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Hive Insulation Valuation Experiment:

Assessing the impacts of thermally improved beehives during the active season in a sub-arctic climate

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Table of Contents

Table of Contents.....	ii
List of tables.....	v
List of figures.....	vi
Abstract.....	1
1 Introduction.....	2
1.1 Potential effects of enclosure thermal conductance on colony health and productivity.....	4
1.2 The Yukon context.....	7
1.3 Location of the study.....	8
1.4 Studies investigating the impact of insulation on bee colonies.....	9
1.5 Aims of this study.....	14
1.6 Study hypotheses.....	14
2 Methods.....	16
2.1 Set up of treatment and control hives.....	16
2.2 Data collection.....	17
2.2.1 Collection of primary data.....	20
2.2.1.1 Frame inspections.....	20
2.2.1.2 Hive weight.....	22
2.2.1.3 Mite counts.....	23
2.2.2 Secondary metrics.....	23
2.2.2.1 Internal hive temperature.....	23
2.2.2.2 Ambient temperature.....	23
2.3 Data screening and analysis.....	24
2.3.1 Missing data and exclusions.....	24
2.3.1.1 Swarming.....	25
2.3.1.2 Queenless period.....	26
2.3.1.3 Confounding interventions.....	26
2.3.2 Frame inspections.....	27
2.3.2.1 Net honey storage rate.....	27
2.3.2.2 Brood rearing rate.....	28
2.3.2.3 Weight.....	28
2.3.2.3.1 Isolating the superorganism weight.....	28

2.3.2.3.2	Step-changes and solution.....	29
2.3.2.3.3	Removal of outlying weight observations.....	30
2.3.2.3.4	Daily weight variation patterns.....	32
2.3.2.4	Temperature and relative humidity.....	33
2.3.3	Varroa mite infestation.....	33
2.3.3.1	Autumn feeding.....	34
3	Results.....	35
3.1	Honey storage.....	37
3.2	Brood rearing.....	38
3.3	Weight gain.....	41
3.4	Varroa mite infestation results.....	45
3.5	Temperature within the hive.....	46
3.6	Humidity.....	49
3.7	Comb building.....	51
3.8	Apiary comparisons.....	52
3.9	Supplemental feed consumption.....	54
3.10	Weight of bees in a hive.....	55
3.11	Swarming.....	55
4	Discussion and conclusions.....	56
4.1	Honey storage.....	56
4.2	Brood rearing.....	57
4.3	Comb building.....	58
4.4	Weight gain.....	58
4.5	Varroa mite infestation.....	59
4.6	Comparisons between study apiaries.....	60
4.7	More swarms from insulated hives.....	60
4.8	Study limitations and recommendations for further research.....	61
4.8.1	Colony and apiary management.....	61
4.8.2	Data collection, preparation, and analysis practises.....	61
4.8.3	Experimental design.....	62
4.9	Conclusions.....	63
5	References.....	64

6	Acknowledgements.....	67
7	Appendix A - Supplementary Tables	68
8	Appendix B – Photos	69

List of tables

Table 1 Selected results from voluntary survey of beekeepers in Yukon.	7
Table 2 Results reported in Villumstads comparison	12
Table 3 Results from Bayburt, Turkey	13
Table 4 Distribution of replicates in five apiaries	17
Table 4 Weather records Whitehorse.....	19
Table 5 Excerpt of spreadsheet used to log results of frame inspections.....	22
Table 6 Data excluded from analysis	24
Table 7 Summary of results.	36
Table 9 Temperatures observed under hives at elemental and river road apiaries.....	53
Table 10 Temperature observations grouped by inspection period and apiary	53
Table 11 Dates of nucleus colony installations and subsequent inspections.	68

List of figures

Figure 1 Climate of southern Yukon.....	8
Figure 2 Temperature profiles for the Bayburt, Hedmark, and Whitehorse areas..	10
Figure 3 Monthly precipitation at Bayburt, Hedmark, and Whitehorse	11
Figure 4 Hive set up	16
Figure 5 Magnitude of errors in visual estimation in the first 4 training runs,.....	21
Figure 6 Magnitude of errors following training.	21
Figure 7 Step-changes occurring on July 18 th	30
Figure 8 Before and after outlier removal.....	31
Figure 9 Example superorganism weights	32
Figure 10 Net daily honey storage rate.	37
Figure 11 Brood rearing rates at each inspection.....	38
Figure 12 Spatial distribution of capped worker brood observed.....	39
Figure 13 Capped brood on outermost frames.....	40
Figure 14 Daily average Superorganism weight (kg).....	41
Figure 15 Weight gain rate 4 th July to 14 th August.....	42
Figure 16 Weight gain rate 4th July to 14th August (41 days).....	43
Figure 17 Superorganism weight	44
Figure 18 Mite drop counts on 17th September.	45
Figure 19 Mean daily temperature box 1 June 1st to September 17th.....	46
Figure 20 Mean daily temperature box 2 June 14th to September 17th.....	47
Figure 21 Boxplot of temperatures in individual hives.	48
Figure 22 Daily average RH	49
Figure 23 Daily RH range observed	50
Figure 24 Comb building rates inspection period 1, 2, & 3.....	51
Figure 25 Elemental comparison.....	52
Figure 26 Sugar syrup consumption August 28 th to September 14 th	54

Abstract

Thermal properties of thin walled wooden hives are inferior to those of tree cavities preferentially chosen by wild and feral colonies of honey bees. Health and productivity of colonies within standard wooden hives were compared with those within insulated hives during the active foraging season. Sixteen hives newly established from nucleus colonies were studied at five apiaries in a subarctic region within 150km of Whitehorse, Yukon, Canada. Eight colonies were housed in standard hives (control group), eight were housed in insulated hives (treatment group). Honey production, brood rearing, weight gain, varroa infestation, temperatures, humidity, comb building rate, and autumn syrup consumption rates were measured.

Insulation changed internal hive conditions; higher mean temperatures, a lower daily temperature range, lower humidity, and a lower daily range of humidity were observed. This was linked to increased early-season honey storage rate, lower Varroa infestation and faster comb building, in comparison to the uninsulated hives. Insulated colonies were also found to have an increased ability to take down and convert supplemental syrup-feed in autumn. Results from the study show that insulated hives allow improved effectiveness and efficiency of thermoregulation and imply that improvements to colony health and productivity can follow. The study found that hive insulation and other management of apiary conditions can increase colony productivity considerably. In particular, that apicultural management with syrup feeding can be more efficiently applied to insulated hives.

Keywords: *Apis mellifera*, hive, insulation, temperature, Varroa, foraging efficiency.

1 Introduction

Since originating in the tropics, the Western honey bee (*Apis mellifera*) has expanded its natural range northwards and southwards as far as Scandinavia and South Africa (Seeley, 1985; Winston, 1987). Although the species' natural range is confined to Europe, Africa, and western Asia, the more productive races of the species have been introduced by humans to successfully colonise most of the rest of the world, including the Americas, east Asia, and Australasia. Large numbers of managed and feral colonies now exist on all continents except Antarctica, including apiaries as far as 68 degrees north in Norway and Sweden (Seeley, 2019). In North America, feral colonies are commonplace below 55° N, but rarer at higher latitudes – although at least one successful overwintered feral colony has been reported above 61° N in Anchorage, Alaska (Malone, 2014; Wolske, 2020). As its natural range extended, the species did not adapt to colder winters at higher latitudes by hibernating like bumble bees, nor through large scale physiological adaptation. Their success in adapting to temperate regions with relatively long, cold winters is in part due to their unique and sophisticated nest thermoregulation capabilities (Seeley, 1985; Winston, 1987; Seeley, 2019).

The primary thermoregulation activities include heat generation and heat retention. The former is modulated as individual bees switch from a resting metabolic rate to a deliberate shivering thermogenesis using thoracic flight muscles (Simpson, 1961; Stabentheiner et al., 2003). The latter is achieved through colony clustering and the adaptive use of propolis to seal the nest. Mitchell (2019a) calculates that a tightly clustered colony reduces the exposed surface area per bee by as much as 60 times compared to a colony dispersed throughout the nest. The outer bees of a cluster also form an insulating layer protecting the core of the cluster (Seeley, 1985; Seeley, 2019; Winston, 1987; Stabentheiner et al., 2003). These thermoregulation mechanisms allow colonies to maintain three minimum temperature setpoints; 34.5 ±1 °C in the brood nest, typically located at the centre of the hive; 18 °C at the core of a broodless cluster, a minimum necessary to allow shivering thermogenesis to continue; and 9-11 °C for all other individuals as this is the temperature below which they may enter a chill coma and

become incapacitated (Stabentheiner et al., 2003; Stabentheiner, Kovac and Brodschneider, 2010; Southwick and Heldmaier, 1987).

Often referred to as a domesticated species, *A. mellifera* could also be considered semi-domesticated (Seeley, 2019). Unlike most other domesticated species utilised by humans, the western honey bee's phenotype has been largely unchanged by human selection (Seeley, 1985). In contrast, the enclosures within which honey bee colonies are commonly housed by commercial beekeepers have many characteristics which are radically different to those chosen by wild or feral colonies. Compared with standard hives (e.g. the Langstroth type), tree cavities typically chosen by wild or feral colonies in temperate climates have lower thermal conductance, higher heat capacity, and predominantly single, smaller entrances located near the bottom of the cavity as opposed to large, or multiple entrances which are common on hives (Mitchell, 2016; Seeley, 2019). Honey bee colonies have been described as superorganisms (Moritz and Fuchs, 1998; Seeley, 1989), the colonies interaction with their nest and combs being so intimate that they could be considered analogous to the organs of the superorganism (Tautz, 2008). In this study the combined weight of bees, comb, stores and brood were treated as the superorganism weight. The enclosure and its associated controlled flows of energy and fluids can be considered an important part of the extended phenotype, Mitchell (2019c; 2019b; 2017; 2019a; 2019d) added detail to this concept by applying a thermofluids approach to examining the effects of the thermal properties of enclosures in which colonies are housed, and successfully made comparisons between impacts on managed colonies and wild / feral colonies. Mitchell proposed that the ratio of colony mass to the lumped thermal conductance of the nest; the Mass Conductance Ratio (MCR), has a strong influence on survival by affecting the success of clustering, humidity regulation, foraging efficiency, honey production, and suppression of *Varroa* mites. Ratios closer to those found in thick-walled tree cavities were found to be beneficial for all the above across all relevant seasons. He concluded that for a managed honey bee colony to control nest temperature, humidity and energy expenditure with similar efficiency of wild and feral nests, their enclosure should have a lumped thermal conductance of less than 0.5 WK^{-1} , and the colony should be of sufficient mass to generate an MCR value of at least $2.0 \text{ kgW}^{-1}\text{K}$.

Behaviours which enable survival over winter in climates colder than the tropics have been extensively studied (Southwick, 1985; Southwick and Heldmaier, 1987; Stabentheiner et al., 2003; Stabentheiner et al., 2010; Seeley, 2019; Watmough and Camazine, 1995; Winston, 1987). Improved understanding of winter survival behaviours has played a role in the development of many beekeeping practises (Hesbach, 2016). Similarly, improvements in understanding how hive thermal properties could be actively managed to support colony's activities during the warmer months, have potential for improving beekeeping practises benefiting colony health and productivity.

1.1 Potential effects of enclosure thermal conductance on colony health and productivity

Wild and feral colonies of *A. mellifera* commonly construct nests in trees within tall, narrow, thick walled cavities high above the ground; by contrast, most operational and research apiculture worldwide is conducted within thin-walled, squat wooden enclosures known as hives (Mitchell, 2016). The characteristics of enclosures chosen by wild and feral colonies have also been identified and contrasted with standard hives by Seeley (Seeley and Morse, 1978).

Reduced thermal conductance can result in obvious improvements in the energy efficiency of thermoregulation where the ambient temperature is lower than the temperature within the enclosure. The quantity of heat lost through the enclosure wall is related to the difference between the brood nest setpoint temperature (34.5 °C) and ambient. In many temperate areas (where beekeeping is widespread), the difference between ambient temperature and brood nest temperature during the active summer season can frequently be >20 °C.

Although previous work on the energy balance of nectar gathering and processing largely ignored the energy cost of nectar desiccation, Mitchell (2016) calculated that over 50% of the energy delivered to a hive by foragers may be used in this activity. Even in exceptionally favourable conditions in temperate climates, the cost of desiccation is at least 25%. When energy consumed during foraging is equal to the

energy contained in the foraged nectar, less all other energy used to process the nectar within the hive, then the foraging has reached a break-even distance. Therefore, improvements in the efficiency of nectar processing within the hive will extend the break-even foraging distance for any given nectar concentration and improve the profitability of foraging at distances below the break-even. This may be particularly relevant in areas where nectar sources are often widely dispersed, as is often the case in Yukon beekeeping (Tardif, 2019). Extended foraging break-even range and improved efficiency of nectar foraging and processing could increase net honey production in managed colonies with associated improvements in overall colony health.

Ambient temperatures low enough to provoke clustering in uninsulated hives are possible year-round in many temperate regions. Southwick (1982) established that clustering can occur in conditions equivalent to a thin-walled wooden hive at 10 °C external ambient temperature, whereas Mitchell (2016) proposes that clustering will not occur in thick walled tree cavities or insulated hives at temperatures above -60 °C or -20 °C, respectively. Clustering interrupts the normal productive summer behaviours of the colony, not least by potentially leaving brood unprotected and vulnerable. Temporary cooling of larvae increases risk of chalkbrood mummification (Flores et al., 1996).

As well as regulating temperature, honey bees have been shown to regulate the relative humidity (RH) in their nests but can “only adjust humidity within sub-optimal limits” due to linkages with other controlled parameters such as CO₂ levels and temperature (Human, Nicolson and Dietemann, 2006). CO₂ concentration inside the nest is regulated between 1000 and 4250 ppm through fanning behaviors which flush CO₂ from the enclosure (Seeley, 1974; Southwick and Moritz, 1987). Increases in metabolic heat generation result in increased CO₂ levels, and as CO₂ is flushed from the enclosure cooler air is drawn into the hive which usually is of lower absolute humidity (AH), potentially driving RH away from an optimal level. RH setpoints within the hive are not uniform, higher RH is desirable in the brood nest than in the nectar stores which are typically above the brood nest. This difference in RH is achieved despite the high quantity of water being evaporated in nectar stores during the honey ripening process (Human, Nicolson and Dietemann, 2006). Condensation on cool surfaces is another

factor which cannot be controlled in isolation by the colony but can drive RH away from setpoints. In a review of nest climate regulation, Simpson (1961) found when insulation is minimal and ambient temperature is low it is necessary to have free ventilation in order to avoid condensation within the hive, whereas insulated walls reduce condensation.

Deviations from optimum temperature and reduced humidity in the brood nest have been linked to increased brood mortality (Doull, 1976) and altered behaviour in resulting adult bees (Groh et al., 2004; Tautz et al., 2003; Stabentheiner et al., 2003; Bonoan et al., 2014).

Better insulation may enable a colony to maintain higher temperatures and a brood nest RH close to 80%, which have both been linked to reductions in disease and parasites (Chen et al., 2012; Flores et al., 1996; Kraus and Velthuis, 1997). Specifically Kraus found that Varroa mites almost never reproduce at RH above 80% while the optimum range for normal honey bee hatching was found to be between 90 and 95% RH (Doull, 1976). Conversely, in comparisons of Varroa fecundity at 40% RH versus 70% RH, Le Conte et al (1990) found higher fecundity at higher humidity levels. Taken together, both results suggest that Varroa fecundity has a left-skewed relationship with RH, fecundity peaking at an RH close to 70%. Le Conte also found that optimal temperature for Varroa development was 32.5 – 33.4 °C, while honey bees typically maintain the broodnest close to 34.5 °C with occasional short duration temperature increases which can moderate the development of Varroa.

1.2 The Yukon context

There are an estimated 100 – 150 hives in Yukon (Tardif, 2020). While honey production and winter loss are not recorded in national statistics, results of voluntary surveys covering 2017/18 and 2019/20 are highlighted in Table 1

Table 1 Selected results from voluntary survey of beekeepers in Yukon, adapted from (Tardif, 2020).

Year	Hives reporting	Honey harvested (kg)	Winter loss (following winter)
2017	108	1461	54%
2019	70	872	43%

It is common if not universal practise in the region to insulate hives during the winter months, the majority of hives are uninsulated for the duration of the active foraging season. Here it was investigated whether reduced thermal conductance of hives is associated with improved health and productivity when compared with standard hives. The research question will be addressed through data collection in southern Yukon and Northern British Columbia, an area of northern Canada, local to Whitehorse, Yukon (59 ° 35' N, 132 ° 42'W to 60°53' N, 135 ° 26' W).

1.3 Location of the study

There is a saying among beekeepers “all beekeeping is local” meaning that local climate and other environmental conditions influence colony development, activity and productivity, and is cause for varying apicultural practices from place to place. While some of the information produced by this study may have relevance for beekeepers in other regions in which daily average temperatures are usually well below brood nest temperatures, the primary aim was to produce information which can be of direct use to beekeepers in southern Yukon.

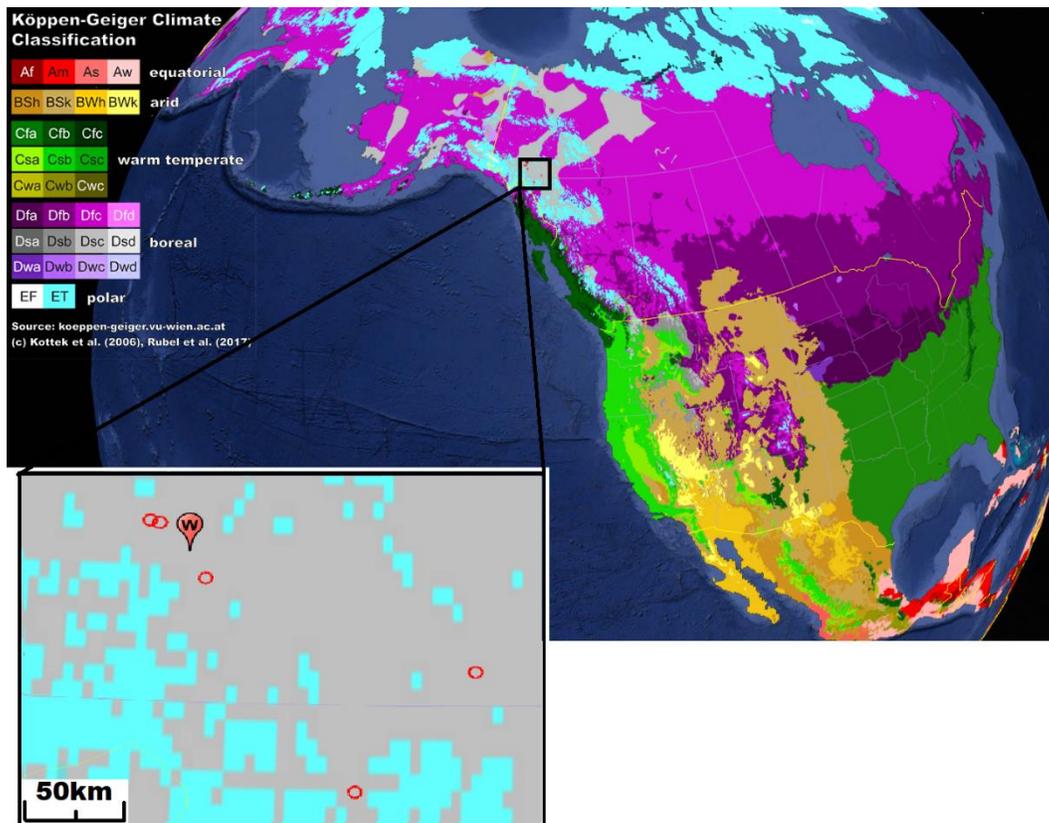


Figure 1 The five apiaries used in the study all share a common climate, as shown by the Köppen-Geiger climate classifications. The W marker indicates Whitehorse, Yukon (60°44' N, 135°5' W). Each small red circle represents a foraging area of c. 28 km² (3 km radius) surrounding each of the 5 apiaries. In the Köppen-Geiger system, this sub-arctic climate is classified as Dsc; meaning a cold continental climate with dry summers and less than three months where the daily average temperature is above 10°C (Peel, Finlayson and McMahon, 2007).

1.4 Studies investigating the impact of insulation on bee colonies

While multiple studies in Egypt, the Arabian Peninsula, and northern Australia have shown significant benefits to insulated hives during the productive seasons in hot climates, where the external temperatures are often higher than inside the nest presenting qualitatively different challenges to colonies and beekeepers. There have been very few studies in colder temperate or subarctic regions where average T_{Δ} during the productive season is negative by 15 °C or more. As illustrated in Figure 2 the daily average temperature in the location of this study is never above 14.5 °C so is always at least 20°C below the brood nest setpoint.

Investigations in Hedmark, Norway (Villumstad, 1974) and Bayburt, Turkey (Erdoğan, 2019) both occurred in regions where climactic temperatures during the productive season are somewhat similar to that of southern Yukon, although the productive season in both is noticeable longer which can be inferred by the longer duration of average temperatures above the 8 °C line in Figure 2. According to the British Columbia Ministry of Agriculture; Plant & Animal Health Branch (2015) 8 °C is taken as a minimum temperature for foraging, with optimum conditions occurring between 16 and 30 °C. Hedmark's climate is classified as Dfb, meaning a cold continental climate, with a humid warm summer and at least four months averaging above 10 °C. Bayburt's climate is classified as Dsb, meaning a cold continental climate with a dry Mediterranean influenced warm summer and at least 4 months averaging above 10 °C.

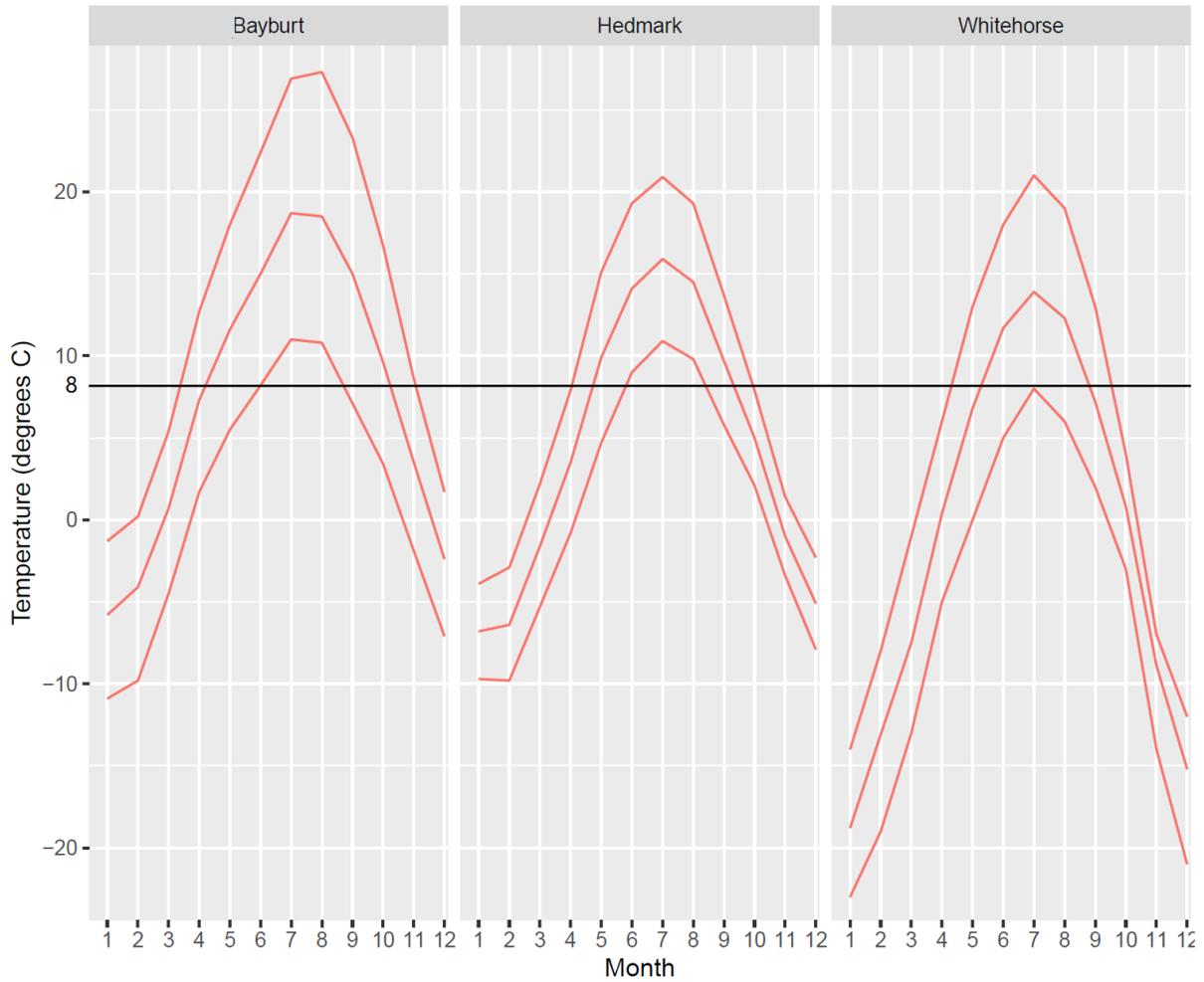


Figure 2 Typical annual temperature profiles for the Bayburt, Hedmark, and Whitehorse areas. The graphs show minimum daily temperature, average daily temperature, maximum daily temperature trends based on 30-year means over the period 1982 to 2012 (Climate-Data.org, 2020). The black horizontal line indicates 8 °C.

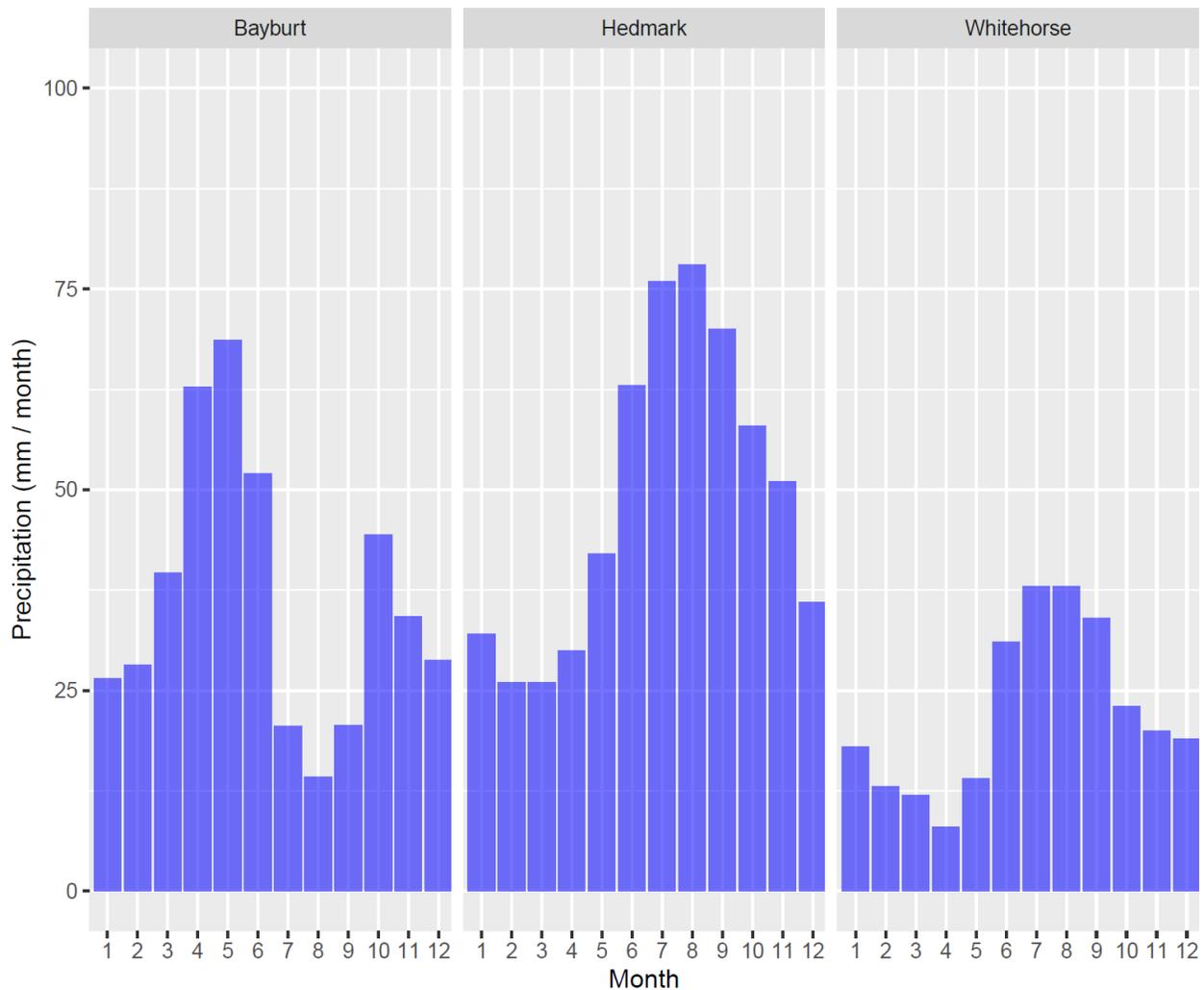


Figure 3 A comparison between for the typical monthly precipitation at Bayburt, Hedmark, and Whitehorse (Climate-Data.org, 2020).

Villumstad reports on three comparisons between the performance of colonies in single-walled hives and double-walled hives conducted at Hedmark, Norway (60° N, 11° W, elevation ~150 m asl) during 1953-55, 1965-66, and 1971-74. Incomplete reporting of thermal properties of the hives prevented inference of either the thermal resistance per unit area (known as R-value) of the hive walls or the lumped thermal conductance of the hive. Wintering and spring development were found to be better in insulated hives than in standard hives. No significant differences in total annual honey yields were detected, however mean honey yield from insulated hives was higher in summer and lower in

autumn when compared with control hives. Villumstad concluded that this is the result of a faster spring development in insulated hives resulting in earlier peaking of colony strength.

Table 2 Results reported in Villumstads 1965 & 66 comparison of insulated (single walled) vs non insulated (double walled) hives

Colony type	Year	Hive type	Average honey yield (kg)		
			Summer	Autumn	Total
Medium-strong	1965	Double walled	29.8	6.9	36.7
		Single walled	25.6	9.3	34.9
	1966	Double walled	21	25.1	46.1
		Single walled	16.8	32.5	49.3
Weak swarm	1971-73	Insulated		3.1	3.1
		Non-insulated		1.9	1.9
Weak overwintered	1973-74	Insulated	16.5	15.8	32.3
		Non-insulated	11	16.3	27.3

In 2018, a study with similar aims was conducted at Bayburt University in Turkey (40° N, 13° E, elevation 2,137 m asl), comparing brood development, nectar flow period, weight gain, bee flight activity, aggression response, and honey yield in wooden, polystyrene, and composite insulated hives over the productive season between 5th June and 30th August 2018. It was not possible to infer either the R-value of the hive walls or the lumped thermal conductance of the hives. “Polystyrene hives” may refer to commercially available brands such as Apimaye, Lyson, or Paradise Honey, all of which have hive wall R-values of 1.2±2 Wm⁻¹K⁻¹. “Composite hives” are described as “a material consisting of insulating foam between two thin wooden sheets”, neither the thickness nor the material used for insulation were specified. Both types of insulated hives significantly outperformed the wooden hive in weight gain, amount of

brood area and honey yield. 35% more honey was harvested from composite insulated hives than from wooden hives (Erdoğan, 2019).

Table 3 Results from Bayburt, Turkey (Erdoğan, 2019)

	Wooden hive			Polystyrene hive			Insulated hive		
	Mean	p Value	SEM	Mean	p Values	SEM	Mean	p Value	SEM
Development of honeybee colonies (pieces frame/colony)									
June	9			9			9		
July	17.97 ^b	.00	0.19	20.18 ^b	.00	0.33	21.28 ^b	.00	0.45
August	24.03 ^c	.00	0.48	25.72 ^c	.00	0.38	27.02 ^c	.00	0.626
Mean	16.43 ^a	.30	1.04	18.30 ^a	.30	1.31	19.10 ^a	.30	1.418
Development of brood area (sealed brood/cm ²)									
June	3195.60 ^a	.00	42.39	3222.40 ^a	.00	53.21	3393.40 ^a	.00	36.58
July	5545.50 ^c	.00	59.75	6913.50 ^c	.00	37.10	7228.10 ^c	.00	74.61
August	4283.67 ^b	.00	47.22	5032.70 ^b	.00	32.07	5529.50 ^b	.00	75.31
Mean	4341.67 ^a	.12	180.50	5056.20 ^{a,b}	.13	280.81	5383.67 ^b	.64	293.56
Weight gain of the application groups (kg/colony)									
	34.86 ^a	.00	0.48	41.75 ^b	.00	0.46	53.40 ^c	.00	0.79
Number of outgoing honeybees									
	80.95 ^a	.00	1.60	93.15 ^b	.00	1.58	103.62 ^c	.00	2.49
Honey yield (kg/colony)									
	17.08 ^a	.07	0.61	20.17 ^{a,b}	.07	0.92	23.04 ^b	.09	1.17
The number of bee sting of the honeybee colonies in the application groups									
	3.04 ^a	.18	0.26	4.05 ^b	.59	0.25	3.69 ^{a,b}	.18	0.25
								.59	
Maximum and minimum temperature and humidity values in the hive									
Max. temperature (°C)	38.61 ^b	.00	0.11	33.90 ^a	.49	0.20	34.24 ^a	.49	0.28
Min temperature (°C)	20.10 ^a	.00	0.26	24.97 ^b	.81	0.14	24.82 ^b	.81	0.11
Max humidity (%)	55.72 ^a	.00	0.75	66.94 ^c	.00	0.71	61.41 ^b	.00	0.84
Min humidity (%)	24.69 ^a	.00	0.27	37.29 ^c	.00	0.56	29.99 ^b	.00	0.51

SEM: standard error of mean.

^{a,b,c}p<.05.

1.5 Aims of this study

As this study aims to inform a beekeeper's decision on whether to use insulated hives, a quantitative hypothesis testing approach within the positivist paradigm was used to investigate the various ways thermal insulation may impact *A. mellifera* colonies during the active season.

1.6 Study hypotheses

Honey bee colonies invest a substantial proportion of their energy in producing honey, a valuable product for the beekeeper. The net quantity of honey stored by a colony determines how much can be harvested and sold. As the quantity of honey stored is affected by overall colony health and size, it can be considered a proxy measurement for overall colony health.

H₁₀ – The mean quantity of honey stored over the June-August period does not differ between colonies in thermally insulated and non-thermally insulated hives.

Productivity of a colony depends on a seasonal build-up of population in preparation for foraging and honey storage. This is particularly true for colonies in regions where the foraging season is short, including southern Yukon. Brood rearing is highly temperature sensitive, temperatures deviating from optimal at egg, larval, and pupal stage have been linked to adverse effects including increased brood mortality (Doull, 1976; Le Conte et al., 1990; Flores et al., 1996; Jones *et al.*, 2005; Simpson, 1961; Tautz et al., 2003). Insulation allows for improved temperature stability and may increase the amount of comb space which a colony can maintain at brood rearing temperature.

H₂₀ – The mean number of capped brood cells observed during each inspection does not differ between colonies housed in thermally insulated and non-thermally insulated hives.

Newly established wild or feral colonies must rapidly build comb, store nectar and pollen, and raise new generations of bees to improve their chances of survival. Similarly, a managed colony must also perform these tasks if it is to survive and produce surplus honey for harvesting by a beekeeper. Weight gain due to these activities can be measured and compared between treatment and control hives. A weakness of this approach is that total weight gain within a hive results from several

non-equivalent parts; nectar, honey, wax, pollen, brood, and adult bees. Weight of nectar and resultant honey storage is influenced by water content, the quantity of pollen stored is partly determined by brood rearing rate, sugar to wax conversion ratios¹ between 1.8 and 13.2 have been reported (Hepburn, Pirk and Duangphakdee, 2014), and increases in weight of bees within the hive are the result of a conversion of pollen and nectar/honey into new bees. Nonetheless, a productive colony is expected to gain weight and more favourable conditions within the hive environment are expected to allow a faster rate of weight gain.

H3₀ – The mean weight gain recorded at each inspection does not differ between colonies in thermally insulated and non-thermally insulated hives.

As outlined in the introduction higher RH expected in insulated hives and improved regulation of temperature in the broodnest are both expected to reduce Varroa reproduction.

H4₀ – The mean number of Varroa mites detected using a sticky board at each inspection does not differ between colonies in thermally insulated and non-thermally insulated hives.

¹ Sugar to wax conversion ratios were defined as the net amount of sugar consumed against wax produced .

2 Methods

2.1 Set up of treatment and control hives

To test the effect of hive insulation, a comparison between treated and non-treated hives was designed. Each treatment hive had 50 mm of high density expanded polystyrene (HD-EPS) attached to all the exterior vertical surfaces, 100 mm of HD-EPS on the top surface, and 50 mm of HD-EPS under the bottom board. Thermal resistance of HD-EPS is inversely related to temperature; R value of 50 mm of HD-EPS is $1.5 \text{ K}\cdot\text{W}^{-1}\cdot\text{m}^2$ at $24 \text{ }^\circ\text{C}$, but increases to $1.74 \text{ K}\cdot\text{W}^{-1}\cdot\text{m}^2$ at $-15 \text{ }^\circ\text{C}$ (ASTM, 2019), R values for 100 mm are double that of 50 mm.

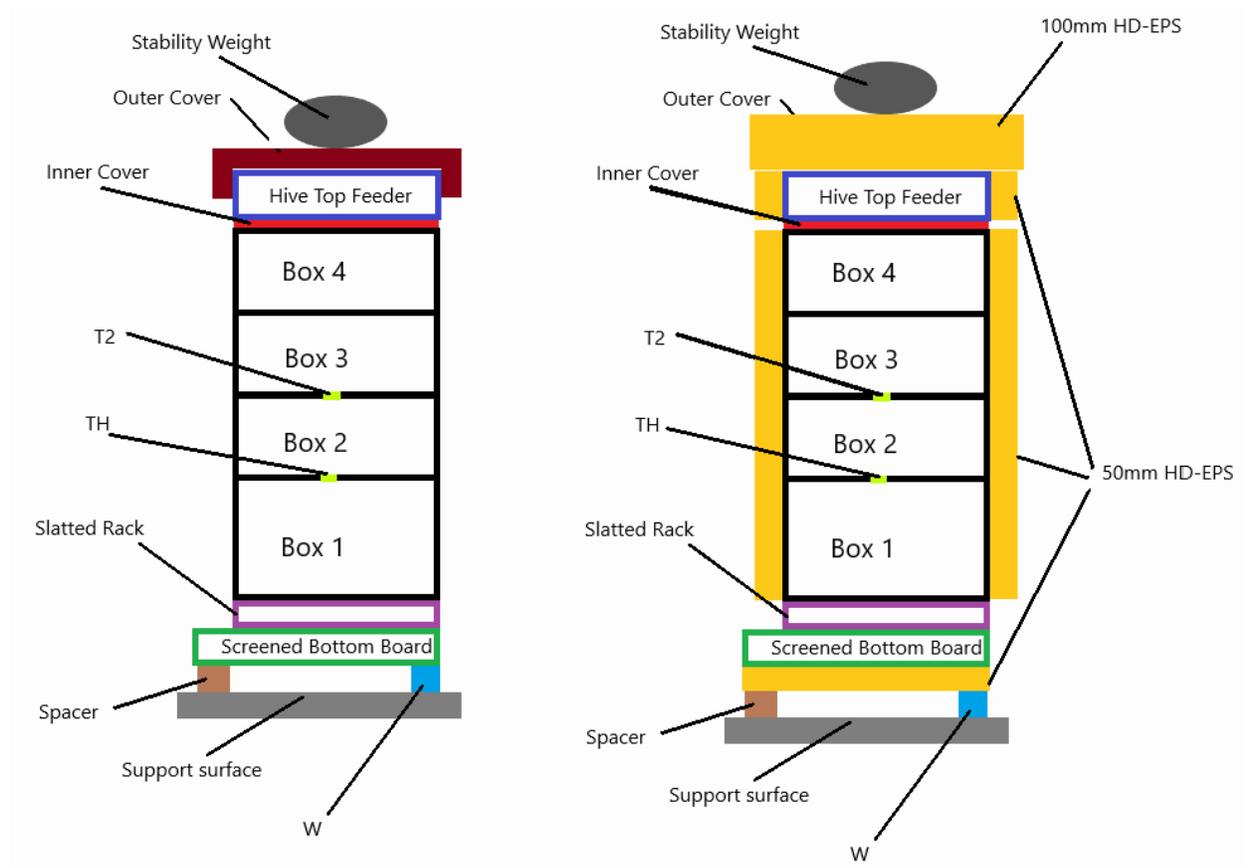


Figure 4 Hives were set up as shown, with a control on the left, treatment on the right. W, TH, and T2 indicate broodminder sensors. Hives were initially set up with Box 1 only, additional boxes were added as colonies grew.

2.2 Data collection

Data describing colony performance were collected from 16 hives; eight treatment and control pairs. The 8 pairs were split between five apiary locations and were managed/inspected by three beekeepers as shown in Table 4. Nucleus colonies were installed in each hive in early summer (May 7th, 28th, and 29th 2020) on frames with undrawn plastic foundation. All hives began as a single deep Langstroth box, additional boxes were added as needed; most hives grew to include two brood boxes and one to two medium honey supers.

Table 4 Distribution of replicates in five apiaries. Control hives were given odd numbers, treatment hives were given even numbers.

Apiary	Beekeeper	Hive numbers
Atlin	Fiona McGlynn	1, 2
Teslin	Gillian Rourke	3, 4
Elemental	Eoin Sheridan	5 - 12
River Road	Eoin Sheridan	13, 14
Wolf Creek	Eoin Sheridan	15, 16

Three main types of data were collected: continuous time-stamped data (superorganism weight, internal temperatures and relative humidity); instantaneous records (worker brood area, mite counts); and net accumulation data (frame area usage demarcation). For instantaneous and net accumulation data, four common inspections were planned for all hives. Period one was planned for 0-14 days, with three more periods of 28 days each.

Weather conditions, individual beekeeper's schedules and preferences along with Covid19 travel restrictions² influenced exactly when inspections were carried out.

² Disruptions to the schedule included excessively cold, windy, or rainy weather; an impassable water logged access road; Covid19 related travel restrictions; personal commitments of beekeepers; a vehicle breakdown; and adjustments made to inspection / intervention schedules based on colony condition and beekeeper preferences.

During the study, inspection schedules were modified to accommodate disruptions while maintaining compatibility between sets of data, actual inspection dates are shown in Table 23 in appendix A.

Table 5 Weather records during the study were sourced from nearby Whitehorse weather station (60°42'34" N, 135°04'02" W, altitude: 706.20 m; (Historical Climate Data - Government of Canada, 2020).

Period	Date Range	Maximum temperature (°C)	Minimum temperature (°C)	Average temperature (°C)	Average daily max. (°C)	Average daily min. (°C)	Average heating (degree days per day)	Average daily precipitation (mm)
1	28 th May - 12 th June	20.2	0.1	10.73	16.48	4.93	7.28	3.5
2	13 th June - 12 th July	22.4	3.2	12.97	18.09	7.78	5.03	1.58
3	13 th July - 17 th August	29.0	2.6	13.42	18.13	8.66	4.62	1.83
4	18 th August - 19 th September	19.8	-3.7	9.94	14.54	5.29	8.06	1.29

2.2.1 Collection of primary data

Metrics suitable for testing the hypotheses are referred to as primary metrics.

2.2.1.1 *Frame inspections*

Frame inspections were performed for testing H1 and H2. Specifically frame comb area (both sides of each frame) was assessed visually to give a semi-subjective value for (a) degree to which the comb had been drawn (0% = bare plastic foundation) and (b) proportional comb area under the following uses; empty drawn cells, capped honey and capped worker brood, pollen stores were observed. Visual inspections of each frame were occurred approximately 2 weeks, 6 weeks, and 11 weeks after nucleus installation.

To limit the effect of the subjective nature of the measurements, one person performed all inspections. The visual assessments were calibrated in the following way. 30 frames from two hives were photographed on both sides resulting in 60 photos of frame sides, which allowed for six rounds of calibration inspections with 10 frame sides inspected three times in each round. The first 10 photographs were visually examined to estimate the four variables. Estimates were restricted to 1%, 2%, 3%, 5%, and up to 100% in 5% increments. After visual inspections were complete, numbers of cells of capped worker brood, capped honey, and pollen were counted to give an accurate measurement. The semi-subjective and accurate measurements were compared and the errors plotted in Figure 5. This learning exercise was used to train estimation skill. Three additional training tests between visual assessment and cell counts were conducted. Between each round a comparison between the two values was studied by the assessor. This training continued until 75% of semi-subjective measurements were within 5% of the counted measurement, and no errors greater than 10%. Estimation improved with each training run, on the fourth the target was achieved. The remaining 2 rounds of photos were inspected to verify consistency, for additional verification the 60 photos were set in a random order and inspected (Figure 6).

Frame inspections were logged in a spreadsheet, each row recording the state of one frame side at the inspection date (Table 6).

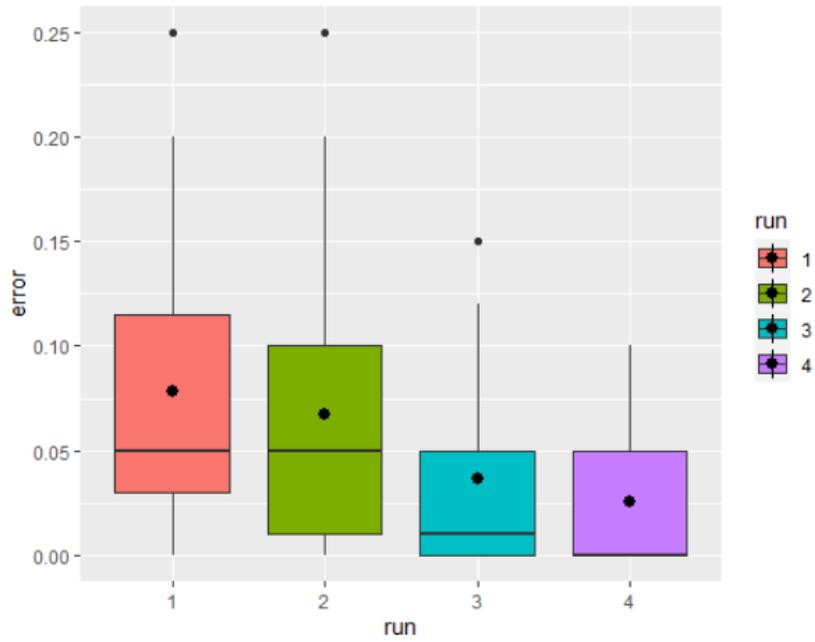


Figure 5 Boxplots showing magnitude of errors in visual estimation in the first 4 training runs, each of which included 10 frame sides. In the fourth run 75% of errors were less than or equal to 5%, maximum error was 10%.

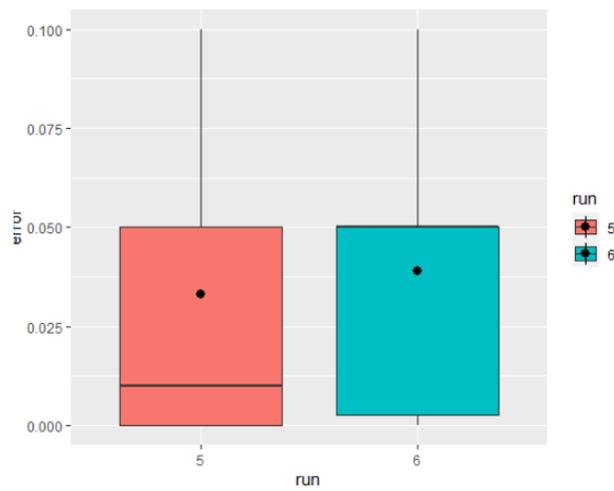


Figure 6 Following training verification runs 5 and 6, errors were within targeted limits.

Table 6 Excerpt of spreadsheet used to log results of frame inspections.

Inspection_date	Nuc installed	hive_num	Box_position	Box_size	Pos_in_box	Frame_side	drawn_comb	Worker_capped_brood	Capped_honey	Pollen
2020-07-12	2020-05-28	7	1	deep	1	L	0.00	0.00	0.00	0.00
2020-07-12	2020-05-28	7	1	deep	1	R	0.30	0.00	0.00	0.15
2020-07-12	2020-05-28	7	1	deep	2	L	1.00	0.50	0.05	0.05
2020-07-12	2020-05-28	7	1	deep	2	R	1.00	0.30	0.10	0.10
2020-07-12	2020-05-28	7	1	deep	3	L	1.00	0.50	0.05	0.10
2020-07-12	2020-05-28	7	1	deep	3	R	1.00	0.60	0.05	0.05

2.2.1.2 Hive weight

The weight of hives and their contents was recorded to test H3 and H5. Weight scales (Broodminder-W, US) were installed under the front of each hive stand supporting roughly half the weight of the hive and its contents. The scales were set to log weight readings at 15-minute intervals.

Scales were calibrated according to manufacturer's instructions (Broodminder, 2019) to take into account that only half a hive's mass was supported. Since comparative changes in mass were important (rather than specific mass), this was deemed acceptable.

For the calculation of hive weight, a separation was made between equipment and contents. Hive equipment was defined as; bottom board, slatted rack, hive boxes, frames, plastic foundation, hive top-feeder, outer cover, and stability weight. Hive contents were defined as; the colony superorganism and any supplemental feed which had not been moved into stores, e.g. sugar syrup in top-feeders or pollen substitute under the inner covers.

Initial weights for hive equipment were recorded and the equipment present at each hive was tracked through the study, enabling the total equipment weight to be calculated for each hive at all times. The calibrated scale weight, less the equipment weight, resulted in the hive contents weight. Except for times when supplemental feeding was applied, hive contents weight was equal to the superorganism weight. On September 17th a single observation was taken of the weight of bees within a hive. All

frames from the selected hive were removed and weighed individually. The weight of bees was then estimated using the formula below.

$$\textit{Weight of bees} = \textit{total weight of hive and contents} - (\textit{frame weights} + \textit{equipment weights})$$

2.2.1.3 Mite counts

Varroa mite infestation levels were measured using the sticky board method as described by Sammataro (Sammataro, Ostiguy and Frazier, 2002).

2.2.2 Secondary metrics

Secondary metrics included climatic data not directly used for hypothesis testing.

2.2.2.1 Internal hive temperature

Hive conditions were measured using Broodminder-T2 (internal temperature) and TH (internal humidity) sensors. At the start of the study, both the T2 and TH sensors were placed at the top of the sole hive box. Once a second box was added, the T2 sensor was moved to the top of the second hive box.

2.2.2.2 Ambient temperature

Temperature was recorded at 15-minute intervals directly under each hive using a Broodminder-W sensor. Temperatures recorded on clear days at the Elemental apiary appeared to be strongly affected by localised solar heating on the steel shipping container structure. At all other locations the temperature recorded was within 1.5 °C of the air temperature as observed using an Acurite 00411CADI digital weather station.

2.3 Data screening and analysis

2.3.1 Missing data and exclusions

Swarming, queen-less periods, and confounding beekeeping interventions affected some colonies in ways that prevented fair comparisons. Table 7 shows the hives, dates, and inspection periods affected. Justification for exclusions and timing are outlined in the following sections.

Table 7 Data excluded from analysis

Hive	Treatment	Cause	Affected metric	Exclusion start	Exclusion end	Inspections affected
1	Control	Confounding interventions	All	12-Jun	-	3
2	Treatment	Confounding interventions	All	12-Jun	-	3
3	Control	Confounding interventions	All	28-May	-	all
4	Treatment	Confounding interventions	ALL	28-May	-	all
8	Treatment	Swarm	BDBR	09-Jul	12-Aug	2
8	Treatment	Swarm	NHSR & Varroa	31-Jul	-	3
10	Treatment	Swarm	BDBR	20-Jul	14-Aug	-
10	Treatment	Swarm	NHSR & Varroa	17-Aug	-	-
10	Treatment	Swarm	Weight	20-Jul	-	3
13	Control	Missing data	Weight	28-Jul	14-Aug	NA
14	Treatment	Missing data	Weight	16-Aug	29-Aug	NA
15	Control	Confounding interventions	ALL	10-Aug	-	3,4
15	Control	Missing data	Weight	28-May	18-Jun	NA
16	Treatment	Swarm	ALL	10-Aug	-	3
16	Treatment	Sensor malfunction	Temperature & Humidity	11-Jul	-	2,3
16	Treatment	Sensor malfunction	Weight	28-May	18-Jun	NA

2.3.1.1 *Swarming*

Several colonies issued swarms during the study. To determine how swarm-affected data should be handled, it was necessary to first examine the generalised effect of swarming on a colony before examining the specific circumstances of a particular swarming event. It is usual for roughly 50% to 70% (Seeley, 2019) of a colony's worker bees to leave with a prime swarm. Worker bees are known to fill their crops with about 36mg of honey before departure (Combs Jr, 1972), typically 40% of the weight of a swarm is honey (Winston, 1987).

Following a swarm departure, it typically takes between 7-9 days for a new queen to emerge, followed by 5-6 days maturing, 1-4 days for mating, then 2 days later she can be expected to begin laying. A total delay of roughly 18 ± 3 days between swarming and a new queen's first egg (Winston, 1987). Additionally, about seven days before the swarm issues, the egg-laying rate of the old queen begins to slow and stops completely shortly before swarming (Winston, 1987). In the days immediately after a swarm has issued, worker brood mortality is higher than usual, pre-swarming mortality has been measured at about 7% whereas post swarming worker brood mortality has been recorded at about 42%, mostly occurring in the egg and larval stages (Winston, Dropkin and Taylor, 1981). Laying is slowed or stopped from seven days before the swarm date to about 18 days after, allowing for a lag of seven days between egg laying and capping of cells, it can be inferred that the number of capped brood cells observed from the date of swarming to 25 days after will be strongly effected. Consequently, observations of capped worker brood during this period were excluded from comparisons. Although swarming could cause a longer-lasting reduction in brood rearing indirectly through reduced numbers of nurse bees, depleted stores, and reduced ability to regulate brood temperature, these affects were expected to be of lesser effect and were not considered reason to exclude brood observations occurring >25 days after a swarm.

The immediate loss of a large portion of worker bees and subsequent egg-laying pause due to swarming was expected to have a lasting effect on nectar foraging and superorganism weight gain. Accordingly, colonies that swarmed were removed from comparisons of honey storage and weight gain from 28 days after swarming date until the end of the season.

Reproduction of Varroa mites within a hive is dependent on brood rearing, indeed inducing a break in egg-laying is recognised as a mite reduction measure (Wagnitz and Ellis, 2010). The brood break caused by swarming or a queenless period can be expected to result in a lower mite infestation level for the rest of the year. Colonies which experienced swarming were therefore excluded from mite-count comparisons from 28 days after swarming until the end of the year.

2.3.1.2 Queenless period

Likewise, queenless periods result in a timespan in which no eggs are laid. Length of time before discovery can be estimated from presence and ratio of eggs, larva, and capped brood. Length of time from discovery to restart of egg laying depends on the requeening method used. If a mated queen is introduced, egg laying could begin with 1-3 days, a virgin queen could take 4–12 days, giving the colony a capped queen cell could take about 15–21 days, raising a new queen from an egg could take about 24–28 days; these estimates assume good weather for mating, poor weather can delay mating indefinitely.

It was decided that a queenless period would exclude a hive from:

- honey comparisons from 21 days + 7 days + 7 = 35 days after estimated last egg laid date until end-of-year.

It takes 21 days after the last egg is laid before the adult worker bee population begins to be affected. The effect gradually increases and there is a lag between population decrease and reduced foraging activity.

- brood comparisons from 9 + 4 days after estimated date of last egg laid until 21 days after estimated re-start of egg laying
- Varroa comparisons from 21 days after estimated last egg laid end-of-year.
- Weight comparisons from 21 days after last estimate egg laid end-of-year.

2.3.1.3 Confounding interventions

In cases where beekeepers removed frames, made splits, combined colonies, or swapped frames from one hive to another the subsequent data were excluded from all comparisons.

2.3.2 Frame inspections

Frame observation data were imported from spreadsheets to R for analysis. Inspections included both deep- and medium-sized frames. Deep frames contained 3,276 cells per side, and medium frames 2,106 cells; differences in frame sizes were standardised using a conversion factor of 1.56 medium-frame sides to a single deep-frame side. All frame inspection metrics were converted to units of Deep Frame Side Equivalent (DFSE). Capped honey, capped worker brood, and pollen store data were converted to number of cells by multiplying the DFSE by 3,276. As the quantity of drawn comb included partially drawn cells, the units for quantity of drawn comb was DFSE. The rate of comb building in each inspection period was also calculated in treatment and control hives.

2.3.2.1 Net honey storage rate

Difference in capped honey observations from one inspection to the next were used to calculate the daily average net number capped over the preceding period. Net Honey Storage Rate (NHSR) was measured in cells per day (c.d⁻¹)

Where HC_n = capped honey cells at inspection n.

$$NHSR = \frac{HC_n - HC_{n-1}}{Date_n - Date_{n-1}}$$

NHSR was recorded after inspection periods two and three. As full frame inspections were not performed when the nucleus colonies were installed it was impossible to calculate the NHSR for inspection period one. During inspection period two, some supplemental feeding of sugar syrup occurred. The last addition of sugar syrup occurred on June 27th and all hive-top feeders were empty by July 4th. Linear models for the null and alternative hypothesis were created for both period two and three. ANOVA was used to compare models.

2.3.2.2 Brood rearing rate

The normal interval between capping of a worker brood cell and the emergence of a new bee is roughly 12 days (Winston, 1987). The Balanced Worker Brood Rate (BWBR) was calculated in units of individuals per day:

$$BWBR = \frac{\text{Number of capped worker brood cells observed}}{12}$$

BWBR must always be less than or equal to the average rate of worker brood cell capping over the preceding 12 days, and greater than or equal to the average daily worker brood emergence over the subsequent 12 days. The BWBR in a healthy colony will be very close to both values. Additionally, if there is a large difference between the preceding capping and the subsequent emergence rates it will be made obvious by an extremely “spotty” brood pattern. As failed brood is uncapped and removed by nurse bees it leaves gaps in an otherwise predictable and contiguous area of worker brood. No extremely spotty brood patterns were observed.

2.3.2.3 Weight

Hive scale weight data were screened prior to analysis to remove misleading information due to disturbances.

2.3.2.3.1 Isolating the superorganism weight

Hive scale weight data were screened prior to analysis to remove misleading information due to disturbances. Scale weight included all hive equipment + stability weight + hive contents. Hive equipment included bottom boards, slatted racks, hive boxes, frames, plastic foundation, feeders, outer covers, stability weight and insulation.

Hive equipment weights were tracked as follows. The equipment included in each hive was logged in beekeeper notes. For each piece of equipment, a generic weight was recorded; three of each item were weighed using a kitchen scale (AccuChef 2305, range 0-15kg, resolution 1g) and an average weight recorded.

The stability weight was subtracted from the total recorded weight. Date-specific equipment weight was then subtracted to calculate the weight of hive contents. Hive contents included the superorganism and any supplemental feed which had not been

stored on the comb. Apart from times when supplemental feeding was present, the hive contents' weight was equal to the superorganism weight.

2.3.2.3.2 Step-changes and solution

Superorganism weight for each hive was plotted for the duration of the study.

Problematic step changes of between 0.5kg and 3kg in both positive and negative directions were observed occurring on some hives during some inspections. Two likely causes were identified:

- Stability weights on top of the hives may have been replaced in slightly different positions, movement toward the scale would increase the force exerted on the scale.
- Variation in equipment pieces (wooden ware in particular).

A procedure was created to correct these step-changes. The procedure was simply to add a value equal to the observed step change multiplied by -1 to the equipment weight table for all subsequent weight observations. In this way all unexpected step changes which occurred during inspections were eliminated. Example plots of affected time periods before and after the corrective procedure are shown in Figure 7.

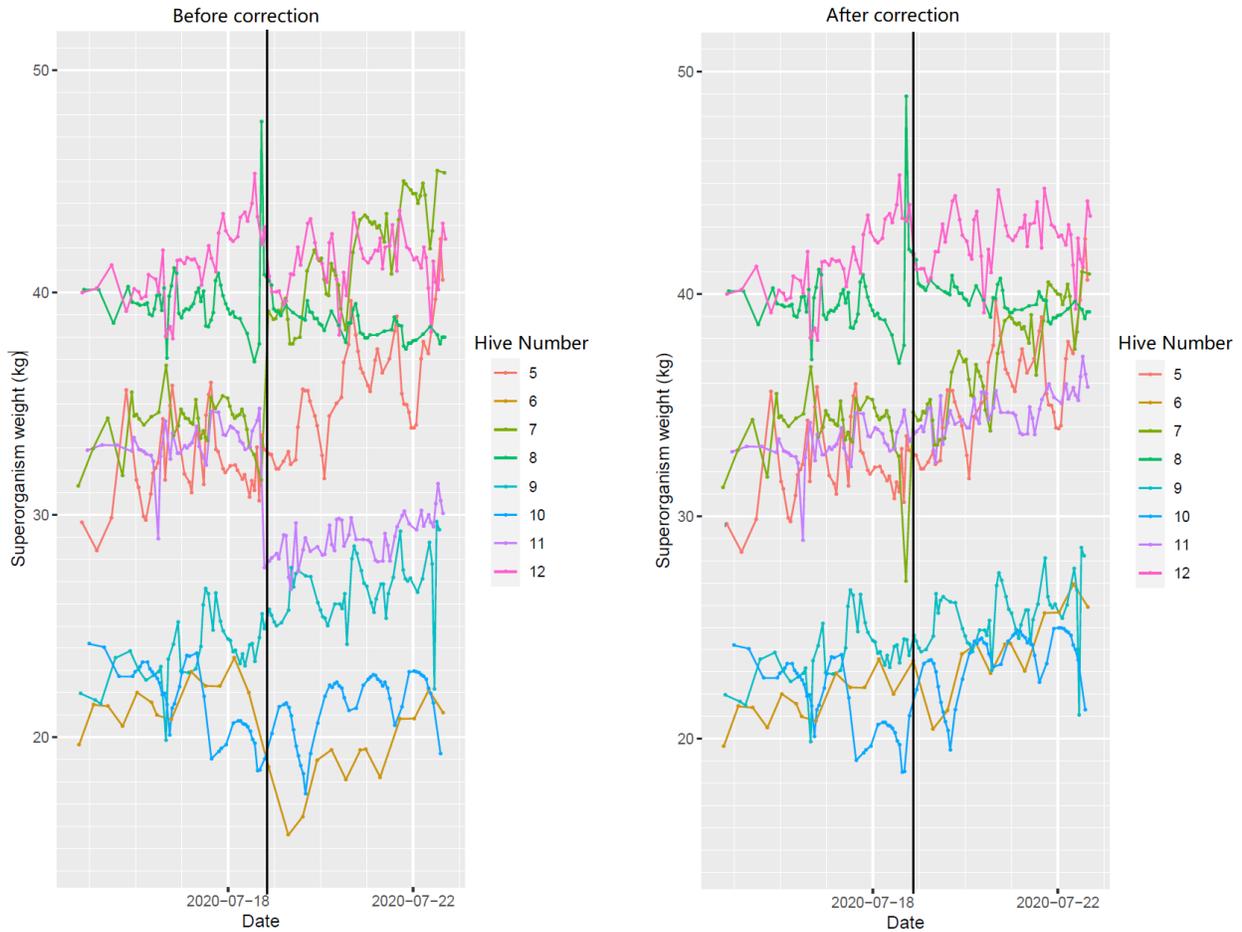


Figure 7 Step-changes of varying magnitude are visible occurring at an inspection on the evening of 18th July. Also visible are dramatic and short duration changes in weight due to equipment being manipulated during an inspection. The step-change has been corrected but outlying values due to disturbance remain.

2.3.2.3.3 Removal of outlying weight observations

Weight datasets for all hives contained a number of outlier values which were sufficiently different from other chronologically-close values to assume the difference was not due to the colony's activities. Some of these outliers occurred at the time of frame inspections, but others were unexplained. To screen outlying weight data for removal, each weight reading was compared to a nine-observation running average (four preceding recorded weights, the weight in question, and the subsequent four recorded data points). Where a difference of greater than 1kg was obtained the data

point was deleted. The above process was performed twice. In this way 4,537 observations were identified as outliers and removed (2.4% of total weight observations).



Figure 8 Before (above) and after (below) outlier removal. Only data from 8 hives shown to improve clarity.

2.3.2.3.4 Daily weight variation patterns

The weight recorded for each superorganism changed over the course of each day. The shape of the plotted changes were similar across almost all days and across all hives, although the magnitude varied considerably. Three causes for this pattern were identified; effect of changes in ambient temperature on the scale (Broodminder, 2019), forager bees departing and returning, changes in superorganism weight due to nectar gathering and subsequent desiccation. There may also be other unknown causes. See example in Figure 9.

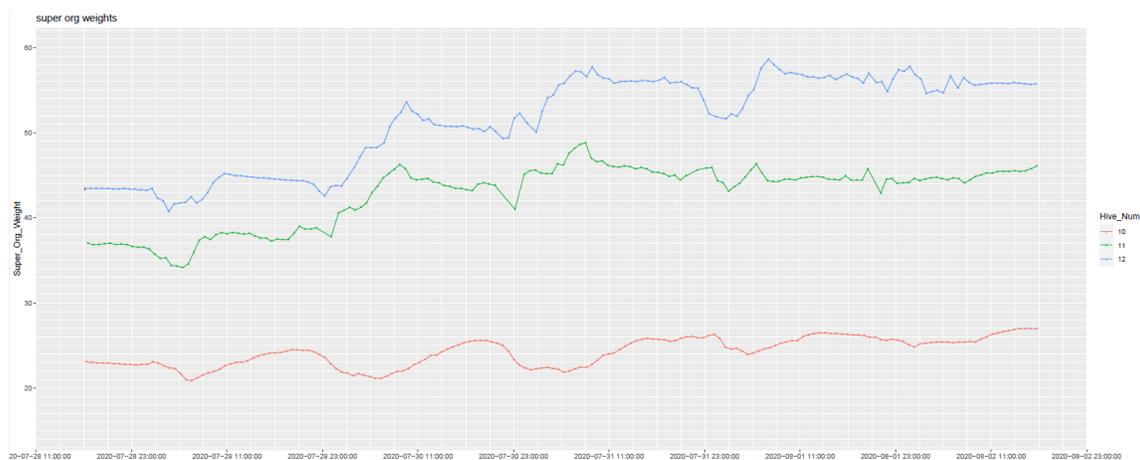


Figure 9 Example superorganism weights for hives 10, 11, & 12 from July 28th to August 2nd.

To enable compare superorganism weight-changes across different hives, average weight for each day was calculated for all hives.

To test hypothesis 3, linear models for the null and alternative hypothesis for total weight gain over the period July 4th to August 14th were created and compared. Weight gain before July 4th could not be reliably measured due to the influence of supplemental feeding, similarly the weight of bees, comb, and stores in each hive at the start of the experiment was unknown. As nucleus colonies from a single supplier in the same shipment were randomly installed in hives numbered five to 16 at the start of the study, initial weights were assumed to be close with the differences randomly distributed

between treatments. The mean weight observed in each treatment on July 4th and August 14th were also compared.

2.3.2.4 Temperature and relative humidity

Each time the hive was opened for an inspection the temperature and humidity were affected. To remove these observations and other unexplained spikes in temperature and humidity each temperature and humidity data point was compared to the average of 9 sequential data point centred on the data point in question. Where the difference was greater than 5°C or 10% RH the data point was removed. Out of 49,859 RH data points, 3.5% were removed. Similarly, 2.1%, 2.7% and 3% of data points for temperature in box-1, box-2 and under-hive, respectively, were removed.

2.3.3 Varroa mite infestation

The number of mites observed in each sticky board test was divided by duration of the test to estimate daily mite drop. Mite counts were very low at inspections one, two, and three, likely because nucleus colonies began with close to zero mites, possibly having been treated by the supplier. Since the sticky board method is less reliable when mite counts are very low (Oliver, 2013), counts from the first three inspections were not included in comparisons. Instead, an additional mite count was conducted on September 13th to 17th, close to the time of year that phoretic mite numbers and natural mite drop are expected to peak (Branco, Kidd and Pickard, 2006). Higher mite counts were observed in September.

To test H4, linear models for the null and alternative hypotheses using data from inspection four in September were created and compared using ANOVA.

2.3.3.1 Autumn feeding

When autumn feeding via hive-top feeders began on August 29th all hives had ample room in stores for nectar. Syrup (prepared using 2:1 ratio) was provided. Feeders were topped up before running dry. The amount of syrup taken from the feeder into stores was measured at each top-up, allowing the total quantity taken from August 29th to September 15th to be calculated.

3 Results

A summary of results is presented in Table 8, including mean and standard deviation of primary metrics and secondary metrics for control and treatment groups. *P* values and *F* statistics resulting from hypothesis testing using ANOVA comparisons are included. Notably, mean NHSR in period two in the treatment group was more than double that of the control group, while in period three the mean in the treatment group was only 10% greater. Mean mite drop was over three times greater in the treatment group than the control group.

Table 8 Summary of results.

	Control hives			F statistic	Treatment hives	
	Mean	sd	p value		Mean	sd
NHSR (cells.day⁻¹)						
Period 2	158	121	0.07 ^a	4.36 ^a	340	246
Period 3	618	531	0.77 ^a	0.09 ^a	680	623
BDBR (individuals.day⁻¹)						
Inspection 1	1271	318	- ^b	- ^b	1313	262
Inspection 2	1461	610	- ^b	- ^b	1475	578
Inspection 3	1133	344	- ^b	- ^b	1086	308
Weight						
(kg.day ⁻¹) 04/07 to 14/08	0.23	0.21	- ^b	- ^b	0.22	0.12
(kg.day ⁻¹) 27/07 to 03/08	1.15	0.55	- ^b	- ^b	0.9	0.71
Weight on 04/07 (kg)	26.2	5.66	0.37 ^a	0.87 ^a	30.5	9.73
Weight on 14/08 (kg)	38.0	8.87	0.48 ^a	0.59 ^a	41.8	7.13
Varroa (natural daily mite drop)						
September 17th	9.2	6.9	0.13 ^a	2.94 ^a	3	2.2
Average daily Temperature (°C)						
Box 1 period 1	27.2	6.4	- ^c	- ^c	27.7	5
Box 1 Period 2	32.8	3.89	- ^c	- ^c	34.1	1.48
Box 1 period 3	30.3	4.81	- ^c	- ^c	32.7	2.42
Box 2 period 2	30.4	4.14	- ^c	- ^c	31.8	2.07
Box 2 period 3	28	4.18	- ^c	- ^c	28.7	4.76
Average daily temperature range (°C)						
Box 1 period 1	7.86	4.18	- ^c	- ^c	4.59	3.49
Box 1 period 2	2.36	2.97	- ^c	- ^c	1.53	1.44
Box 1 period 3	2.67	2.93	- ^c	- ^c	2.44	1.94
Box 2 period 2	3.7	2.55	- ^c	- ^c	2.9	2.08
Box 2 period 3	3.7	2.26	- ^c	- ^c	3.7	2.55
Average daily RH						
Box 1 period 1	55.5	8.81	- ^c	- ^c	53.8	6.32
Box 1 period 2	53	5.51	- ^c	- ^c	52.7	5.63
Box 1 period 3	61.9	9.02	- ^c	- ^c	54	6.56
Daily RH range						
Box 1 period 1	16.3	8.97	- ^c	- ^c	15.6	9.37
Box 1 period 2	14.4	6.09	- ^c	- ^c	9.9	6.3
Box 1 period 3	13.1	8.08	- ^c	- ^c	9.9	4.92
Comb building rate (DFSE.day⁻¹)						
Period 1	0.218	0.057	- ^c	- ^c	0.354	0.066
Period 2	0.405	0.123	- ^c	- ^c	0.445	0.261
Period 3	0.155	0.139	- ^c	- ^c	0.1	0.088
Autumn syrup consumption (L)						
29/08 to 15/09	12.3	4.6	- ^c	- ^c	16.1	1.1

^a result of ANOVA comparison to test hypothesis

^b observations in treatment and control hives obviously similar, p-values and F statistics were not calculated

^c interpretive data, calculation of p-value and F statistics were not appropriate

3.1 Honey storage

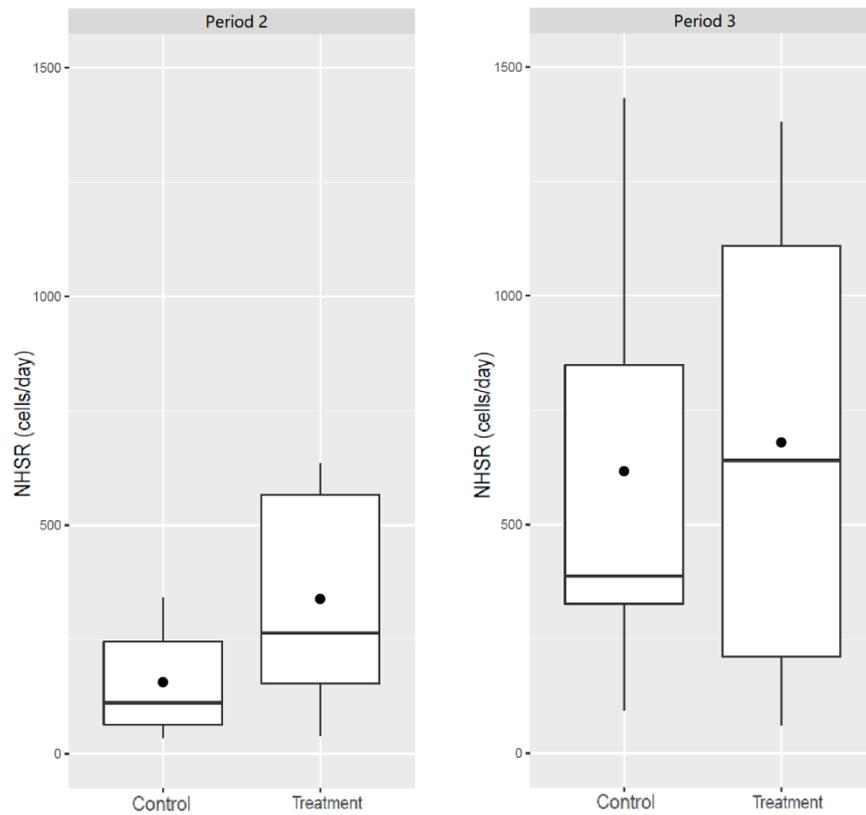


Figure 10 Box plot showing net daily honey storage rate in units of 5.4 mm cells per day. The period 13th June to 12th July is shown on the left, 13th July to 17th August.

3.2 Brood rearing

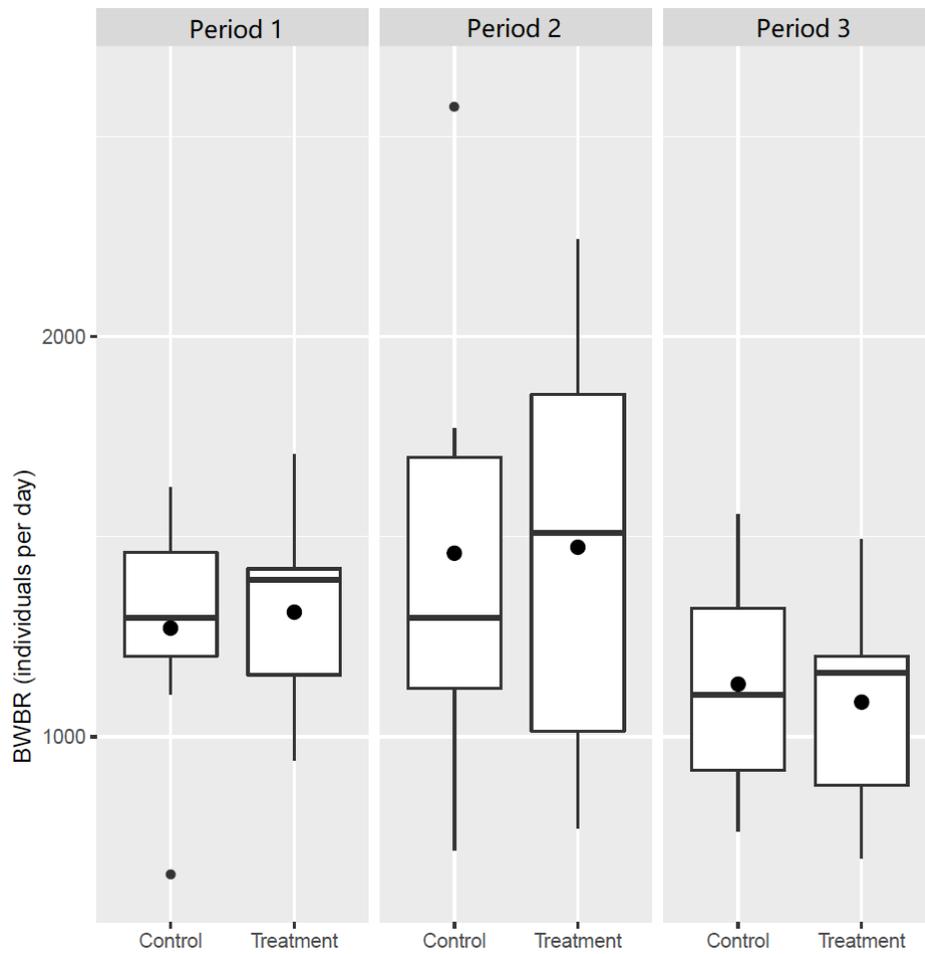


Figure 11 Box plots showing brood rearing rates at each inspection.

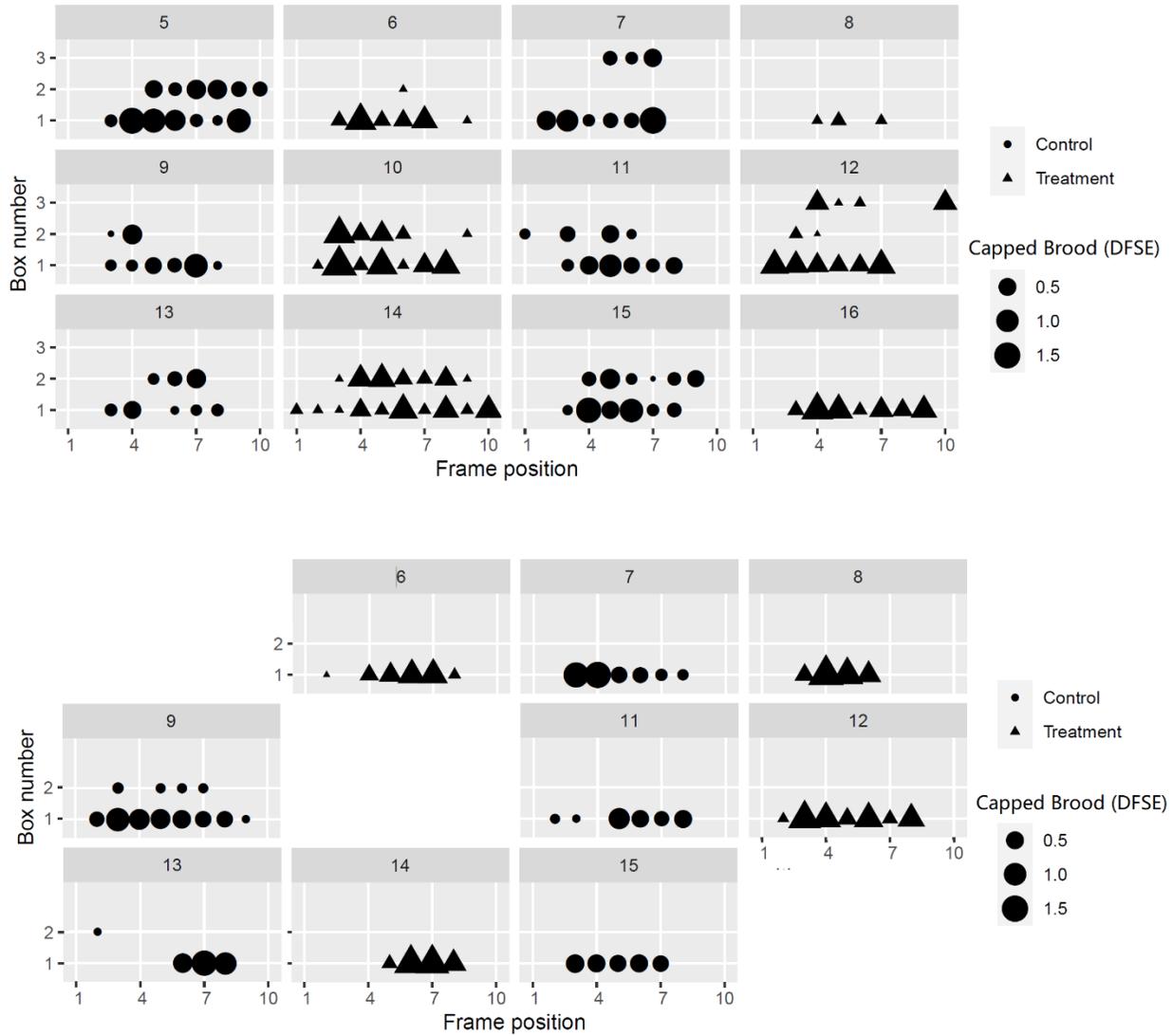
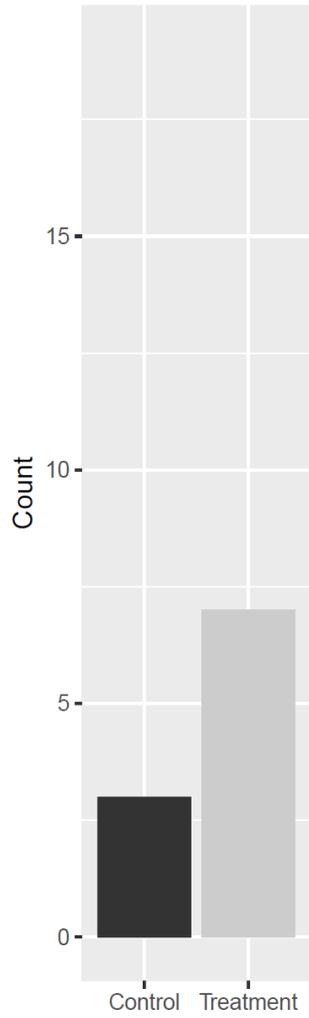


Figure 12 Visualisation of the spatial distribution of capped worker brood observed at inspection two (upper grid) and inspection three (lower grid). Horizontal axis indicates frame position, vertical axis indicates box number, the numbers above each plot indicate the hive number, size of symbol indicates area of capped brood in units of DFSE.

Capped Brood on frames 1 or 10



Capped Brood on frames 2 or 9

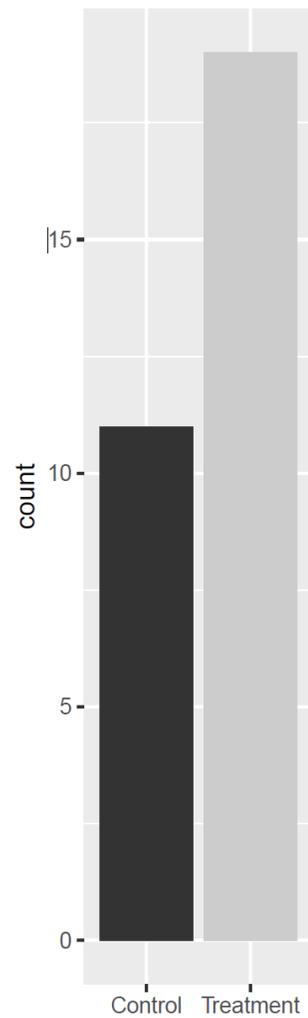


Figure 13 Observations of capped brood on outermost frames (frames numbered 1 and 10) are counted on the left bar chart. Observations of capped brood on the second from outermost frames (frames numbered 2 and 9) are counted on the right

3.3 Weight gain

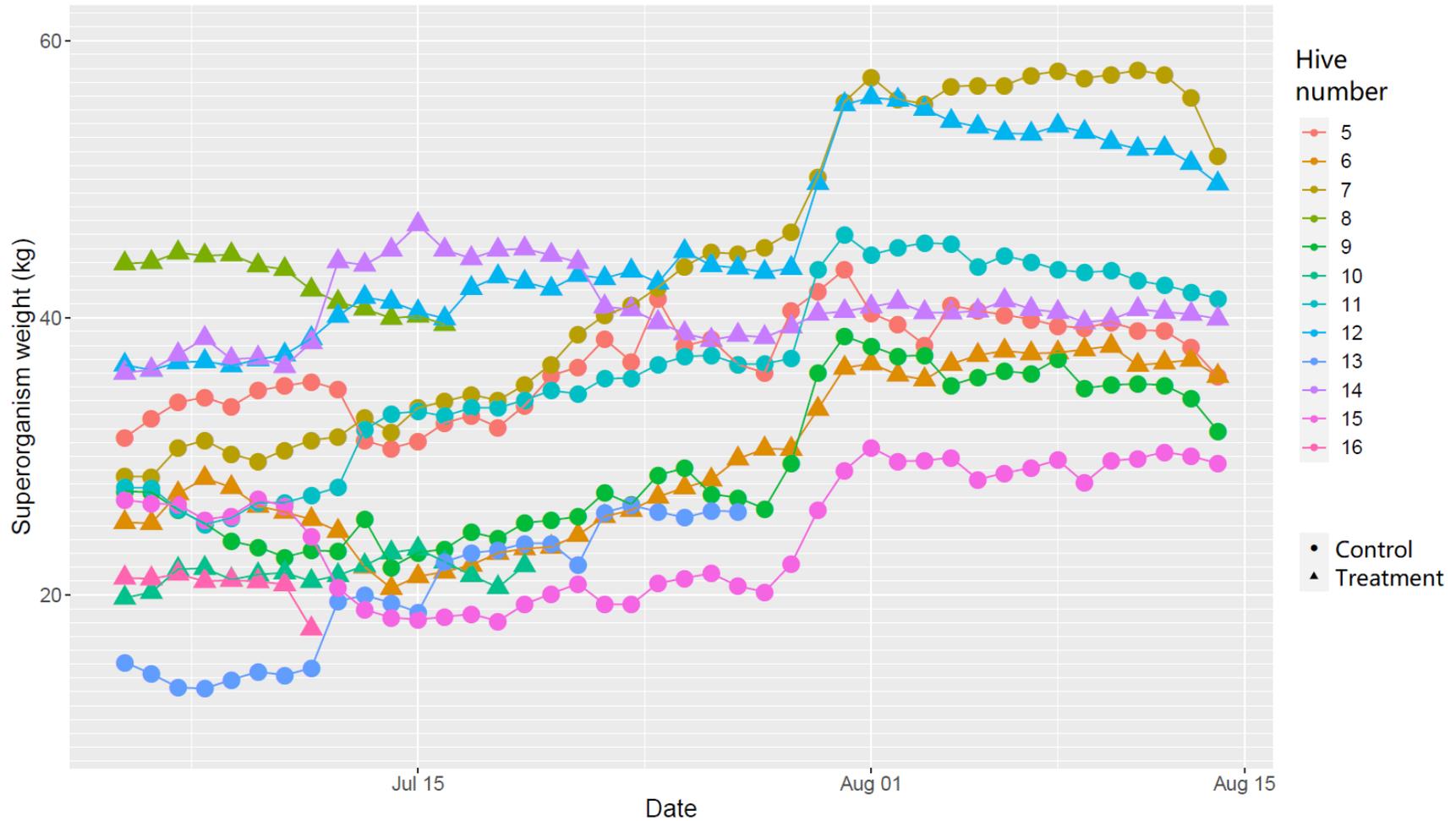


Figure 14 Daily average Superorganism weight (kg) plotted from 4th July to 14th August. Weights do not include any unstored supplemental feed.

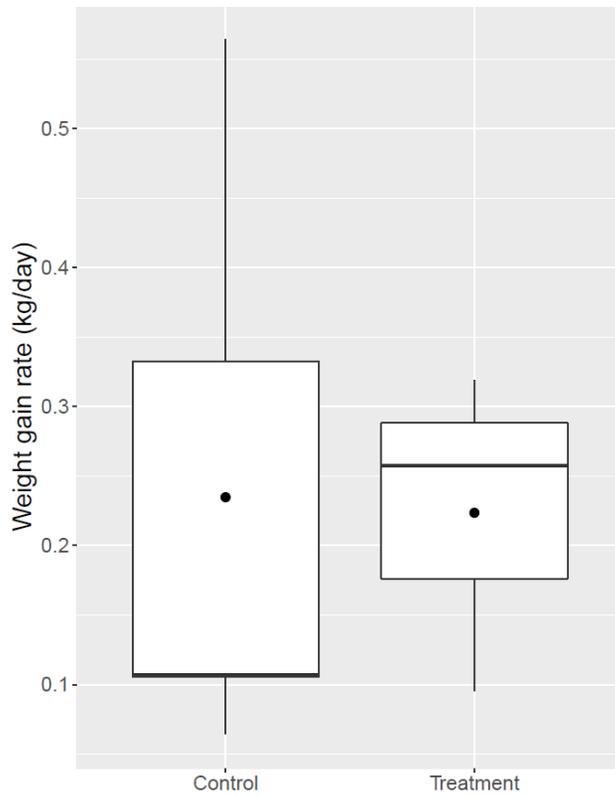


Figure 15 Weight gain rate (kg day⁻¹) for control and treatment hives from 4th July to 14th August 14th (41 days). A single control hive (number seven) achieved a gain rate of 0.56 kg.day⁻¹ whereas the median rate for the control group was 0.10 kg.day⁻¹

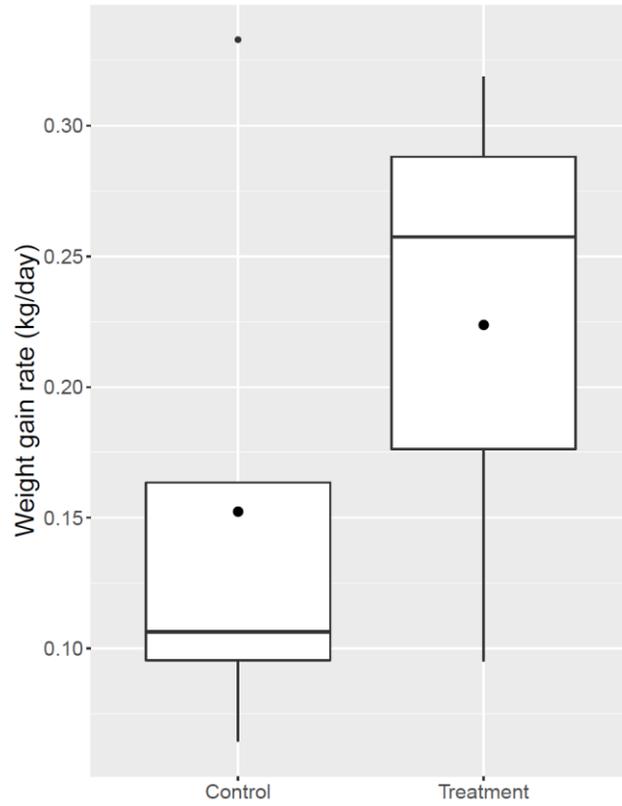


Figure 16 Weight gain rate (kg day⁻¹) for control and treatment hives from 4th July to 14th August (41 days) with hive seven removed.

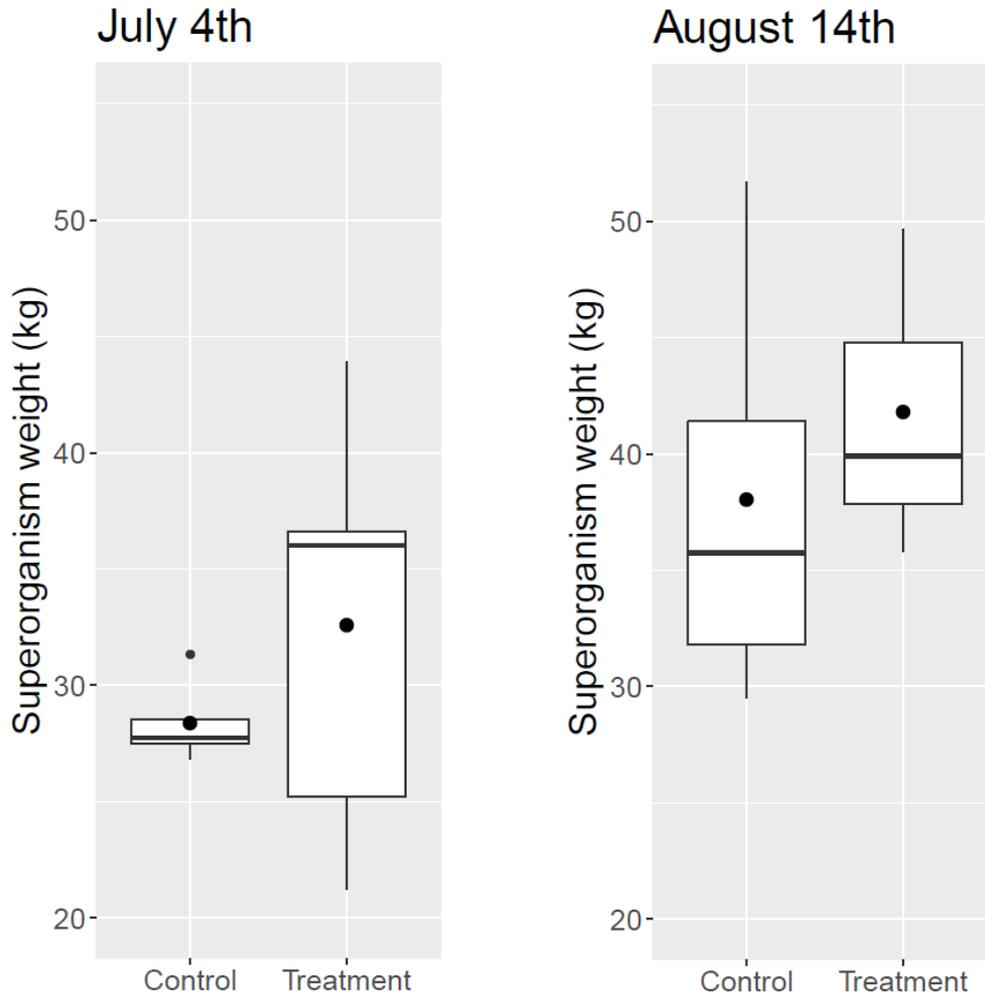


Figure 17 Superorganism weight (kg) observed in each group, July 4th on the left, August 14th on the right

3.4 Varroa mite infestation results

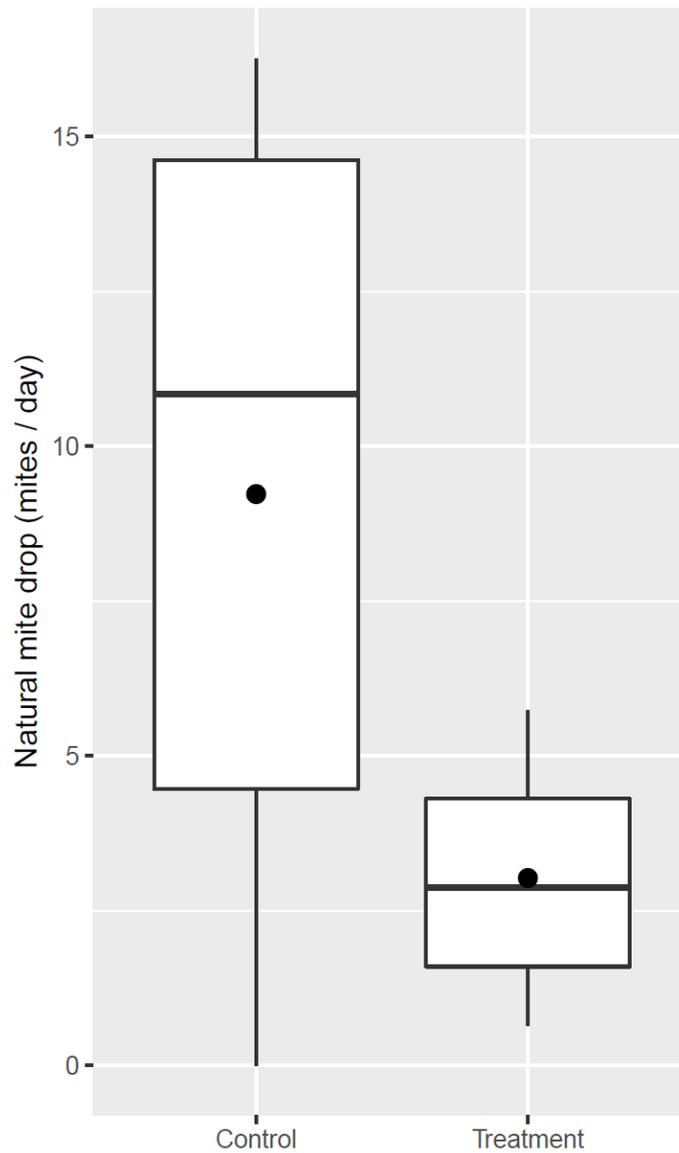


Figure 18 A large difference is visible in mite drop counts from control and treatment hives on 17th September.

3.5 Temperature within the hive

The temperatures observed at the top of box 1 are closest to the centre of the brood nest in all hives at all inspections, whereas temperatures observed at the top of box 2 are farther from the centre of the brood nest and closer to honey stores.

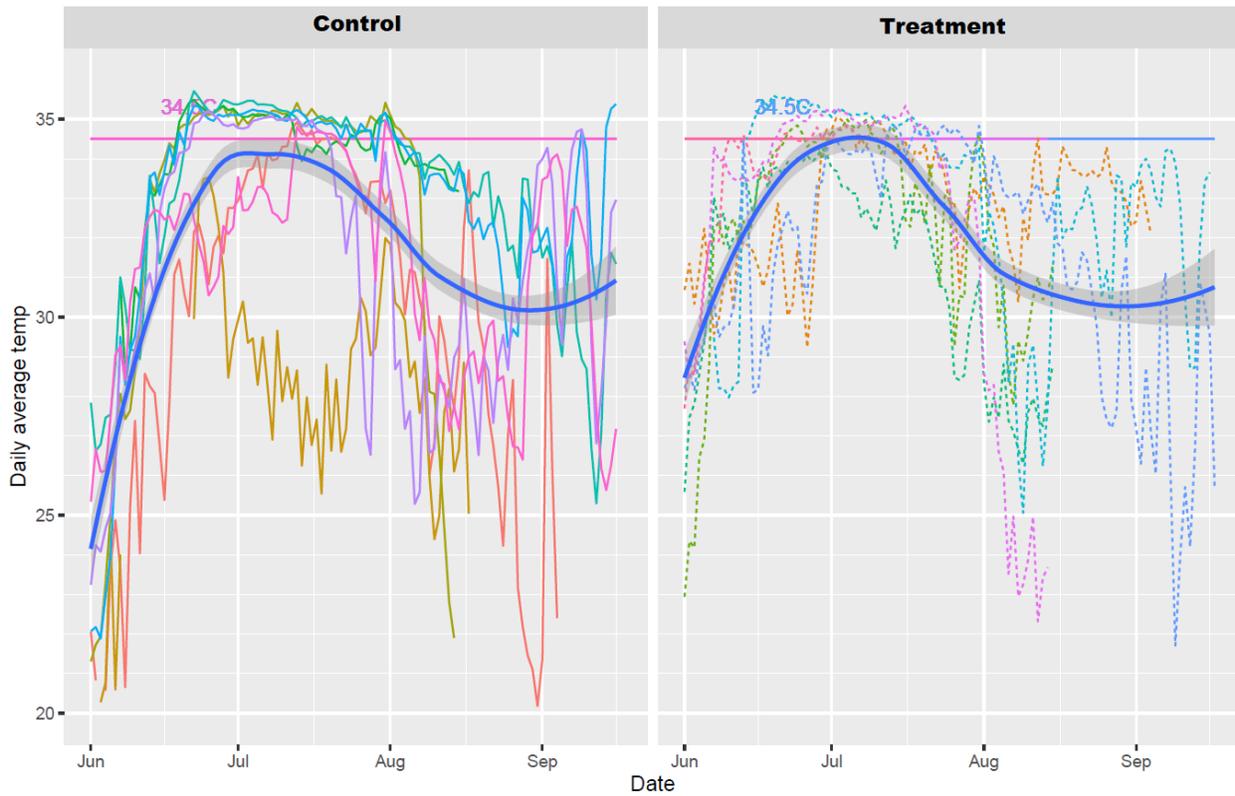


Figure 19 Mean daily temperature observed at the top of Box 1 from June 1st to September 17th. Control hives are shown on the left, treatment hives on the right

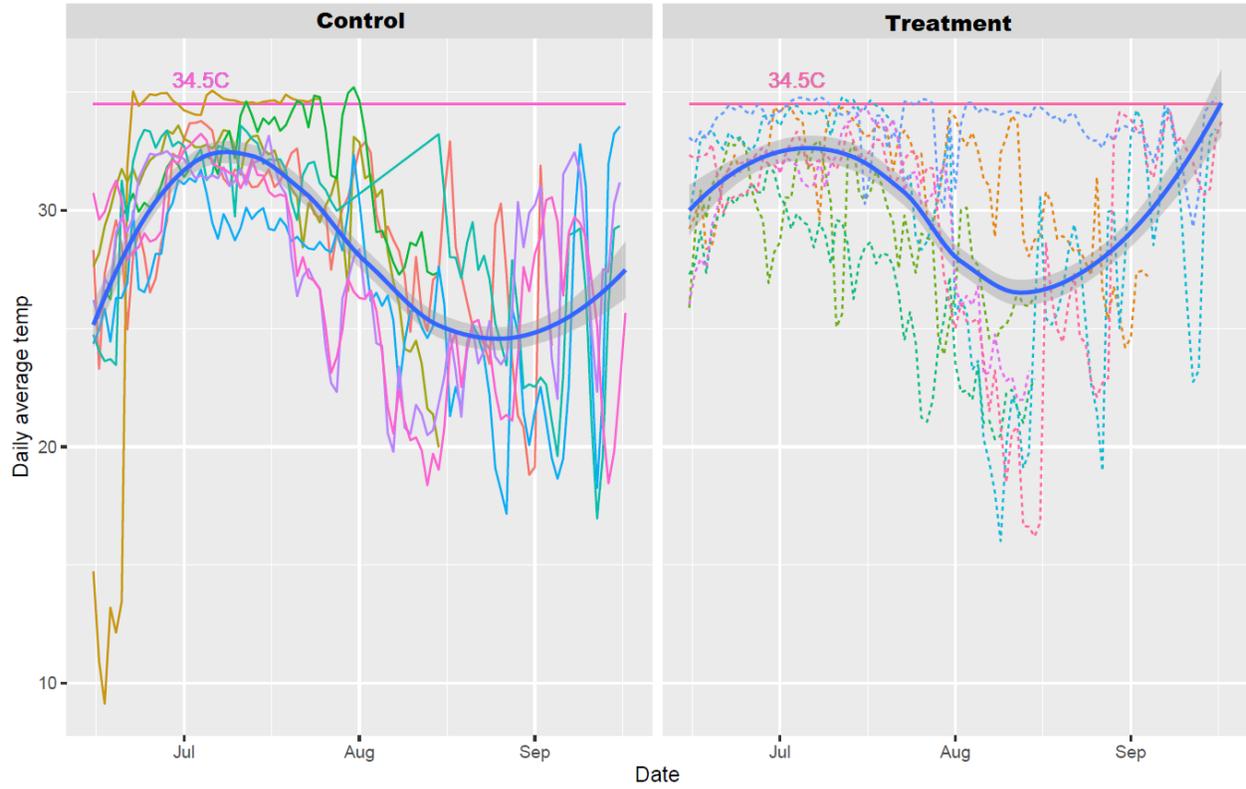


Figure 20 Mean daily temperature observed at the top of box 2 after second boxes were installed. June 14th to September 17th. Control hives are shown on the left, treatment hives on the right

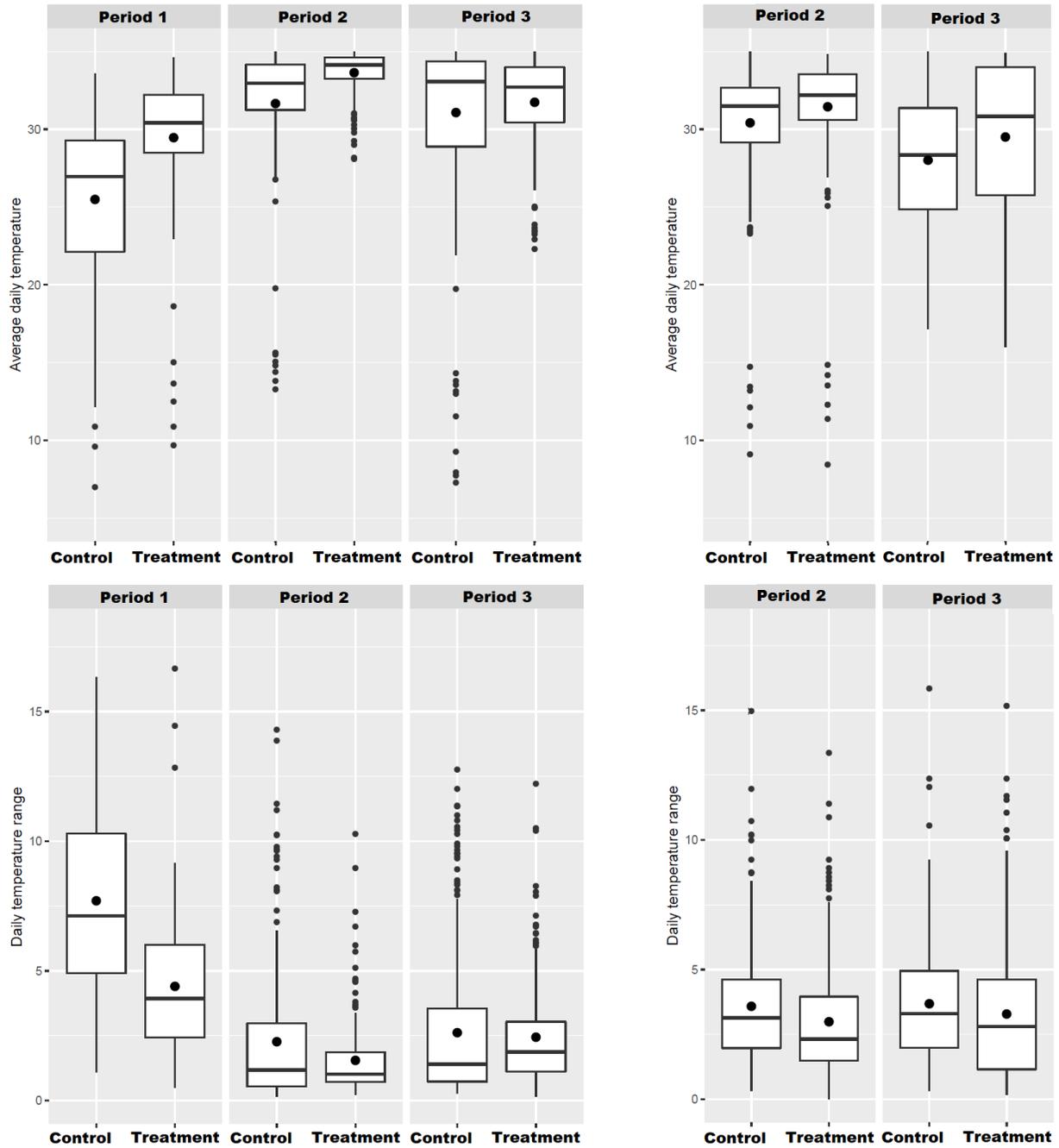


Figure 21 Boxplot of temperatures in individual hives. Top row shows daily average temperatures in °C. Bottom row shows daily temperature range in °C. Observations from the top centre of Box 1 are shown on the left, top centre of Box 2 on the right

3.6 Humidity

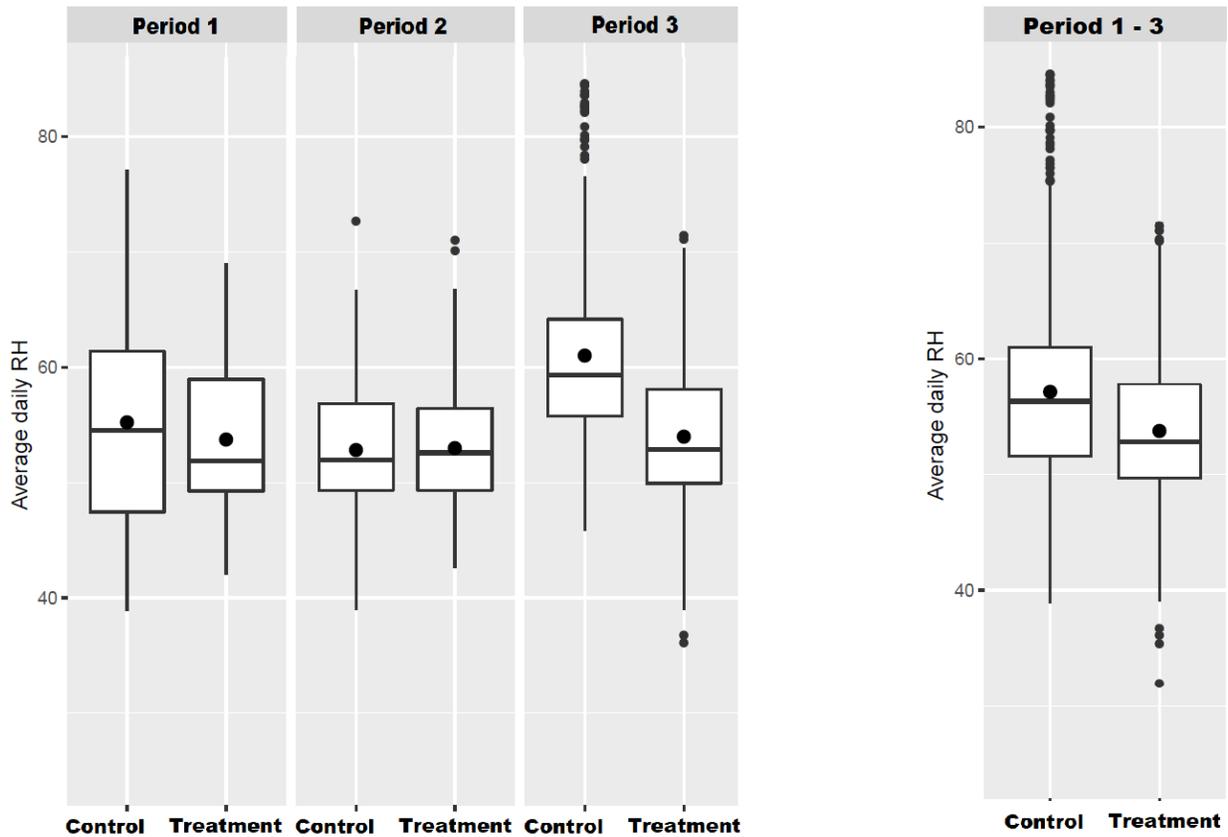


Figure 22 Daily average RH observed at the top centre of Box 1 in individual hives grouped by treatment. Three plots on the left are also grouped by inspection period, the rightmost plot includes all observations from periods one – three. Mean RH across all periods are 58% in the control group, 54% in treatment group

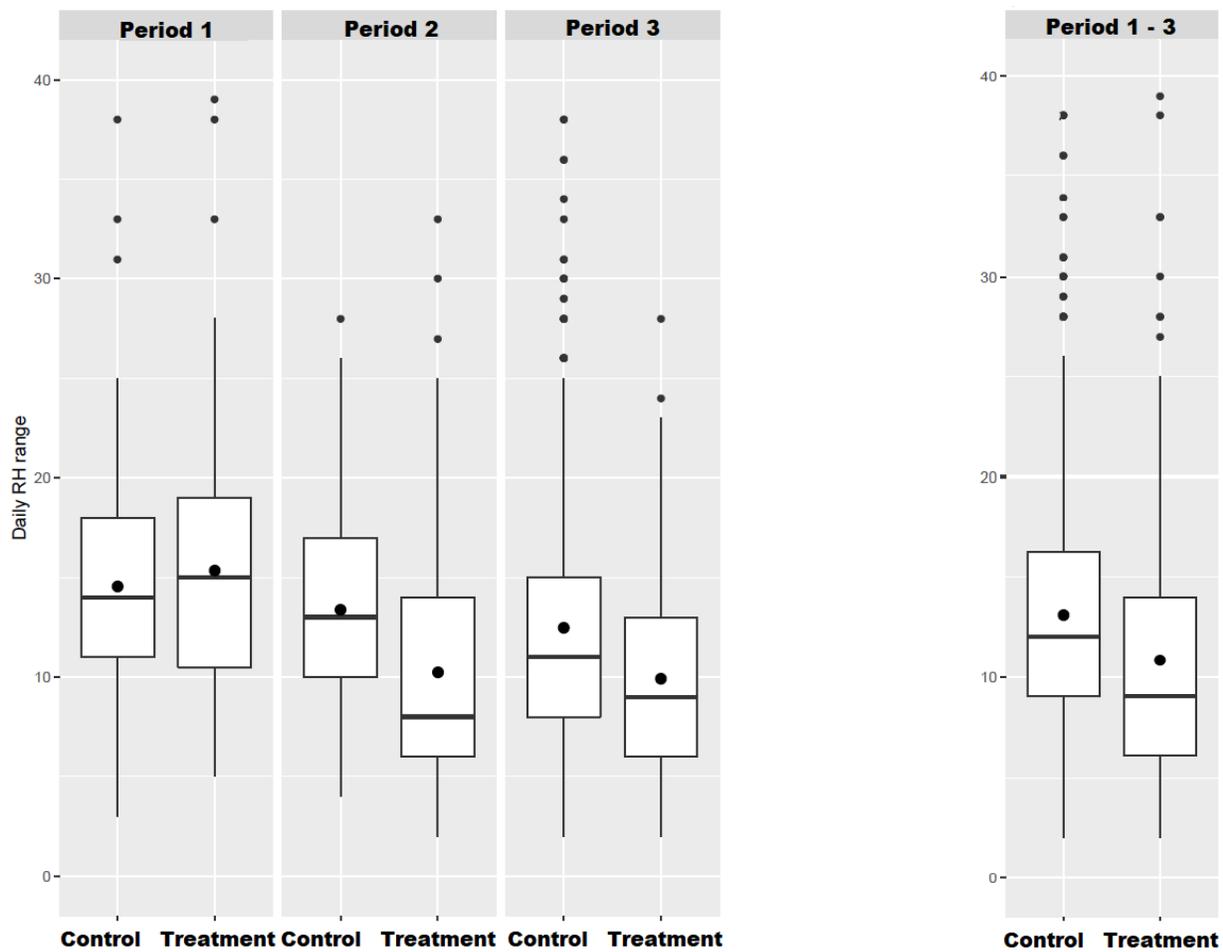


Figure 23 Daily RH range observed at the top centre of Box 1 in individual hives grouped by treatment. Three plots on the left are also grouped by inspection period, the rightmost plot includes all observations from periods 1 – 3. Mean daily range of RH across all periods are 14% in the control group, 11% in treatment group.

3.7 Comb building

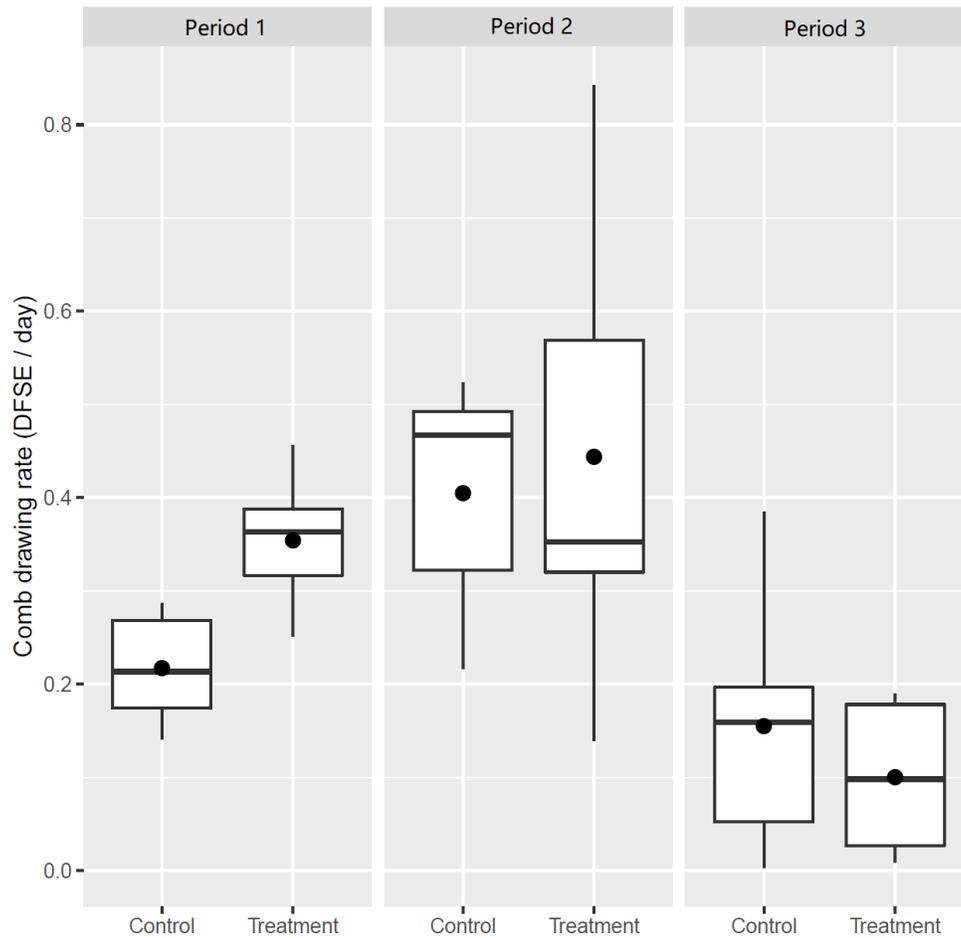


Figure 24 Comb building rates in treatment and control hives for inspection period 1, 2, & 3. Mean comb building rate was higher in the treatment group during the first inspection period, roughly equal in the second and lower in the third inspection period.

3.8 Apiary comparisons

During the study a distinct micro-climate effect was noticed at Elemental apiary which may have created more favourable conditions. To investigate, comparisons were made between Elemental and other apiaries. The box plots below show colonies at Elemental stored more honey, raised more brood, and built more comb than other apiaries.

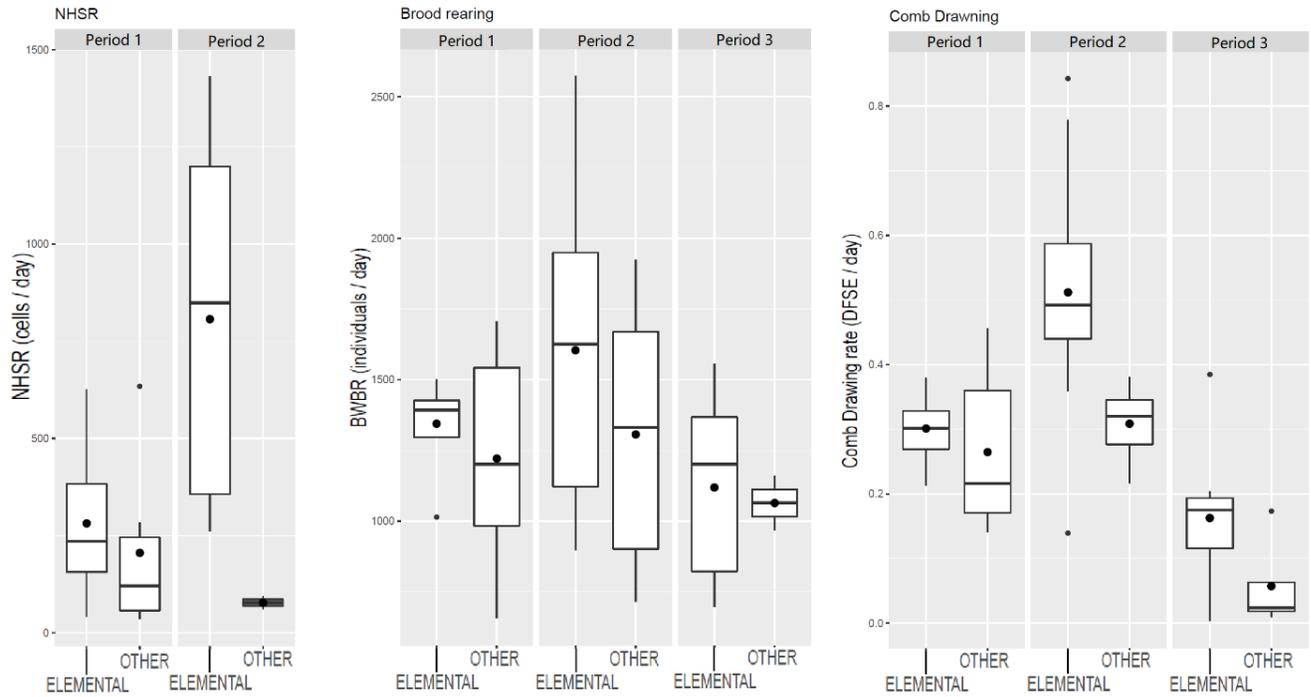


Figure 25 Honey storage rate, brood rearing rate, and comb building rate at Elemental apiary and all other apiaries in inspection periods one, two, and three

Table 9 Daily minimum, mean, and maximum ambient temperatures observed under hives at elemental and river road apiaries. The mean value for each inspection period is reported.

Inspection period	Apiary	Min T_a	Mean T_a	Max T_a
1	ELEMENTAL	7.9	13.2	18.5
1	RIVER ROAD	6.9	12.5	17.3
2	ELEMENTAL	8.5	14.6	20.6
2	RIVER ROAD	7.5	13.5	18.4
3	ELEMENTAL	7.4	13.9	21.7
3	RIVER ROAD	7.6	12.7	17.7

Table 10 Percentage of temperature observations above 7°C, 10 °C, 13°C, 16°C , 19 °C , 22 °C , and 25°C grouped by inspection period and apiary

Inspection period	Apiary	>7C	>10C	>13C	>16C	>19C	>22C	>25C
1	Other	85%	69%	50%	32%	18%	2%	0%
1	Elemental	80%	65%	50%	32%	19%	8%	4%
2	Other	95%	79%	56%	34%	9%	1%	0%
2	Elemental	93%	78%	61%	46%	30%	13%	4%
3	Other	94%	76%	45%	23%	9%	2%	1%
3	Elemental	92%	76%	52%	34%	20%	10%	3%

3.9 Supplemental feed consumption

Post-harvest autumn feeding of insulated and non-insulated hives was recorded. The results presented in Figure 26 cover the first 17 days of feeding while observations showed all hives had ample comb space for processing and storage. Subsequent feeding which may have been limited by reduced comb space is not reported.

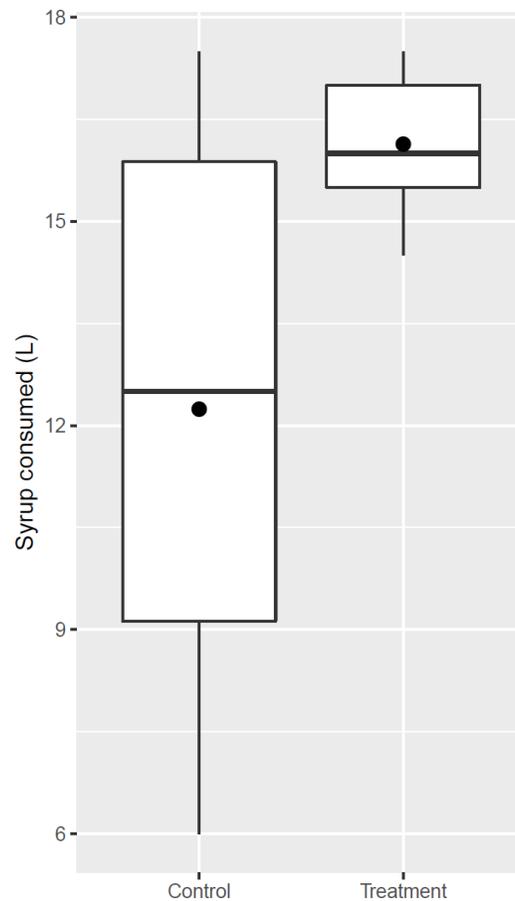


Figure 26 Sugar syrup taken from hive top feeders in treatment and control hives between 28th August and 14th of September.

3.10 Weight of bees in a hive

Weight of bees in Hive 7 on September 17th was estimated at 1.82kg. This estimate is not considered reliable as it is derived by subtracting reasonably reliable weights (equipment, stability weight, comb and contents) from a total weight reading of 83kg. Small relative errors known to be possible in the subtracted weights and total weights could result in very large relative errors in the resulting weight of bees.

3.11 Swarming

Swarming activity was reliably recorded for hives five – 16, incomplete information was available for hives one to four. Among hives five - 16, swarms were observed in three treatment hives (hives eight, ten, and 16) and no control hives.

4 Discussion and conclusions

The dramatic reductions in daily ranges of temperature and RH observed in the treatment group gives additional support to the idea that insulation enhances the colonies ability to control their environment independently of ambient conditions.

4.1 Honey storage

Honey storage (NHSR) was observed in periods two and three. The quantities observed in treatment hives during period two (which included two weeks when colonies were being fed with sugar syrup), was approximately double that in control hives, providing some support to the thesis that honey storage rate is positively influenced by hive insulation. Statistical comparisons (ANOVA) indicated the results fell short of significance and didn't fully justify rejection of the null hypothesis ($F_{4,35}$, $p=0.07$). In period three, when there was no supplemental feeding, the mean value observed in treatment hives was only 10% greater than that observed in control hives. The observed results in this period therefore indicate that NHSR appeared not to be influenced by hive insulation.

It is worth noting that if hive seven is removed from the comparison mean NHSR was 64% higher in the treatment group.

At least two potential causes for the large differences between periods two and three are suggested by other information. Insulation may advance the timing of the peak in colony strength, without necessarily providing a benefit when examined over a longer period. Also, colonies in insulated hives may have greater ability or propensity to take down supplemental sugar syrup. Further studies are needed to confirm the contribution of either case experimentally. The specific climatological and phenological conditions of the season during the study (i.e. annual variation) are likely also to have affected the result.

In the case of advanced timing Villumstad (1974) observed a similar temporal pattern, where colonies within insulated hives stored more honey than those within wooden hives in the early part of the season, but the reverse was observed later in the season. Villumstad suggests that the colonies in wooden hives developed more slowly and culminated in strength later in the season. Erdogan's (2019) more recent study reported increased honey yield as a total for the year, so it is unknown whether a similar temporal pattern occurred. If verified, the effect of hive insulation on timing of colony strength peaking could become a useful tool for beekeepers, creating an additional degree of control to improve targeting of specific nectar flows or pollination crops. In Yukon, fireweed (*Chamaenerion angustifolium*) which provides a heavy nectar flow later in the season is considered highly desirable by many beekeepers so the later colony strength peaking of non-insulated hives may be preferred whereas haskap (*Lonicera*

caerulea) pollination occurs in early spring in which case early build up would be preferred.

In observations of autumn feeding, the mean quantity of sugar syrup used in insulated hives was approximately 30% higher than in control hives. This again suggests a potential inverse relationship between hive thermal conductance and supplemental feed consumption. This may have been driven by insulated feeders retaining more heat from the colony below, coupled with forager bees' known preference for warmer syrup (Johansson and Johansson, 1976), and enhanced by the improved desiccation efficiency suggested by Mitchell. The rate at which stored syrup can be cured and the resources required for curing are influenced by hive insulation in two relevant ways; warmer syrup takes less energy to bring it up to the temperature of the stores, and desiccation after initial warming has been completed is more efficient in an insulated enclosure. Calculations based on Mitchell's (2019c) thermal energy efficiency of nectar desiccation formulae show >12% reduction in energy required to desiccate 2:1 syrup taken from a feeder at 25°C as compared to 5°C, with additional reductions dependent on ambient temperature and lumped thermal conductance of the hive.

The effect of Mitchell's proposed increased efficiency of combined nectar foraging and storage would be larger with a bigger difference between hive and ambient temperatures (i.e. when air temperatures were lower). The difference between brood-nest temperature and out-side air temperature was 21.5°C in period 2 and 21.1°C in period 3, a reduction of <2%. As energy expended to maintain temperature within a hive is largely proportional to $T\Delta$, the slight increase in average ambient temperature between periods two and three is unlikely to have played a large role in creating such differing results. Consequently, this proposed increase in efficiency is unlikely to have had a strong effect on the observed difference in honey storage in period two.

4.2 Brood rearing

There is no evidence to suggest that colonies in insulated hives reared more brood than colonies in uninsulated hives. Brood rearing rates were similar in treatment and control hives, although a slightly higher mean rate was observed in the treatment group during inspection one, no significant differences were observed in any inspection period. Brood was observed more frequently on frames closer to the sides in treatment hives than in control hives. It is possible that insulated hives provided a greater amount of suitable frame space for brood rearing but did not result in greater brood-rearing rates because of other limiting factors. Additional frame space available for brood rearing due to insulation could become relevant in hives with two-queen systems or with a particularly prolific queen.

Brood rearing is one of the more temperature-sensitive activities within a hive. Additionally, the thermoregulation energy requirements of frames closer to side walls are influenced by sidewall thermal conductance temperature differential with the exterior. The increased presence of brood on the outer frames could be evidence of reduced lateral temperature gradients within insulated hives, which would offer the

colony greater freedom in positioning and expanding the broodnest. Observations of extents and densities of brood nests show that colonies within the insulated hives spread the brood nest over a greater number of frame sides, though external foraging conditions may not have allowed exploitation of this efficiency during this particular season. Subsequent experiments could be designed to better understand the implications of variations in brood-distribution patterns in insulated and non-insulated hives.

The spatial distribution of other activities within hives may also have been affected by the use of insulation. Management of nucleus colonies includes the movement of frames within the hive to encourage comb building, in this study the only limitation placed on this movement was that frames with eggs, larva, or capped brood were never moved to the two outermost positions. A useful extension of this study would be to examine the distribution of broodnest, stores, and comb building in the complete absence of frame movement. Separately an additional extension of the study would be to examine the effectiveness of frame-swapping as a management tool to hasten the buildup of broodnest, stores, and comb building within an insulated hive environment, since less steep temperature gradients within an insulated enclosure allow more movement than would otherwise be practical.

4.3 Comb building

The observed mean comb-building rate during period one in insulated hives was 59% higher, but relatively similar in periods two and three. Tardif (2020) speculates that this result relates to the competing energy demands of wax secretion and temperature regulation. During period one the challenge of maintaining temperature within the hive is at its greatest for reasons discussed in the introduction. Production of wax is energy intensive, at this stage of development when the foraging force is relatively small and energy consumption for thermoregulation is relatively high it is plausible that reduced thermal conductance could free up more resources for wax production and comb building. To examine this a further study should investigate colonies specifically during times when wax production is likely to be a high priority e.g. the period immediately after a nucleus colony or swarm has been installed into a larger/new hive.

4.4 Weight gain

In the period 4th July to 14th August, mean weight gain in control hives was slightly higher than that of treatment hives. Mean weight in treatment hives on July 4th was 10.8% higher than in control hives, suggesting that insulated hives may gain weight faster in the early part of the season. However the large degree of variation meant the difference was not significant ($p=0.37$). The above provides additional support to Villumstads conclusion that insulation benefits the colony more during the early part of the productive season than the later part.

The method for determining total weight of bees within a hive would need to be improved and validated before the resulting weights could be relied on for any comparison.

4.5 Varroa mite infestation

Observed Varroa drop-rate in September was three times higher in control hives than treatment hives, giving some support to the idea that insulation affects colony's interactions with Varroa. However, a large degree of variation and low number of replicates meant the difference in means was not significant and the null hypothesis was not rejected ($p=0.13$).

The hypothesis partly originated from observations that Varroa mite reproduction was severely reduced at RH above 75-80% (Kraus and Velthuis, 1997) taken with Mitchell's suggestion that insulation should allow RH above this threshold to be maintained in the broodnest. However, observations in this study do not support this mechanism.

Observed mean RH was 4% lower in the treatment group (54% vs 58%), additionally daily average RH over 75% was only seen in four hives and only on very few days in those hives. The majority (>96%) of all daily average RH observations in both insulated and non-insulated hives were under 75%. Optimum humidity for reproduction of Varroa ranges from 55% to 70% according to Nazzi and Le Conte (2016). Thus, the RH observations in this study were in the vicinity of the lower limit of the optimum range, where Varroa fecundity can be expected to be positively related to RH. Mitchell's proposed negative relationship occurs at and above the upper limit of the optimum range which may be more relevant in regions which experience higher ambient RH such as the temperate oceanic climate covering much of Northern Europe and less relevant in dry continental regions. The relationship between RH and Varroa is clearly not a simple one.

Temperature observations suggest that colonies within insulated hives maintained higher daily average temperatures in the vicinity of the broodnest, with a distinctly-reduced daily temperature range both in the brood nest and honey stores. In line with Le Conte's (1990) observations, the higher temperatures observed in insulated hives may have reduced Varroa fecundity. While the single temperature sensor close to the broodnest centre cannot reliably determine the frequency or magnitude of localised short duration temperature spikes, the observed higher mean temperature and reduced temperature gradient may have allowed more efficient and effective temperature peaking behaviours of the type described by Villa, Gentry and Taylor (1987) and shown to be unfavourable to the development of Varroa by Le Conte (1990). Additionally, Varroa mites preference for cooler cells favours drone brood which is typically located at the periphery of the broodnest (Levin and Collison, 1990; Winston, 1987), the reduced temperature gradients within the insulated hives may have provided less opportunity for optimum Varroa reproduction.

4.6 Comparisons between study apiaries

In period two colonies at the Elemental apiary stored 37% more honey, raised 23% more brood, and built 65% more comb than colonies at other apiaries. Similar differences were recorded in periods one and three (Figure 25). At Elemental the hives were situated on top of a dark coloured 2.4m x 6.1m shipping container (height 2.5m) with a 1.1m high black windbreaking fence around the perimeter. The shipping container was oriented with its long side on a north-south axis. As a result, both the shipping container walls and the windbreak caught early morning and late evening sunshine creating a relatively warm microclimate driven by the solar gain. Minimum, mean, and maximum daily temperatures observed under hives at the Elemental apiary were higher than those observed at River Road, which was the closest at a distance of only 5.3km.

Security from bears and other wildlife was the reason for situating the hives on a shipping container, the resulting micro-climate and its associated benefits were unexpected.

4.7 More swarms from insulated hives

Swarming activity generally indicates that a colony's circumstances are sufficiently good for reproduction to occur (all other circumstances being equal). It is less common during the first year of nucleus colony development. The outcome where insulated colonies grew stronger and filled hive space faster than uninsulated controls, adds further evidence to indicate the positive impact on colonies from insulated hives. Additionally, as the swarming events caused hives to be excluded from subsequent comparisons, it is possible that greater differences between control and treatment hives may have been recorded had swarming been prevented. The three hives that issued swarms between inspection two and three had large brood nests at inspection two, ranked 2nd, 3rd, and 7th largest.

4.8 Study limitations and recommendations for further research

4.8.1 Colony and apiary management

“All beekeeping is local beekeeping” – colony responses to reduced thermal conductance of hives is likely to be influenced by many local factors such as interannual climate variation, forage species present, seasonal and daily timing of nectar and pollen availability etc. While the outcomes of this study are most relevant to beekeepers in the subarctic southern Yukon region, they may have relevance in any region where air temperatures in the foraging season are consistently lower than the optimum brood nest temperature of 34.5°C.

Varroa mite infestation levels can be influenced by multiple factors which could potentially be controlled in future experiments. Namely, the level of infestation at the start of the experiment was unknown in all hives; the date and magnitude of possible mite introduction from external sources was unknown; genetic traits of mites were unknown and substantial differences between mites in each hive or apiary were possible; natural mite drop rate on which the sticky-board method relies includes substantial randomness. A future experiment could reduce the effect of these factors by performing repeated oxalic acid vapour treatments at the start of a study followed by controlled introduction of mites from a heavily infested colony, more accurate assessments of mite infestations could then be obtained through the alcohol wash method and lead to a more accurate characterisation of how varroosis and colony strength are related.

4.8.2 Data collection, preparation, and analysis practises

Multiple results from this study provide some support for Villumstad’s suggestion that insulated hives allow for an earlier peaking of colony strength with little or no improvement in total productivity over the whole year. Certainly, there is variation in the effect of insulation at each stage of colony development. A future study examining the relationship between insulated hives and temporal patterns of population buildup could result in the development of management practises assisting beekeepers’ control of colony strength timing, with associated benefits in targeting specific nectar flows and crops requiring pollination. Economically important examples in the region include haskap berry pollination in early summer and fireweed honey production in late summer.

Overwintering success rates which were not included in this study could also be influenced by differences in timing and magnitude of colony development due to insulation during the preceding summer. Hives five to 16 have undergone identical winter preparation and should be assessed for survival and strength in spring 2021.

4.8.3 Experimental design

Eight replicates for each treatment were used in the study. The numbers of colonies excluded from comparisons due to confounding factors severely reduced replication and experimental power. There will always be practical reasons limiting replicate numbers but closer management would maintain higher numbers and reduce the likelihood of type-2 errors. Results from this study will be useful in estimating experimental power for other studies in the region.

The random signal in all analyses could be reduced by ensuring that queens used are genetically similar, sister queens mated at the same place and time are recommended. Where possible all replicates should be located at the same apiary, while taking care not to introduce excessive foraging competition as another confounding issue. If nucleus colonies are used then a complete set of data points should be collected as they are installed.

4.9 Conclusions

Results from this study demonstrated that insulated hives allow colonies a greater control over their environment and supported a reduction in energy demanded by key thermally driven colony activities, in turn leading to minor improvements in colony health and productivity. While increased honey production in insulated hives is mostly confined to the early part of the active foraging season, increases in the latter part may be relatively minor, neutral, or possibly even negative. Over a full season, productivity gains of 0 – 30% are possible with the lower end of that range being more likely. Beekeeping operations which primarily target later nectar flows may see smaller gains than those primarily targeting early flows. Improvements in comb building rates are similarly biased towards the early part of the season. The full impact of early season efficiency and productivity, as well as potential health benefits, cannot be fully assessed until the following season when over-wintering success can be considered.

Insulation also led to a consistently altered spatial distribution of brood, although experimental circumstances didn't allow a full exploration into the reasons for the changed behaviour. However, this wider distribution within the brood-nest space appeared to be an opportunity exploited by colonies as soon as internal conditions allowed so it is likely to be associated with some efficiency perceived by the colonies.

Insulation may also help to reduce Varroa infestation levels. Although there is much uncertainty around the mechanism through which this is achieved, hive insulation has potential to assist in the battle against Varroa when used alongside other mite management practises.

In addition, insulation can allow beekeepers to encourage faster and more controlled application of supplemental syrup feeding through hive top feeders, particularly when ambient temperature is below minimum foraging temperature.

Apiary layouts designed to create favourable microclimates may deliver similar benefits of equal or greater magnitude, as such any beekeeper considering investing time and money in insulated hives should also consider and compare improvements to apiary micro-climate.

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7 Appendix A - Supplementary Tables

Table 11 Dates of nucleus colony installations and subsequent inspections.

Inspection	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16
Nuc Install	29- May	29- May	07- May	07- May	28- May	29- May	29- May									
1	11-Jun	11-Jun	21-Jun	21-Jun	12-Jun	14-Jun	14-Jun	14-Jun	14-Jun							
2	11-Jul	11-Jul	-	-	13-Jul	13-Jul	12-Jul	12-Jul	12-Jul	12-Jul	12-Jul	12-Jul	15-Jul	15-Jul	12-Jul	12-Jul
3	-	-	-	-	18- Aug	18- Aug	17- Aug	17- Aug	16- Aug	16- Aug	16- Aug	16- Aug	17- Aug	17- Aug	17- Aug	17- Aug

8 Appendix B – Photos



Figure 27 Elemental apiary located on top of a shipping container



Figure 28 Elemental apiary after the first snow in October 2020



Figure 29 Insulated hive box



Figure 30 Four insulated and four standard hives immediately before installation of nucleus colonies



Figure 31 Example set-up of insulated and standard hive, each with a slatted rack, 1 deep box, 1 medium box, and a hive top feeder.