stress/Heat treatment

To test the effects of constant exposure treatments versus disrupted exposure treatments, I had one treatment type with constant exposure to elevated temperatures and one treatment type with disrupted exposure to elevated temperatures, both lasting for 14 or 21 days. The 14 day treatments were called short treatments, while the 21 day treatments were called long treatments. Ten boxes were used in each treatment type: long constant, short constant, long disrupted and short disrupted (Table 1). These 4 different treatment types were tested at 3 temperatures 34˚C, 36˚C and 38˚C. Ten boxes were placed in a chamber with room temperature to act as control.

Continuous exposure to elevated temperatures: The long treatment remained in the climate chamber for the entire thermal stress period (21 days) and the short treatments remained in the chambers the first 14 days before being moved into climate chamber with room temperature for the remainder of the exposure period (Figure 3 and Table 1)

Disrupted exposure to elevated temperatures: After 3 days of exposure to elevated temperatures all boxes were moved to the chamber with room temperature. After 4 days with room temperature the boxes were moved back to the climate chambers with elevated temperatures for another 3 days of exposure. This was repeated for the entire experimental period, except for the short treatments. They were moved from the elevated climate chambers after 14 days and into the climate chamber with room temperature (Figure 3).

Table 1 An overview of the different temprature treatments. A) describes the constant exposure treatments, B) the disrupted treatments while C) is the control.

	Treatment codes	Temperature	Days of exposure period	Total days exposed to elevated temperatures
A)	34˚C Long	34°C	1-21	21
	34 ˚C Short	34°C	1-14	14
	36 ˚C Long	36°C	1-21	21
	36 ˚C Short	36°C	1-14	14
	38 ˚C Long	38°C	1-21	21
	38 ˚C Short	38°C	1-14	14
B)	34 ˚C Long	34°C	1-3, 8-10, 15-17	9
	34˚C Short	34°C	1-3, 8-10	6
	36 ˚C Long	36°C	1-3, 8-10, 15-17	9
	36 ˚C Short	36°C	1-3, 8-10	6
	38 ˚C Long	38°C	1-3, 8-10, 15-17	9
	38 ˚C Short	38°C	1-3, 8-10	6
C)	Control	22°C	0	0

Experimental Protocol

1) Before thermal stress

Two and a half weeks prior to the experiment, fifth and fourth instar nymphs were separated from the stock cultures based on their distinct character. This was done to guarantee that we would have experimental animals of the same age for the experiment. The fifth and fourth instar nymphs were fed once and then left for 2 weeks to moult into adult and fifth instar nymphs. Adults were separated by sex and placed into new boxes, and fifth instar nymphs were separated from the fourth instars that did not moult during the 2 weeks and placed into another box. Two days prior to the treatment, all the adults (male and female) were fed until

fully engorged. Nymphs were not fed. The experimental bed bugs rested for another day prior to the experiment.

2) During thermal stress

Mortality: Mortality was checked and noted every day during the period with thermal stress (Figure 8) by using a stereomicroscope (Leica MZ16 A – Leica microsystems, Switzerland Ltd) and observing activity in all the boxes. If the bed bug was laying on the dorsum and did not move limbs or antennas it was considered as dead.

3) Right after thermal treatment:

Egg production and quality: All the eggs that were produced during the treatment were counted. I also noted the condition of the egg, if it was hatched, unhatched or disfigured (Figure 2). The eggs were then placed in collection boxes and then frozen for storage.

Figure 2. Eggs five days after ovipositioning. Upper eggs are healthy and unhatched, while lower eggs are deformed and non-viable (Hosokawa et al. 2010). The arrow indicate red eyespots which is a sign of healthy and viable eggs.

After counting the eggs, the survivors from each treatment was reorganized in a matter that we ended up with "complete" boxes (Figure 3) with 3 females, 3 males and 6 nymphs like in the beginning of the experiment. All dead bed bugs were discarded. This was done so that I would have comparable units after the population recovery period and also have an equal sex

ratio to reduce unwanted copulations between fifth instar nymphs/males and other conspecific males. All nymphs that would hatch from now on would originate from eggs deposited after the thermal stress period and in the population recovery period. A new filter paper was placed in the boxes, but this time the filter paper was folded in half to give the bed bugs refuge and to limit stress.

Feeding ability after treatment: The bed bugs were given 3 days to recover from the thermal stress before they were fed. Feeding ability was judged based on whether or not they were fully engorged after given the opportunity to feed. The bed bugs were then to be fed once every second week for eight weeks during the population recovery period (Figure 3). Due to the increasing numbers of bed bugs in the units, it was difficult to observe and note how many bed bugs that fed after the first feeding. Feeding ability was therefore not observed in the remaining feedings in the population recovery period.

4) Eight weeks later

Fifth instar: During the 8 week long recovery period the bed bugs were only fed 4 times (Figure 3). Two days after the last feeding all the remaining boxes with bed bugs were killed by freezing. With only 4 possible feedings, the hatched nymphs would maximum have reached the fourth nymphal instar, and I was therefore able to separate them from the initial fifth instar nymphs that were treated with thermal stress.

Long term effects on egg production and quality: The total amount and the quality of eggs produced were also registered 8 weeks after the terminated thermal stress (Figure 3). This was to see if there were any negative effects on eggs observed after the thermal treatment was sustained in each box. The number of eggs in the boxes was registered as hatched, unhatched or deformed.

Offspring development: After the 8 weeks population recovery period, the number of total nymphs was counted and it was noted which instar the individual nymphs had reached during the recovery period (Figure 3). The different instars were identified by using a

stereomicroscope (Leica Motor Focus System, Leica MZ16 A – Leica microsystems, Switzerland Ltd) to count the rows of spines on the abdominal tergite on the nymphs. The number of casted exuvia were also counted and noted. The number of exuvia can imply whether or not the population is able to progress and develop normally after being exposed to the different treatments.

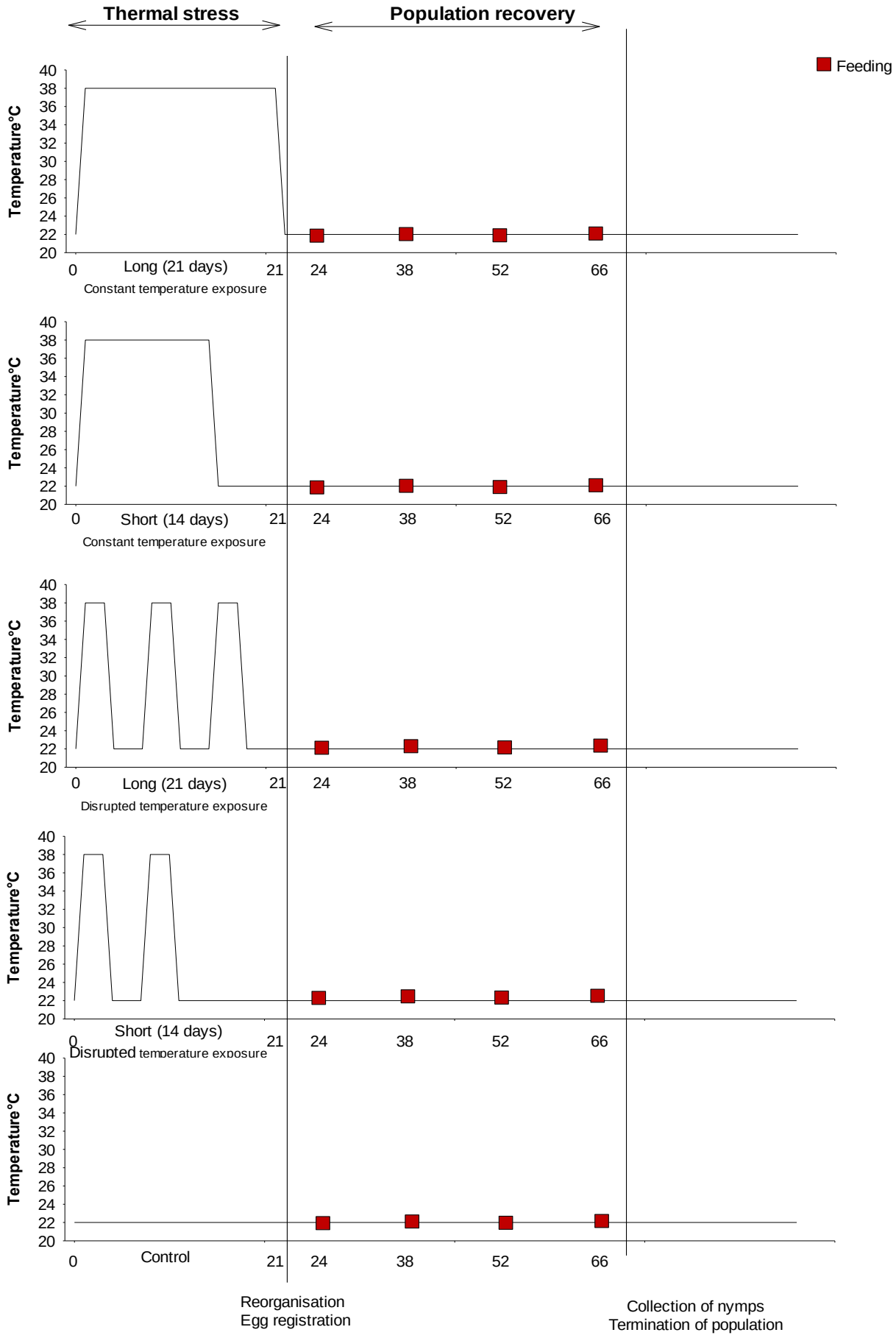


Figure 3 Experimental design showing bed bug treatment during thermal stress period (exposure time and temperature) and timing of feeding events after ended thermal stress.

Statistical analysis

All data was analysed by using SigmaPlot 12 (Systat Software Inc. Son Jose, California,USA) and JMP pro 11.1.1 (SAS institute, Cary, NC, USA). The data was tested for normality, and multiple comparisons were performed using analysis of variance (ANOVA). Pairwise comparisons were tested by using t-test. The level of significance was set to 0.05. If normality failed the nonparametric Kruskal-Wallis One Way Analysis of Variance on Ranks was used to test for differences. In multiple comparisons versus control group, Dunnett's method or Dunn's pairwise comparison was used to identify the group or groups that differed from the control. Kaplan-Meier product limit method with the log-rank test between groups was used in survival analyses. Averages are always given with ± standard error (SE).

Results

Progress in survival among adults and nymphs during thermal stress

Constant exposure: the survival in all treatments differed between all treatments both regarding adults and nymphs (Kaplan-Meier survival analysis: 36°C Short vs 34°C Short: χ^2= 16.9, df=1, p<0.001 (only least significant test shown)). Adults in the 38°C treatment had its first mortality at day 2 or 3 and reached 50% survival at day 7 or 9 (Figure 4 A and B) while the nymphs had its first morality at day 1 and reached 50% survival at day 5 (Figure 5 A and B). In the 36°C treatments the first adult mortality was registered 3 or 4 days later compared to 38 C and 50% survival was reached at day 19. First mortality for the nymphs was observed at day 1 or 2 and 50% survival was reached at day 15 (Figure 5 A). At 34°C the first adult mortality occurred at day 19 or 21 and first nymph mortality at day 3 or 4. Both 34°C treatments remained well above 50% survival.

Disrupted exposure: All of the disrupted treatments remained well above 50% survival, but they still showed the same temperature dependent connection to thermal stress with higher mortality at the highest temperatures. There was only significant mortality at 38°C (Kaplan-Meier survival analysis; 38°C Long vs 36°C Long: χ^2=9.6, df=1, p=0.0019 (only least significant test shown)). At 38°C the first adult mortality occurred at day 6 (Figure 4 A) and the first nymph mortality at day 2 or 3 (Figure 5 B). The treatment of 34°C and 36°C had some mortality after 9 days of treatment.

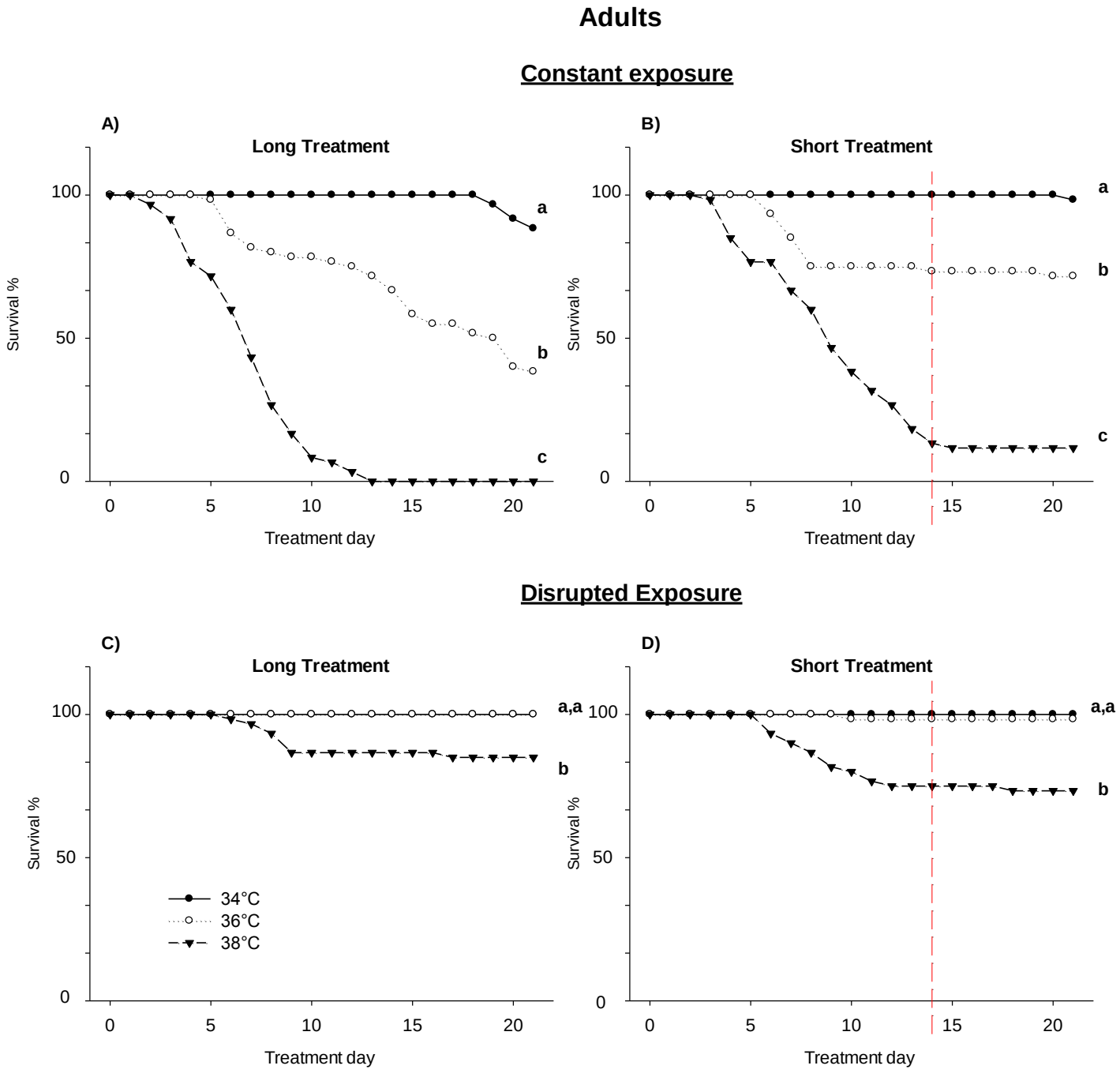


Figure 4 Survival of adult bed bugs during thermal stress period showing A) long and B) short treatments with constant exposure to thermal stress and C) long and D) short treatments with disrupted exposure to thermal stress. The dotted red line indicates where thermal stress was terminated after 14 days in the short treatments. Different letters a, b and c denote significant differences in survival between treatments ($p<0.05$)

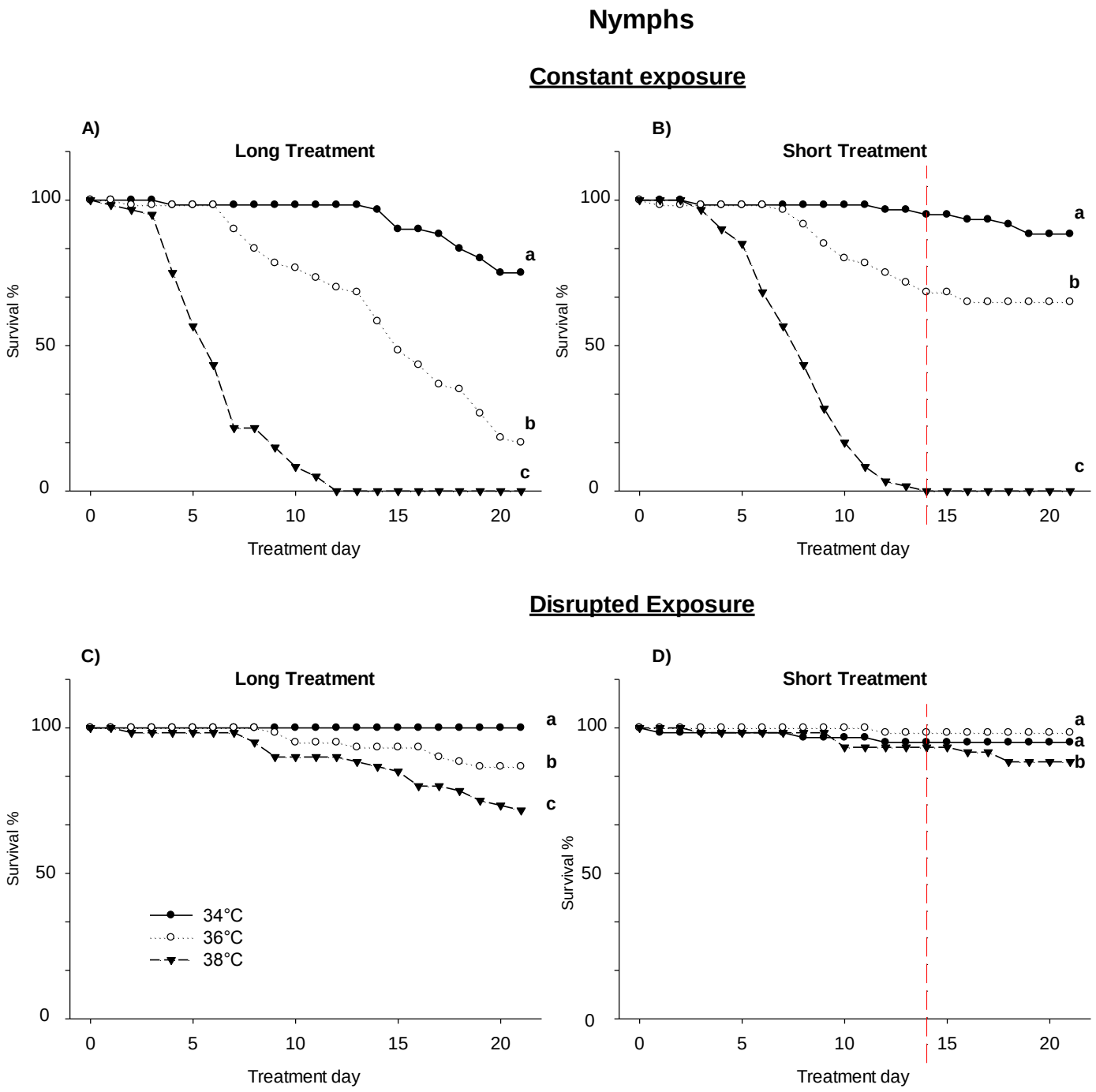


Figure 5 Survival of bed bug nymphs during thermal stress period showing A) long and B) short treatments with constant exposure to thermal stress and C) long and D) short treatments with disrupted exposure to thermal stress. The dotted red line indicates where thermal stress was terminated after 14 days in the short treatments. Different letters a, b and c denote significant differences in survival between treatments ($p<0.05$).

Overall Survival after exposure to elevated temperatures

There was no significant difference in male and female survival when compared across all heat treatments (Paired t-test; t=1.20, n=12, p=0.25). The sexes were thus pooled together and analysed as adults. The nymphs, however, showed a significantly lower survival when compared to the adults (Paired t-test; t=2.4, n=12, p=0.03). On average the survival was 7% lower, but the maximum difference was as large as 22% (Table 2). The survival data for adults and nymphs are and analysed separately.

Constant exposure: All control animals survived for 21 days (Table 2C) while elevated temperatures induced a significant mortality among the adults (Kruskal-Wallis; H= 62.4, df= 6, $p<0.001$) and the nymphs (Kruskal-Wallis; H=59.9, df=6 $p<0.001$). Compared to the control, survival at 38°C and 36°C was found to be lower for both adults and nymphs, while not in the 34°C treatments (Table 2A). The long treatment of 38°C had no adult survivors, while an average of 11.7 ± 6.6% of the adults survived the short treatment. Further reduction of thermal stress increased survival from 38.3 ± 4.3% in the long treatment at 36°C and to 98.3 ± 1.7% in the short 34°C treatment. In terms of significance the nymphs experienced the same effects on survival, but survival was generally lower than the adults (Table 2A).

Disrupted exposure: Compared to constant exposure the survival in the disrupted treatments was higher and it ranged from 73% to 100% survival among the adults and 72% to 100% survival among nymphs. Disrupted temperature treatments affected survival significantly in both adults (Kruskal-Wallis; H=48.1, df= 6, $p<0.001$) and nymphs (Kruskal-Wallis; H=33.9, df=6, $p<0.001$), but the 38˚C treatments different from the control (Table 2B). The significant effect among adults was found at short exposure to 38°C with an average survival of 73.3 ± 6.7% while the significant effect among the nymphs was found at long exposure to 38°C with an average survival of 73.3 ± 6.7%. The remaining treatments showed less than 15% mortality.

Tabel 2 Average±SE percentage survival of adults and nymphs after ended thermal stress and the difference between adults and nymphs. * = significant differences<0.05 as compared to the control. A) describes the constant exposure treatments, B) the disrupted treatments while C) is the control.

	Treatment	Adults	Nymphs	Difference
A)	38°C Long	0.0 ± 0.0%*	0.0%*	0
	38°C Short	11.6 ± 6.6%*	0.0%*	12
	36°C Long	38.3 ± 4.3%*	16.7 ± 7.1%*	22
	36°C Short	71.6 ± 5.0%*	65.0 ± 4.6%*	7
	34°C Long	88.3 ± 4.3%	75.0 ± 6.7%	13
	34°C Short	98.3 ± 1.6	88.3 ± 0.0%	12
B)	38°C Long	85.0 ± 3.9%	71.7 ± 5.0%*	13
	38°C Short	73.3 ± 6.7%*	88.3 ± 2.6%	-15
	36°C Long	100.0%	86.7 ± 5.9%	13
	36°C Short	98.3 ± 1.7%	98.3 ± 1.7%	0
	34°C Long)	100.0%	100.0%	0
	34C Short	100.0%	95.0 ±3 .6%	5
C)	Control	100.0%	100.0%	0

Egg production and hatching success during thermal stress

Whereas mortality was strongly reduced in the disrupted temperature regimen compared to the control, egg production and hatching success appeared to most rely on the maximum temperature experienced. An average of 14.6 ± 2.5 eggs per box was produced in the control during the heat treatment period. Elevated temperatures significantly reduced egg production of 38°C and 36°C in both constant (Kruskal-Wallis; H=48.6 df =6, p<0.001) and disrupted treatments (Kruskal-Wallis; H=40.6 df =6, p<0.001), while 34°C treatments did not (Table 4A). Elevated temperatures also had significant effect on hatching success in both constant (Kruskal-Wallis; H=58.3 df =6, p<0.001) and disrupted treatments (Kruskal-Wallis; H=58.3 df =6, p<0.001), and as for the egg production, the treatments of 38°C and 36°C were different from the control while both the 34°C treatments where not (Table 4A). The two highest temperatures produced less than half the amount of eggs and the hatching success was below 5%.

Table 4: Average±SE egg production, hatched eggs and hatching success during thermal stress. * = significant differences (p<0.05) as compared to the control. A) describes the constant exposure treatments, B) the disrupted treatments while C) is the control.

	Treatment	Total egg production	Hatched eggs	Hatching success%
A)	38°C Long	-	-	-
	38°C Short	0.4 ± 0.3*	0*	0
	36°C Long	3.0 ± 1.2*	0.3 ± 0.3*	0
	36°C Short	5.2 ± 1.4*	0.1 ± 0.1*	0.7 ± 0.7
	34°C Long	10.7 ± 1.6	7.6 ± 1.4	63.2 ± 8.8
	34°C Short	11.4 ± 1.7	8.8 ± 1.5	76.6 ± 4.9
B)	38°C Long	1.6 ± 0.7*	0*	0
	38°C Short	2.2 ± 0.7*	0*	0
	36°C Long	8.6 ± 1.6	0.4 ± 0.5*	3.8 ± 1.7
	36°C Short	5.2 ± 0.9*	0.2 ± 0.1*	3.8 ± 2.7
	34°C Long	10.2 ± 1.5	7.2 ± 1.6	69.9 ± 8.5
	34C Short	13.7 ± 2.2	9.9 ± 1.9	73.3 ± 7.7
C)	Control	14.6 ± 2.5	11.9 ± 2.1	81.9 ± 3.2

Feeding ability after thermal stress

The control had an average of 80.8 ± 9.2% bed bugs feeding after the thermal stress period. Constant exposure to elevated temperatures significantly influenced the feeding ability (Kruskal-Wallis; H=33.5, df =6, p<0.001). The short treatment of 38°C and the long treatment at 36°C reduced significantly the feeding to below 20% (Figure 6A). None of the treatments with disrupted temperature exposure were different from the control (Kruskal-Wallis; H=7.6, df= 6, p=0.27, Figure 6B).

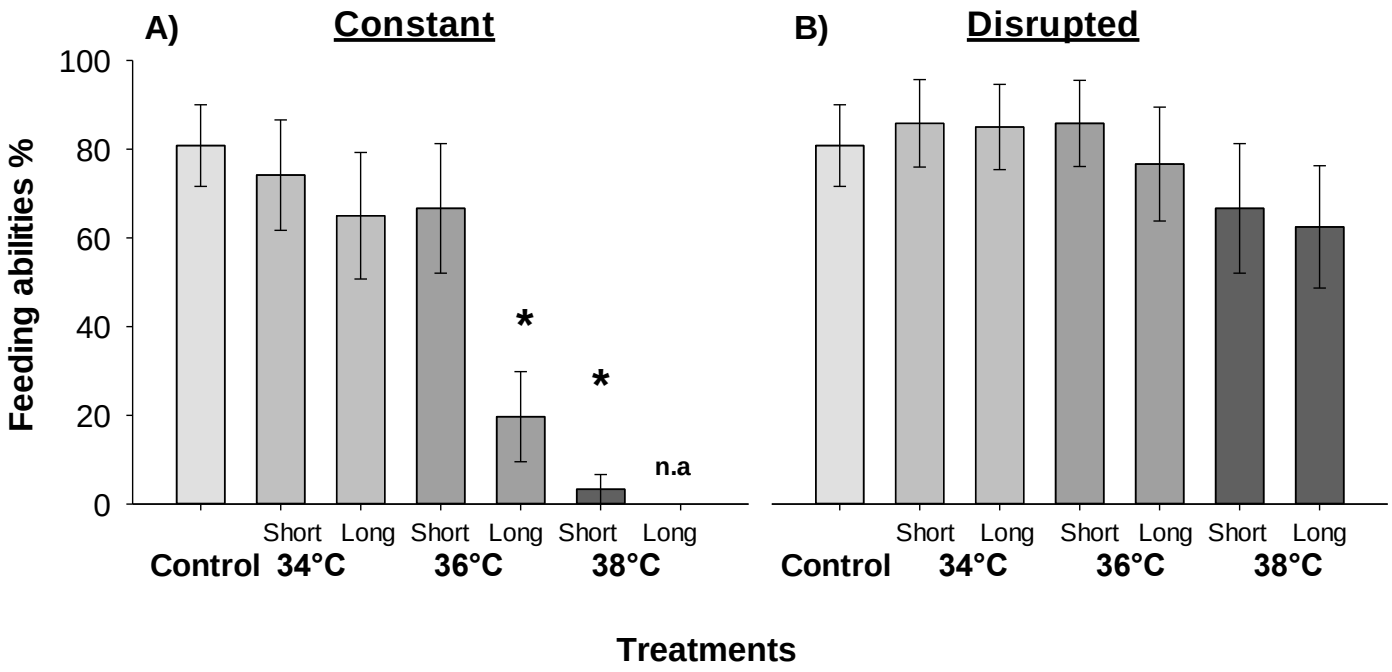


Figure 6 Feeding ability for the temperature treatments in both A) constant and B) disrupted exposure after thermal stress. * denotes significant difference from control (p<0.05). "n.a." – not applicable.

Development of fifth instar nymphs after thermal stress

The constant exposure treatments had significant effects on the development of the fifth instar to imago (Kruskal-Wallis; H=39.1, df=6, p<0.001). Compared to the control, progression to adults was reduced in both treatments of 38°C, 36°C and in the long treatment of 34°C, while the short treatment of 34°C was not reduced (Figure 7A). The 38°C treatments and long 36°C treatment had none or just a few nymphs developing into (Figure.7A). The disrupted treatments were not significantly different from each other (Kruskal-Wallis; H=12.5, df=6, p=0.053, Figure 7B). However the disrupted treatments show the same trends; decreased feeding with higher temperatures (Figure.7B). Although not statistically different from the control, the average number of nymphs developing into adults is half of what the control has and the p-value is very close to being significant.

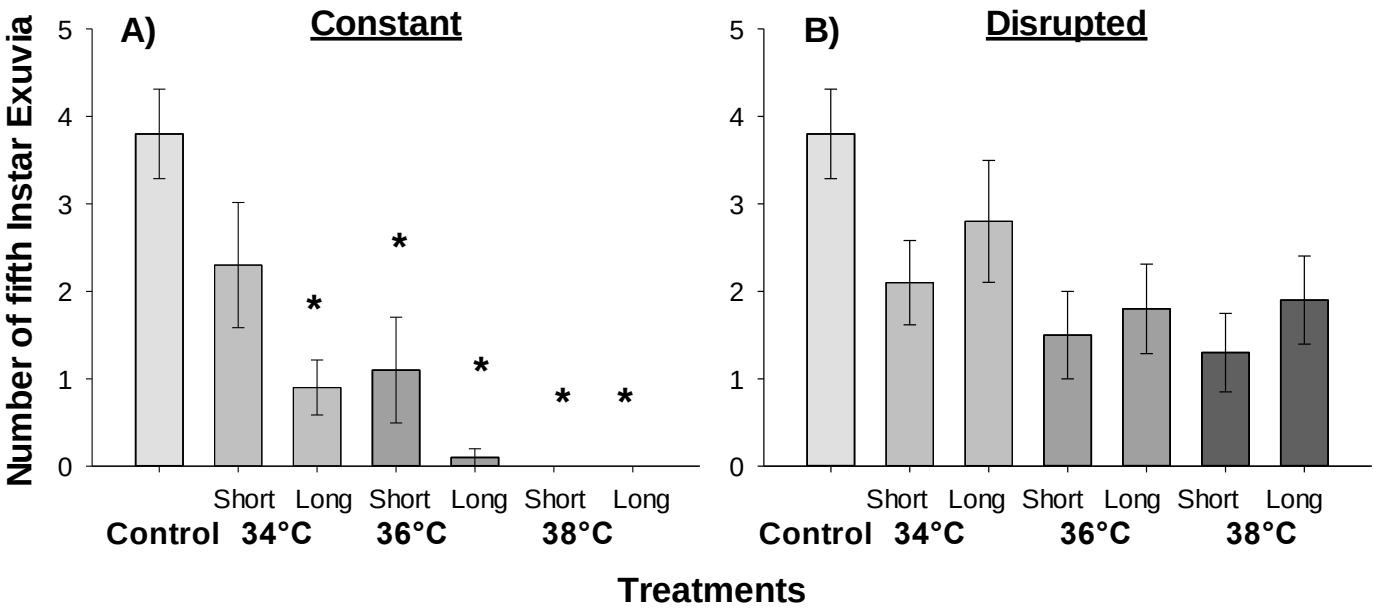


Figure 7 Number of fifth instar nymphs developing into adults after heat treatment and population recovery period for both A) constant and B) disrupted treatments.

Effects on egg production and hatching success after thermal stress

An average of 43.6 ± 6.13 eggs per box was produced in the control treatment during the 8 weeks long population recovery period (Table 5C). Previous, constant elevated temperature exposure had a significant effect on long-term egg production (Kruskal-Wallis; H=43.2, df =6, $p<0.001$) and hatching (Kruskal-Wallis; H=44.6, df =6, $p<0.001$). The comparison to the control identified all treatments of 38°C and 36°C and the long treatment of 34°C to be different from the control while the short 34°C treatment was not (Table 5A). Compared to the control the significantly affected treatments produced in average one fourth of the eggs and hatching success was far less than that of the control. Opposed to this the total production of eggs in the disrupted treatments was not reduced (ANOVA; F=1.9, df=6 p=0.085). An effect on hatching success however was present (Kruskal-Wallis; H=14.9, df =6, p=0.021), with reduced hatching at 38°C and 36°C (Table 5B).

Table 5: Average±SE of egg production , hatched eggs and hatching success after population recovery period * = significant differences p<0.05 as compared to the control." –22" no available data on eggs due to high mortality among adults. A) describes the constant exposure treatments, B) the disrupted treatments while C) is the control.

	Treatment	Total egg production	Hatched eggs	Hatching success%
A)	38℃ Long	-	-	-
	38℃ Short	-	-	-
	36℃ Long	2.3 ± 1.8*	0.3 ± 0.3*	1.7 ± 1.7
	36℃ Short	16.7 ± 5.6*	10.7 ± 3.9*	39.7 ± 12.9
	34℃ Long	11.8 ± 3.8*	6.5 ± 2.5*	36.2 ± 9.1
	34℃ Short	25.9 ± 7.2	17.8 ± 4.3	58.6 ± 11.2
B)	38℃ Long	20.5 ± 5.4	8.3 ± 2.9*	27.7 ± 7.5
	38℃ Short	24.6 ± 7.2	15.5 ± 4.0	49.9 ± 12.4
	36℃ Long	22.6 ± 4.3	10.5 ± 2.7*	36.3 ± 7.7
	36℃ Short	28.1 ± 6.3	15.5 ± 4.8*	50.9 ± 9.1
	34℃ Long	33.6 ± 6.2	22.4 ± 5.5	62.4 ± 8.7
	34C Short	23.5 ± 4.6	17 ± 3.9	69.5 ± 3.8
C)	Control	43.6 ± 6.1	31.7 ± 5.7	71.2 ± 6

Population development after thermal stress

As the direct effects of temperature and exposure intensity are clearly manifested on the individual level, the effect on the population development was also evaluated according to these two criteria. The average number of nymphs produced after 8 weeks and the number of casted exuvia in each treatment was used as response measure that was compared to the control population. Elevated temperatures with constant exposure had a significant effect on the total number of nymphs produced (Kruskal-Wallis; $H=45.4$, $df=6$, $p<0.001$) and the number of casted exuvia (Kruskal-Wallis; $H=38.0$, $df=6$, $p<0.001$). Disrupted exposure of elevated temperatures also had similar effects on the total number of nymphs in the different treatments (Kruskal-Wallis; $H= 17.8$, $df=6$, $p=0.007$) and on the number of exuvia casted (Kruskal-Wallis; $H=32.13$, $df=6$, $p<0.001$). The total population effects (Figure 8) could thus be assigned as; 1) **disaster**, if all adult bed bugs were dead or had not managed to produce any

eggs or offspring (dark red), 2) **strong**, if both the number of nymphs and casted exuvia was significantly different from the control (red), 3) **intermediate**, if one of the responses were different from the control (yellow) and 4) **no effects**, none of the responses were different from the control (green). The control had a high count of both total numbers of nymphs and casted exuvia. High counts of exuvia imply that the population is able to progress into higher levels of development. Higher temperatures and higher intensity in exposure gave a lower count of hatched nymphs and casted exuvia than treatments with lower temperature exposure and lower intensity (Figure 8).Although the three treatments with the lowest intensities with exposure to 34°C did not differ significantly from the control, it is worth noticing that they approximately had half the amount of offspring and exuvia compared to the control (Figure 8). The average instar among all the 34°C treatments was 2.5 ± 0.3 (Figure 8). In the 36°C treatments average instar was 2.0 ± 0.4. The two treatments with the highest intensity with exposure to 38°C did not have any living adults left to produce any offspring in the population recovery period and the average instar for all four treatments was 1.0 ± 0.6. The control had nymphs that progressed into the fourth instar.

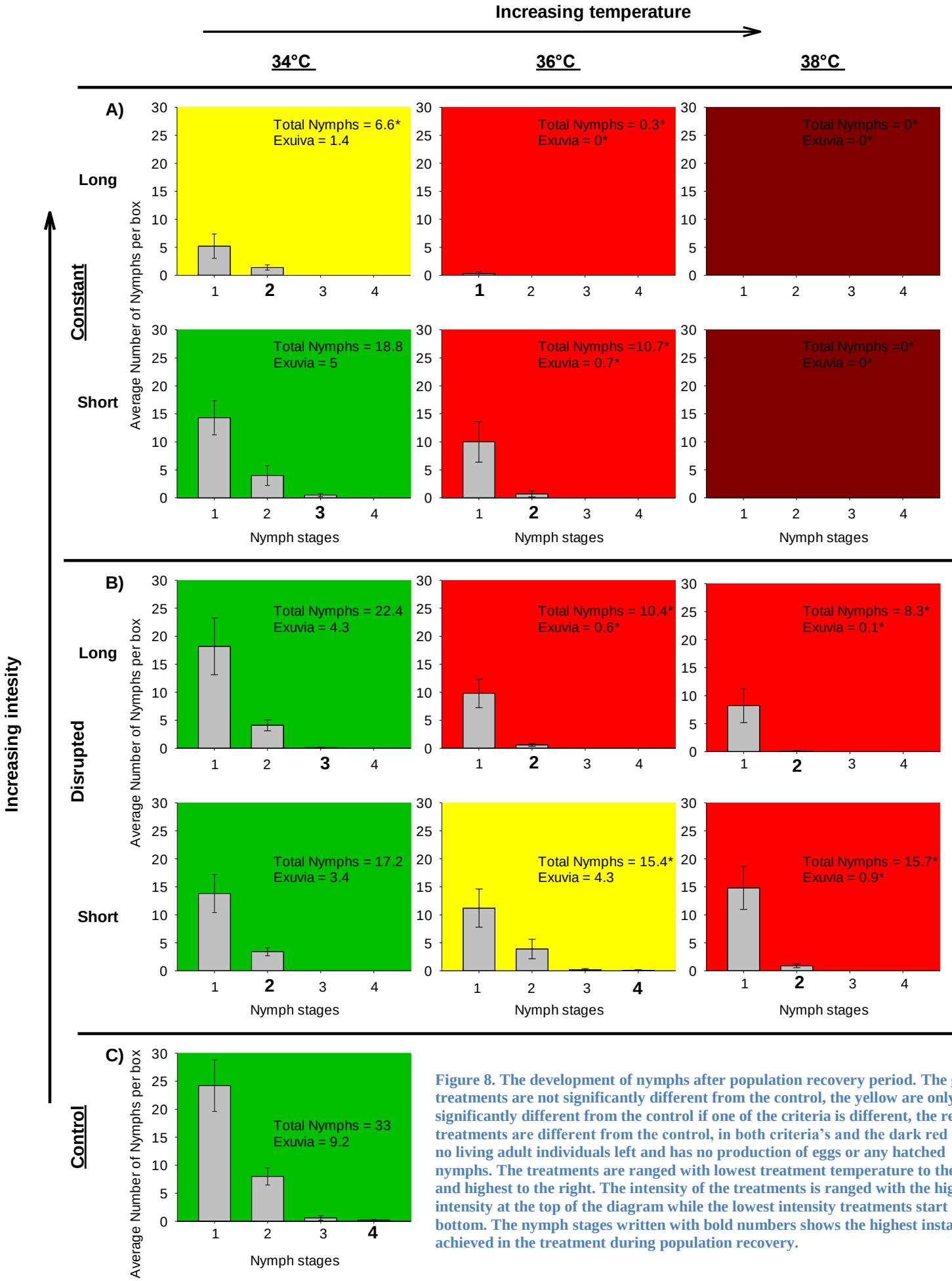


Figure 8. The development of nymphs after population recovery period. The green treatments are not significantly different from the control, the yellow are only significantly different from the control if one of the criteria is different, the red treatments are different from the control, in both criteria's and the dark red has no living adult individuals left and has no production of eggs or any hatched nymphs. The treatments are ranged with lowest treatment temperature to the left and highest to the right. The intensity of the treatments is ranged with the highest intensity at the top of the diagram while the lowest intensity treatments start at the bottom. The nymph stages written with bold numbers shows the highest instar achieved in the treatment during population recovery.

Discussion

This study has revealed that the use of sub lethal temperatures for an extended period of time can cause negative effects on bed bug populations in terms of increased adult mortality, lower feeding abilities, reduced production of eggs and nymphs, and lowered ability for the offspring to progress to the next instar. The two highest temperature treatments had more detrimental impacts, during thermal stress and right after thermal stress and in the later population recovery period. The lowest treatments had some the negative long term effects related to moulting disabilities, egg production and hatching success. This will cause direct impact on the bed bug population as it will reduce an established population size immediately after or during treatment while the long term effects, such as lowered egg production and nymph development, mostly reduces the rate of population growth in the future. This could consequently limit reoccurring infestations in bed bug populations.

The treatment temperatures 38°C, 36°C and 34°C were selected based on information from previous studies (Benoit, 2011, Rukke et al 2015). In Rukke et al (2015) study, they examined the direct effects of the temperature range 34°C-40°C with exposure for 3, 6 and 9 days and also at the long term effects on feeding, fertility and development of offspring's of bed bugs exposed to 35.5°C-38.5°C. My thesis examined the temperature range 34°C-38°C with even longer exposure time and also involved examination of the long term effects on the second generation. In their experiment, bed bugs that were exposed to 38.5°C for 3 or 6 days significantly lowered their offspring's feeding and moulting ability. In my study, bed bugs exposed for a longer period produced offspring's with defects in even in the 36°C treatments and some in the 34°C treatments as well. There was a clear difference in mortality between the three temperatures even with a modest change of 2°C. This indicates that the selected temperatures indeed are close to the critical range and are actually well above the expected tolerance level for the bed bugs if exposure time is sufficiently prolonged. Three weeks of exposure to 36°C and 38°C induced high levels of mortality. Even at 34°C treatments mortality occurred at the end of thermal stress period, in addition to mortality all treatments suffered from reduced fertility with reduced production of eggs and reduced hatching ability in the long run. In Rukke et al (2015) bed bugs exposed to 38.5°C had 100% mortality after 9 days, in my study the bed bugs exposed to 38°C, 0.5°C lower than Rukke et al (2015) reached 100% after 13 days. This confirms that these insects have little variation of resistance to high temperatures (Chown & Nicolson 2004).

The constant exposure treatments had higher mortality among adults and the nymphs than in the disrupted treatments during the thermal stress period. When comparing the long treatments to the short treatments, both are identical when it comes to progress in mortality. Even though the heat exposure stopped after 14 days in the short treatments, a few bed bugs continued to die in the following week. This effect was observed even in the lowest temperature treatments. It is obvious that the bed bugs are deeply affected by the temperatures used in this experiment and that it takes time before the repairing effects begins. Repairing abilities seem to be less effective during exposure in the thermal stress period. This supports that the temperatures, especially 36°C and 38°C do have negative effects on bed bugs if exposure time is long enough, and that these temperatures are not below their critical temperature range after all.

The bed bugs exposed to disrupted temperature treatments were not affected by thermal stress in the same degree as the bed bugs that were exposed to constant temperature treatments; even the highest temperature that induced 100% mortality in 21 days with constant exposure has little effect on mortality when disruption in exposure occurs. In the disrupted treatments, the bed bugs seem to be able to recover from the damages caused by the thermal stress during the 4 days with room temperature between the heat exposures. The total experienced exposure time is also severely reduced compared to the total exposure time in the constant exposure treatments which also explains the reduced effects. Since bed bugs are thought to originate from bat caves with stable temperature, adaptations to temperature extremes may be scarce. However, feeding on humans induce a short time thermal stress on the bed bugs as the blood has a temperature of 37°C and insects have consequently evolved physiological responses to heat to avoid thermal wounding in short term exposures (Lahondre & Lazzari 2012). This study confirms that bed bugs are indeed able to handle temperatures well beyond what is found in their natural habitat if long disruptions occur during exposure. Production of heat shock proteins limit mortality among the bed bugs in the disrupted treatments as these proteins prevents unfolding of proteins and structures within the bed bug and minimizes harmful effects (Chown & Nicolson 2004). Three days with exposure and 4 days of disruption is sufficient enough for the bed bug to be able to recover from the damages caused by the thermal stress. It has been shown that one hour exposure to 44°C would yield a upregulation of heat shock proteins (Hsp70 and Hsp90) already 2 hours after exposure (Benoit et al. 2009a). It is not tested whether or not it would be the same in what was considered as

temperature well below critical range of short term exposure. My thesis do however imply that the temperature range 34°C-38°C is high enough to trigger the response to produce heat shock proteins to withstand mortality when exposed to sub lethal temperatures for 3 days at a time. Producing these proteins is very energy demanding for the bed bugs (Chown & Nicolson 2004). It could be that shorter disruptions (less than 4 days) but more frequent exposures could have more damaging effects due to increased stress due to up and down regulation of heat shock proteins during exposures and disruptions (Chown & Nicolson 2004).

The bed bugs with constant exposure to high temperatures had severely reduced feeding ability when fed 3 days after ended thermal stress. Disrupted exposure to sub lethal temperature did not have any significant effects on feeding ability, but there were some reductions in feeding for the 38°C treatments. It is not known exactly why feeding ability is affected by exposure to thermal stress. Not being able to feed is an important factor for bed bug control. If the disability to feed is permanent, the surviving population is not able to produce any new offspring's and the nymphs won't be able to develop. This will have a great effect on the population and the population growth. In Rukke et al (2015) the offspring's of the exposed bed bugs were able to feed, but could not moult into the next instar. The explanation for why feeding was low right after constant exposure to thermal treatment could be that the bed bugs needed more days to recover from the damages during thermal stress. The disrupted treatments had much better feeding ability, possibly due to lower levels of heat stress and were therefore able to recover from the damages. It would have been interesting to follow the feeding abilities throughout the recovery period, but due to increasing moulting of bed bugs and high activity caused by increased number of bed bugs in the experimental boxes it became too difficult to keep track of which bed bug you already counted. It was also difficult to notice the difference between adults, sex and nymphs after being feed. Physical handling of the bed bugs could also cause physical wounding and stress. The folded filter paper and other practical limitations thus led to the decision to not pursue further measurement of feeding ability throughout the experiment (Figure 3).

During thermal treatment negative effects on egg production and hatching success appeared to mostly rely on the maximum temperature experienced and not duration or disruption in temperature exposure. The hatching success during high thermal stress in both constant and

disrupted treatments are similar and well below 5% whereas the treatments with exposure to 34°C had a hatching success above 60%. That is an enormous increase for just a temperature reduction of 2 degrees Celsius. During the population recovery period of 8 weeks the adults from the constant exposure treatments were still producing a low count of eggs and the hatching success was reduced by half. The control approximately produced 4 times as many eggs in average. This would lead to a severe reduction in growth for the treated bed bug population for both during and after thermal stress as fewer new individuals would hatch. Even the 34°C treatments have negative effects if exposure time is prolonged. Previous studies has shown that high mortality does occur among eggs with long exposure to 34°C-37°C (Johnson 1941; Rukke et al. 2015). My study also confirms this effect. The adults from the disrupted treatments were doing much better producing eggs during the recovery period than the constant exposure treatments. Even the treatments with the two highest temperatures had a high production of eggs and hatching success in the recovery period. Rukke et al (2015) experienced similar effects when exposing eggs to thermal treatment with temperatures 34°C to 40°C, all the laid eggs died during exposure to the highest temperatures and high levels of mortality occurred in the lower treatment temperatures. However, eggs laid after thermal exposure had higher survival rate. This coincides with the results from my study were there was an increased production of eggs and increase in hatching success during population recovery than during thermal stress.

After the 8 week recovery period and 4 potential feedings the fifth instar nymphs that were exposed to thermal stress had severe difficulties in moulting into adults. High heat intensity treatments had the largest impact on moulting success, but in all the treatments, including the disrupted exposure treatments, less than half the nymphs or less were able to moult into adults. The test for disrupted treatments returned not significant with a p-value equal to 0.053, nonetheless, I would say it is biologically significant as it would have an effect on the population development. If the ability to progress from nymphs to adult is reduced permanently, the surviving population will have few new adults to produce any new offspring. This moulting arrest will have a great effect on the population and the population growth. The exact mechanisms for why the fifth instars are not able to develop into adults are not known, but lack of B vitamins is most likely the cause.

The moulting ability of the nymphs can indicate how the population is progressing and partially determine the growth rate in the long run. A high count of nymphs in a population does not matter if the nymphs cannot progress towards the adult stage or if development is delayed with several months like it was in this study. There was a clear link between temperature, intensity of the treatment, nymph count and the number of moults (Figure 8). Even the offspring's of the bed bugs that had experienced intermediate sub lethal heat clearly struggled to progress over to higher stages during the population recovery. At the same time the fecundity of the surviving adults and the progress of the surviving fifth instar nymphs are dramatically reduced. The consequence of these 3 elements combined could have severe effects on the population dynamics over time. Even if some adults mange to survive the thermal treatment, the offspring they produce will not be able to develop normally and would not be able to reproduce and create new offspring's of them self, at least not for a long time. This could be good news for bed bug management as these delayed population effects will slow the infestation rate and reduce the possibility to further contaminations of nearby sites. Combined with other control measurements this could lead to the collapse of the population, and treating the bed bugs with thermal stress in addition to traditional treatments could be more efficient than conventional methods used today.

Other insect such as aphids, ants, weevils and cockroaches are also associated with the *Wolbachia* symbiont that provides them with essential nutrient such as B vitamin. Several studies have revealed that high temperature treatment can lead to loss of symbionts in the mycetomes of bed bugs and that the balance of the host-symbiont relationship is disturbed (Chang 1974; Li et al. 2014; Nikoh et al. 2014; Wernegreen 2012). Based on the results from my study, I might suggest that the thermal limit for the *Wolbachia* may be found between 34°C and 36°C if exposure time is long enough. Rukke et al (2015) also confirms this theory. In addition to fecundity, the knockdown of symbionts can also explain the reduced development on the offspring's from the treated bed bugs. If high temperatures gives a greater reduction of symbionts than lower temperatures it would explain why the highest temperature treatments had the most under developed nymphs compared to the control and the lower temperature treatments. With low amounts of the symbionts the nymphs can't have a normal development and would also be malnourished. In Rukke et al (2015) the nymphs hatched after thermal stress were able to feed, but suffered with severe moulting arrest. Without symbionts

to produce any B vitamins, development slows down or is completely stopped (Hosokawa et al. 2010).

The temperature range of 34°C -38°C is a realistic temperature that could easily be reached in a room with the use conventional heaters and fans that most households may already own (Rukke et al. 2015). Most pest managing companies could argue that a 2-3 weeks treatment or more would be impractical and that most people would think that closing off a room for 3 weeks is too long. However the time it takes from the first suspicion of a bed bug infestation to when the actual treatments is over may as well take 3 weeks. First of all you need to book an appointment with the pest managers to come in for an inspection which could potentially take 1 week. When the suspicion is confirmed, all the preparations before the treatments can start has to be made; removal, and treatment of infested items and sealing of the room. It is first after this that the various treatments methods could be applied. The process of treating a bed bug infestation is long and it probably needs to be for the method to be efficient. An alternative or supplemental approach to defeat a bed bug infestation could be to turn up all the heaters to a maximum and simultaneously go about normal bed bug control routines with the help of professionals. If the temperature in the room is around 38°C during the treatments period and remains at this temperature for a period of 3 weeks most of the bed bugs and the eggs would die and any surviving individuals would be severely reduced and weakened and thereby making it more efficient to deal with the remaining individuals by using a combination of pesticides, desiccant dust, essential oils, entomopathogenic fungi, traps, bariers and alarm- or host signals for activation (Aak et al. 2014; Benoit et al. 2009b; Koganemaru 2013). Some studies have shown that bed bugs are more susceptible to pesticide after being stressed, either by hunger or other stressful factors like sub lethal heat treatment (Doggett et al. 2012). This approach, based on the results of this study, could have the potential to be more efficient than conventional methods and it does not require expensive equipment's like high capacity heaters. This method has the potential to thrive as it has the potential to kill of all or nearly all individual bed bugs are high. Any surviving bed bugs would be severely reduced. Sealing the infested room is highly recommended as this method still contains the risk of spreading the infestation to neighbouring rooms. Anecdotal reports point out that bed bugs may disperse to other rooms when exposed to elevated temperatures. What precise temperature exposure that triggers this response has not yet been identified. The disruptions that would occur during the treatment should be limited to the minimum to avoid

drop in treatment temperature. As shown in this study, bed bugs are less affected by thermal stress when given time to recover from thermal wounding and frequent disruption in treatment will reduce the effect of the treatment. It is important to note that the tested temperature range is harmful for *C. lectularius* which is adapted to temperate regions. The *C. hemipterus* which is adapted to tropic and sub-tropical regions has a higher temperature tolerance and the critical temperature range for this species may be higher than 34-38°C. It is important for pest controllers to identify the bed bug to species level when inspecting the infected room to be able to adjust treatment method. Exposing *C. hemipterus* to 38°C for 2 weeks may not have similar detrimental effects like it was for *C. lectularius* and this method would perhaps not work at all on this species.

Future studies

Sub lethal heat treatment has the potential to be a part of integrated pest management method. But before being able to utilize sub lethal heat treatment with the combination of other treatment methods as an approach to control bed bugs, it has to be tested in a field study. It is important to test how the heat will be distributed in the room and if high temperatures are reached inside the walls as well. This could easily be tested with the use of temperature loggers placed in different parts of the room. There are few studies of how the behaviour of the bed bugs is effected during sub lethal heat exposure, will sub lethal heat cause dispersal of bed bugs as a response to the heat? And if so, how long time would it take before the response is triggered? This study only examined the long term effects of sub lethal heat exposure for 2 months after exposure. It would be interesting to see how long it would take for the populations to reach normal development after being treated with thermal stress. How would the situation be in 6 months for example, would the population still have a large proportion of nymphs in the early instars or are they progressing faster and into a normal development rate? All these question needs to be answered for us to understand the impact of heat treatment on bed bug. We can use this information to develop a bed bug control method that is efficient and can withstand the bed bugs ability to develop resistance.

Conclusion

This method is not a stand-alone solution against bed bugs, but could be an efficient method if used in combination with IPM. The results of this study highlight the importance to also study the long term effects when evaluating pest management methods. As it is with a lot of other thing, too much of anything can be dangerous. Sub lethal temperatures, even if not deadly in short term exposures, it can cause detrimental effects on bed bugs if exposure is long enough.

Acknowledgments

First of all I would like to thank my supervisors Anders Aak, Tone Birkemoe and Bjørn Arne Rukke for valuable help and guidance during the whole process of this thesis. I would also like to thank the Norwegian Institute of Public Health, Department of pest control for help and supplying equipment supporting this study, and of course for letting me feel like a part of the team. Thanks to my family for supporting me during this period and for encouraging me to pursuing this.

References

Aak, A., Rukke, B. A., Soleng, A. & Rosnes, M. K. (2014). Questing activity in bed bug populations: male and female responses to host signals. *Physiological Entomology*, 39 (3): 199-207.

Austin, D. (2013). Bed bug suplement, avoiding hot water. *Pest Control Technology* (41): 42-44.

Bacot, A. W. (1915). LXXXII. Notes on the development of Bacillus pestis in bugs (Cimex lectularius) and their power to convey infection. *The Journal of hygiene*, 14 (Suppl): 777-792.3.

Benoit, J. (2011). Stress Tolerance of Bed Bugs: A Review of Factors That Cause Trauma to Cimex lectularius and C. Hemipterus. *Insects*, 2 (2): 151-172.

Benoit, J. B., Lopez-Martinez, G., Teets, N. M., Phillips, S. A. & Denlinger, D. L. (2009a). Responses of the bed bug, Cimex lectularius, to temperature extremes and dehydration: Levels of tolerance, rapid cold hardening and expression of heat shock proteins. *Medical and Veterinary Entomology*, 23 (4): 418-425.

Benoit, J. B., Phillips, S. A., Croxall, T. J., Christensen, B. S., Yoder, J. A. & Denlinger, D. L. (2009b). Addition of alarm pheromone components improves the effectiveness of desiccant dusts against Cimex lectularius. *Journal of Medical Entomology*, 46 (3): 572-579.

Brown, A. W. A. P., R. (1971). Insecticide Resistance in Arthropods. *WHO Monograph Series*, 38.

Busvine, J. R. (1957). Recent progress in the eradication of bed bugs. *Sanitarian*, 65: 365-369.

Chang, K. P. (1974). Effects of elevated temperature on the mycetome and symbiotes of the bed bug Cimex lectularius (Heteroptera). *Journal of Invertebrate Pathology*, 23 (3): 333-340.

Chown, S. L. & Nicolson, S. W. (2004). *Insect Physiological Ecology*. United States: Oxford University Press.

Cooper, R. (2011). *Mallis Handbook of Pest Control*. Richfield, Ohio: The Mallis Handbook Company.

Davies, T. G. E., Field, L. M. & Williamson, M. S. (2012). The re-emergence of the bed bug as a nuisance pest: Implications of resistance to the pyrethroid insecticides. *Medical and Veterinary Entomology*, 26 (3): 241-254.

Doggett, S. L., Dwyer, D. E., Peñas, P. F. & Russell, R. C. (2012). Bed bugs: Clinical relevance and control options. *Clinical Microbiology Reviews*, 25 (1): 164-192.

Folkehelseinstituttet. (2015). *Skadedyrovervåkning 2007–2014*. http://www.fhi.no/dokumenter/6afb40ede6.pdf.

Fountain, T., Butlin, R. K., Reinhardt, K. & Otti, O. (2015). Outbreeding effects in an inbreeding insect, Cimex lectularius. *Ecology and Evolution*, 5 (2): 409-418.

Harlan, H. J. (2006). Bed Bugs 101: the basics of Cimex lectularius. *American Entomologist*, 52: 99-101.

Hosokawa, T., Koga, R., Kikuchi, Y., Meng, X. Y. & Fukatsu, T. (2010). Wolbachia as a bacteriocyte-associated nutritional mutualist. *Proceedings of the National Academy of Sciences of the United States of America*, 107 (2): 769-774.

Hwang, S. W., Svoboda, T. J., De Jong, I. J., Kabasele, K. J. & Gogosis, E. (2005). Bed bug infestations in an urban environment. *Emerging Infectious Diseases*, 11 (4): 533-538.

Johnson, C. G. (1941). The ecology of the bed-bug, Cimex lectularius In Britain. 345-461 pp.

Kells, S. A. (2006). Nonchemical Control of Bed Bugs. *American Entomologist*, 52 (2): 109-110.

Kells, S. A. & Goblirsch, M. J. (2011). Temperature and Time Requirements for Controlling Bed Bugs (Cimex lectularius) under Commercial Heat Treatment Conditions. *Insects*, 2 (3): 412.

Koganemaru, R. M., D. M. (2013). The bed bug problem: Past, present, and future control methods. *Pesticide Biochemistry and Physiology*, 106: 177-189.

Lahondre, C. & Lazzari, C. R. (2012). Mosquitoes cool down during blood feeding to avoid overheating. *Current Biology*, 22 (1): 40-45.

Lehane, M. J. (2005). *The Biology of Blood-Sucking in Insects*. second ed. ed. Cambridge, United Kingdom: Cambridge University Press.

Leulmi, H., Bitam, I., Berenger, J. M., Lepidi, H., Rolain, J. M., Almeras, L., Raoult, D. & Parola, P. (2015). Competence of <italic>Cimex lectularius</italic> Bed Bugs for the Transmission of <italic>Bartonella quintana</italic>, the Agent of Trench Fever. *PLoS Negl Trop Dis*, 9 (5): e0003789.

Li, Y. Y., Floate, K. D., Fields, P. G. & Pang, B. P. (2014). Review of treatment methods to remove Wolbachia bacteria from arthropods. *Symbiosis*, 62 (1): 1-15.

Mellanby, K. (1939). The physiology and activity of the bed-bug (Cimex lectularius L.) in a natural infestation. *Parasitology*, 31 (02): 200-211.

Nikoh, N., Hosokawa, T., Moriyama, M., Oshima, K., Hattori, M. & Fukatsu, T. (2014). Evolutionary origin of insect-Wolbachia nutritional mutualism. *Proceedings of the National Academy of Sciences of the United States of America*, 111 (28): 10257-10262.

Olson, J. F., Eaton, M., Kells, S. A., Morin, V. & Wang, C. (2013). Cold tolerance of bed bugs and practical recommendations for control. *Journal of Economic Entomology*, 106 (6): 2433-2441.

Pereira, R. M., Koehler, P. G., Pfeister, M. and Walker, W. (2009). Lethal Effects of Heat and Use of Localized Heat Treatment for

Control of Bed Bug Infestations. *Journal of Economic Entomology*, 102 (3): 1182-1188.

Potter, F. M., Romero, Alvaro., Haynes, F. Kenneth. (2006). Battling Bed Bugs in Apartments. *Pest Control Technology Magazine*.

Potter, M. F. (2011). The history of bed bug management - with lessons from the past. *American Entomologist*, 57: 14-25.

Reinhardt, K. & Siva-Jothy, M. T. (2007). *Biology of the bed bugs (Cimicidae).* Annual Review of Entomology, 52. pp. 351-374.

Romero, A., Potter, M. F., Potter, D. A. & Haynes, K. F. (2007). Insecticide Resistance in the Bed Bug: A Factor in the Pest’s Sudden Resurgence? *Journal of Medical Entomology*, 44 (2): 175-178.

Romero, A., Potter, M. F. & Haynes, K. F. (2010). Circadian rhythm of spontaneous locomotor activity in the bed bug, Cimex lectularius L. *Journal of Insect Physiology*, 56 (11): 1516-1522.

Rozendaal, J. A. (1997). Bedbugs, fleas, lice, ticks and mites. *Vector Control: Methods for Use by Individuals and Communities, Chapter 4*: 237-241.

Rukke, B. A., Aak, A. & Edgar, K. S. (2015). Mortality, Temporary Sterilization, and Maternal Effects of Sublethal Heat in Bed Bugs. *PLoS ONE*, 10 (5): e0127555.

Salazar, R., Castillo-Neyra, R., Tustin, A. W., Borrini-Mayorí, K., Náquira, C. & Levy, M. Z. (2015). Bed bugs (Cimex lectularius) as vectors of Trypanosoma cruzi. *American Journal of Tropical Medicine and Hygiene*, 92 (2): 331-335.

Sharpe, R. M. & Irvine, D. S. (2004). How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? *BMJ : British Medical Journal*, 328 (7437): 447-451.

Susser, S. R., Perron, S., Fournier, M., Jacques, L., Denis, G., Tessier, F. & Roberge, P. (2012). Mental health effects from urban bed bug infestation (Cimex lectularius L.): A cross-sectional study. *BMJ Open*, 2 (5).

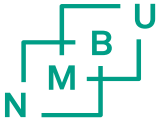
The Bedbug, F. (2013). *European Code of Practice Version 2 Bedbug Management*. Januray 2013 ed. http://bedbugfoundation.org/#https://dl.dropbox.com/u/3217659/ECoPv2%20WebVersion%20Jan%2013.pdf.

Usinger , R. L. (1966). *Monograph of Cimicidae (Hemiptera-Heteroptera)*. College Park, MD: Thomas Say Foundation.

Wang, C., Gibb, T. & Bennett, G. W. (2009). Evaluation of Two Least Toxic Integrated Pest Management Programs for Managing Bed Bugs (Heteroptera: Cimicidae) With Discussion of a Bed Bug Intercepting Device. *Journal of Medical Entomology*, 46 (3): 566-571.

Weeks, E. N., Birkett, M. A., Cameron, M. M., Pickett, J. A. & Logan, J. G. (2011). Semiochemicals of the common bed bug, Cimex lectularius L. (Hemiptera: Cimicidae), and their potential for use in monitoring and control. *Pest Management Science*, 67 (1): 10-20.

Wernegreen, J. J. (2012). Mutualism meltdown in insects: Bacteria constrain thermal adaptation. *Current Opinion in Microbiology*, 15 (3): 255-262.



Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no