The effects of geographic orientation on shrub microclimates related to Australian pipit nesting

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Abstract

Refugia with mild microclimates are central for the survival of many alpine species. Alpine shrubs create microclimates with conditions that can benefit birds, such as the Australian pipit, *Anthus novaeseelandiae* (Gmelin, 1789). Pipit populations in harsh climates utilise specific behavioural techniques to increase their nesting success: the Snowy Mountains *A. novaeseelandiae* population nest underneath shrubs at orientations between NNE and SE as identified by Norment and Green (2004). We wanted to determine whether conditions were significantly milder under a shrub between 22° and 125° in orientation. Recordings of abiotic conditions were taken under shrubs in Kosciuszko National Park and then compared to ambient conditions. We found that alpine shrub microclimates are less extreme than ambient conditions, their stability is independent of their aspect and that the microclimate between 22° and 125° remains more humid as ambient conditions become drier. This could add to our understanding of alpine bird ecology and play a role in conservation.

Key Words

Abiotic conditions, Kosciuszko National Park, pipit nesting

Introduction

Microclimates provide refuge for animals in harsh environments, such as those that characterise alpine regions. Australian alpine conditions are extreme and variable; ambient temperatures range from below freezing to 45°C, winds reach over 80 km/h and relative humidity can drop as low as 15 per cent (Martin and Wiebe 2004). Microclimates tend to differ from the surrounding climate, due to blocking and insulating factors. A study by Scheffers *et al.* (2014) examined the rate of vegetative microhabitat temperature change compared to ambient conditions. Their results show that these microhabitats had a buffering effect, slowing microclimate temperature change and decreasing the occurrence of extreme temperatures.

Many alpine birds make use of milder microclimates created by vegetation to avoid harsh ambient conditions. Selective pressure on microhabitat preferences has been demonstrated by Martin (1998), with birds exhibiting greater nesting success at preferred microhabitat sites than non-preferred ones. The use of microhabitats is especially important during the nesting season, as offspring must be kept in optimal conditions for maximum nest success (Mayfield 1975). Extreme conditions are correlated with decreased fitness or mortality in nestlings (Grisham *et al.* 2016). This study will consider microclimate temperature, wind chill, wind speed, relative humidity and light intensity, as these variables are known to affect nestling growth and survival (Greño *et al.* 2008; Kosicki 2012; Sicurella *et al.* 2015).

The Australian pipit (*Anthus novaeseelandiae* Gmelin, 1789) is an example of a ground-nesting bird species that makes use of the microclimates provided by small shrubs. The species is migratory, following food sources and suitable conditions throughout the year. One Australian pipit population moves between Kosciuszko National Park (New South Wales, Australia) in summer and the surrounding tablelands in winter (McEvey 1952). During an observational study on the Australian pipit's breeding ecology, Norment and Green (2004) discovered that their nests were non-randomly oriented in the shrub, always positioned between 22° and 125° relative to magnetic north. Norment and Green (2004) hypothesised that the Australian pipit's nest selection was determined by weather, as the nest placement avoids incoming cold fronts and storms. This study will build on this platform,

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considering the idea that microclimate conditions at different orientations under a shrub may also impact pipit nest site selection (Rauter and Reyer 2000).

This project aims to determine whether shrubs create a microclimate milder than ambient alpine conditions and, specifically, whether the microclimate between 22° and 125° is more mild than ambient conditions and microclimates at other orientations. It is predicted that shrub microclimates will be more mild than ambient conditions when considering temperature, wind chill, wind speed, relative humidity and light intensity. It is also predicted that the microclimate between 22° and 125° will be milder or more consistently mild than other orientations. Answering these predictions will allow us to determine which abiotic signals the Australian pipits might be responding to when choosing nest location.

Methods

The abiotic conditions associated with alpine shrub orientation microclimates were measured during early summer (December 2016) at Charlotte Pass, NSW, Australia. The species sampled from were *Grevillea australis* (R.Br.), *Kunzea muelleri* (Benth.) and *Prostanthera cuneata* (Benth.) Species name was not recorded when taking data as differences between or within species were not the focus of this experiment.

Field

A curved transect was conducted in Kosciuszko National Park that followed the path from the Charlotte Pass carpark to Mt Stillwell. At the end of the path closest to the road, two shrubs were sampled, one on either side of the transect. A 5 m line was measured perpendicular to the transect on either side and the closest isolated shrub on each side was used. This was repeated every 50 m along the transect for 500 m, for a total of 35 times. Two shrubs selected with this method were measured towards the other end of the trail, making a total of 37 shrubs. The standardised data from these two shrubs were included in the analysis because elevation was not a factor considered in this experiment.

Using a compass (Handy GPS app), the orientations of 30°, 120°, 210°, 300° relative to magnetic north were located. At each of these orientations, abiotic conditions were measured using a Kestrel 3000, with the tool held upright on the ground and facing outwards of the shrub. Abiotic conditions were also measured directly over the shrub, with the Kestrel 3000 facing into the wind, to record ambient conditions. The abiotic conditions measured were temperature, wind chill, wind speed, relative humidity and light intensity. The initial measurements were taken on a clear, warm day and repeated at the same points on the same shrub on the following day, which was overcast and windy, to help standardise the data.

Statistical analysis

Section one: T-tests

T-tests were run using the statistical software program R (version 1.0.44) to compare shrub conditions with ambient conditions for each of the abiotic factors measured (Appendix 1). This was done using the difference between the shrub conditions and ambient conditions using the formula $C_x - C_a = condition_x - condition_a$, where $condition_x$ is the condition measured in the shrub microclimate and $condition_a$ is the ambient condition, those being temperature, wind chill, wind speed, relative humidity or light intensity.

Section Two: Blocked ANOVA

Blocked Analysis of Variance (ANOVA) tests were run in R to determine if microclimate abiotic variables differ with orientation. Individual shrub number was blocked, as it was not the variable of interest (Appendix 2).

Section Three: $C_x - C_a$ *relationship*

Analysis of Covariance (ANCOVA) and linear models were run in R to test whether ambient humidity has an impact on the difference in relative humidity between the shrub microclimate and ambient conditions; the same was also done for temperature data (Appendix 3a and 3b). ANOVA tests were used to summarise the ANCOVA results (Appendix 3a and 3b).

Results

Section One: *T*-tests

The shrub microclimate conditions differed considerably from ambient conditions. We found highly significant differences between ambient conditions and shrub microclimate conditions for each of the abiotic variables measured (Table 1). Shrub microclimates were found to be warmer, have a warmer apparent temperature, be less windy, be more humid and darker.

Table 1: *T*-test results comparing ambient and shrub microclimate conditions

	Average microclimate condition	*Average ambient condition*	*p-value*	*n*
Temperature (°C)	19.05	18.42	≤0.0001***	148
Wind chill (°C)	18.98	18.05	≤0.0001***	148
Wind speed (km h^{-1})	1.29	5.12	≤0.0001***	148
Relative humidity (%)	45.76	39.85	≤0.0001***	148
Light intensity (kl)	21.10	77.06	≤0.0001***	140

Note: * represents *p*-values < 0.0001**

Section two: Blocked ANOVA

The results of the blocked ANOVA indicate that geographic orientation does not have a significant effect on the difference between ambient and shrub microclimate conditions ($C_x - C_a$) for most variables. Non-significant results were seen for temperature ($C_x - C_a$) ($F_{3,108} = 1.24$, $p = 0.299$, n = 37 shrubs), wind chill ($F_{3,108} = 0.900$, $p = 0.444$, n = 37), wind speed ($F_{3,108} = 0.782$, $p = 0.507$, n = 37) and relative humidity ($F_{3,108} = 1.005$, $p = 0.394$, n = 37). However, a highly significant result was found for light intensity ($F_{3,108} = 4.482$, $p = 0.005$, n = 35).

Section three: $C_x - C_a$ relationship

Temperature

The summarising ANOVA of the ANCOVA (see Appendix 3) revealed that neither T_a nor nest orientation significantly affect $T_x - T_a$ (Table 2). Also, nest orientation does not significantly affect the relationship between T_a and $T_x - T_a$.

Table 2: Analysis of variance of ANCOVA test on temperature and orientation; response: $T_x - T_a$

	Df	**Sum Sq**	**Mean Sq**	***F*-value**	***p*-value**
T_a	1	2.21	2.2098	0.7413	0.3907
Orientation	3	5.95	1.9829	0.6652	0.5748
T_a: Orientation	3	16.99	5.6639	1.9000	0.1324
Residuals	139	417.34	2.9810		

Linear modelling showed no significant relationships between $T_x - T_a$ and T_a for any of the orientations. At 30°, there was a weak (R^2 = -0.0286), non-significant (p = 0.995) relationship between $T_x - T_a$ and T_a. At 120°, there was a weak (R^2 = -0.0001), non-significant (p = 0.325) relationship. At 210°, there was a weak (R^2 = -0.0198), non-significant (p = 0.586) relationship. At 300° there was a weak (R^2 =

0.0488), non-significant (p = 0.100) relationship. These tests reported negative adjusted R^2 values, indicating non-significance of explanatory variables due to a small sample size.

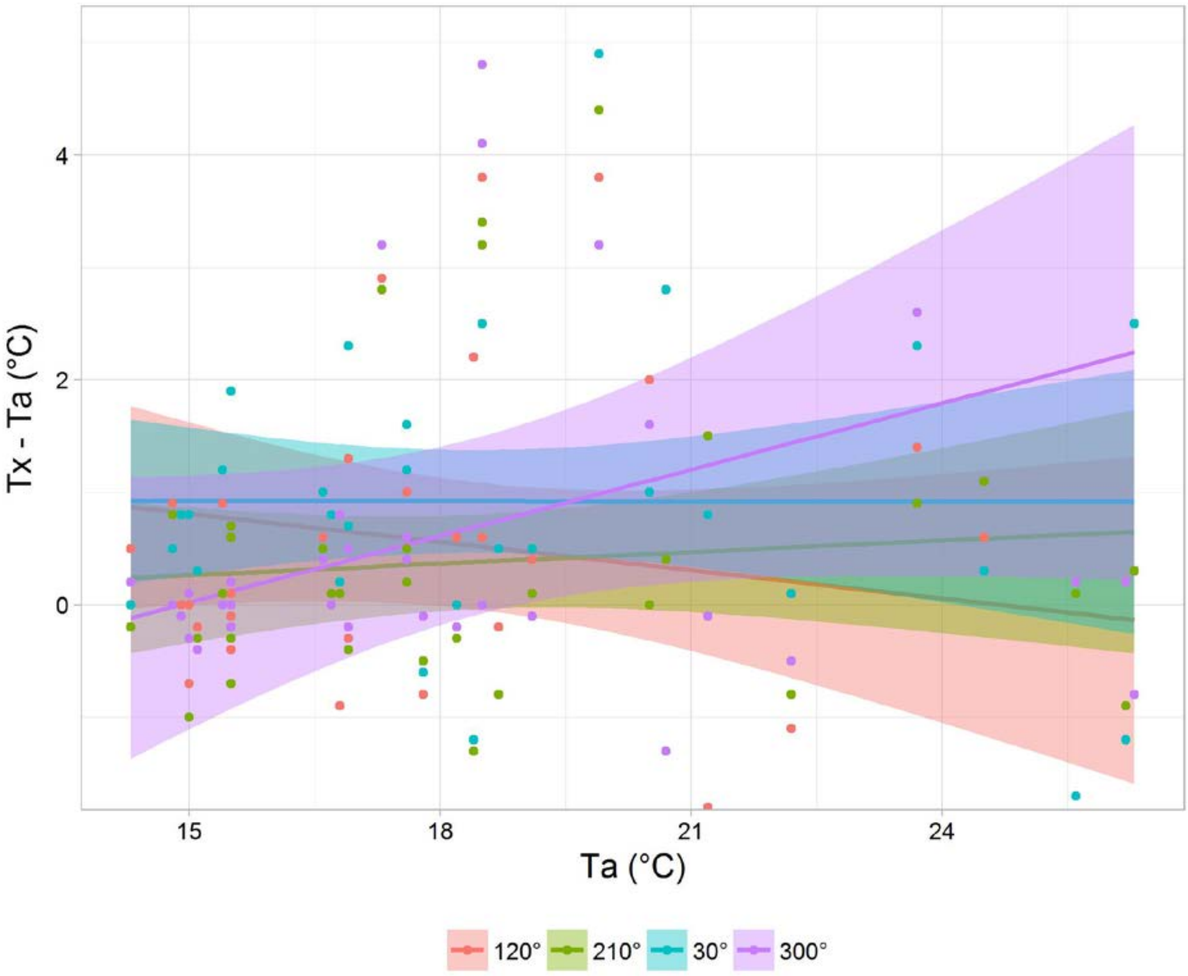

Figure 1: Line graph displaying the correlation between ambient temperature (T_a, °C) and the difference between microclimate temperature and ambient temperature ($T_x - T_a$, °C) for each of the studied geographical orientations. Each graph includes a 95 per cent confidence envelope (α = 0.05, n=35).

Relative humidity

The ANOVA of the ANCOVA revealed that nest orientation significantly affects the relationship between $RH_x - RH_a$ and RH_a (Table 3).

Table 3: Analysis of variance of ANCOVA test on relative humidity and orientation; response: $T_x - T_a$.

	Df	Sum Sq	Mean Sq	*F*-value	*p*-value
RH_a	1	748.1	748.11	16.3566	≤0.0001***
Orientation	3	93.8	31.27	0.6836	0.5635
RH_a: Orientation	3	404.4	134.81	2.9475	0.0350*
Residuals	140	6403.3	45.74		

Note: * represents *p*-values < 0.0001.**

Linear modelling showed significant negative relationships between RH_x – RH_a and RH_a for orientations 30° and 120° but not 210° or 300° (Figure 1). At 30°, there was a weak (R^2 = 0.2666), significant (p = 0.0006) relationship between $RH_x - RH$ and T_a. At 120°, there was a weak (R^2 = 0.1878), significant (p = 0.0043) relationship. At 210°, there was a weak (R^2 = -0.0113), non-significant (p = 0.4449) relationship. At 300°, there was a weak (R^2 = 0.2103), non-significant (p = 0.7360) relationship. The linear modelling tests returned weak adjusted R^2 values for 30° and 120°, indicating that ambient temperature can only explain some of the variation; and negative adjusted R^2 values for 210° and 300°, indicating non-significance of explanatory variables due to a small sample size.

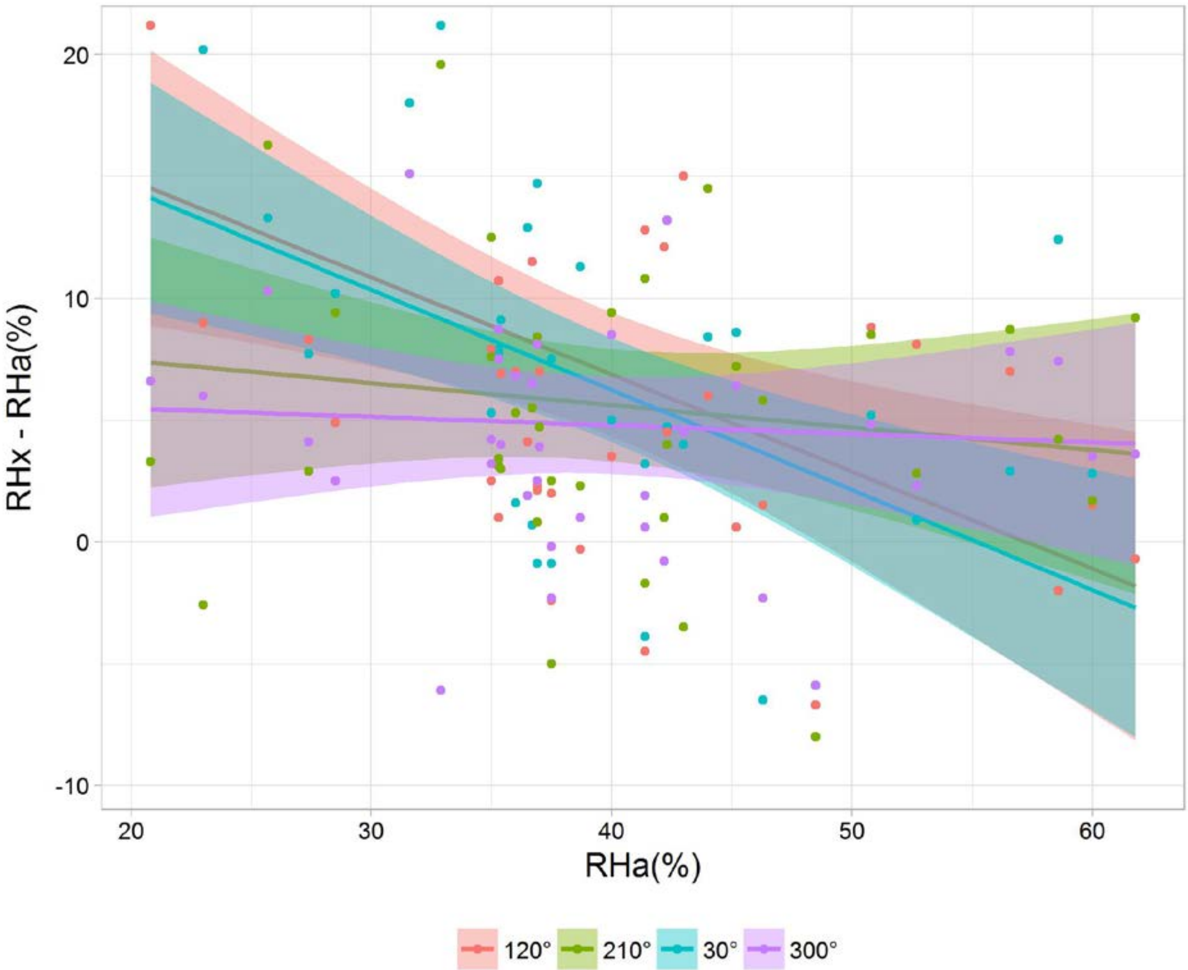

Figure 2: Correlation between ambient relative humidity (RH_a, %) and the difference between microclimate relative humidity and ambient relative humidity ($RH_x - RH_a$, %) for each of the studied geographical orientations. Blue represents an orientation of 30°; orange, 120°; green, 210°; and purple, 300°. Each graph includes a 95 per cent confidence envelopes (α = 0.05, n = 35).

Discussion

How do shrub conditions compare with ambient conditions?

This study found shrub microclimates to be significantly warmer, less windy, more humid and darker than ambient conditions. This microclimate would be more favourable for many alpine species compared to ambient alpine conditions. Shrub microclimates may help prevent conditions such as hypothermia and dehydration in animals, and temperature stress and desiccation in plants that would otherwise occur due to the extreme conditions of the alpine environment. The migratory Australian pipit is sensitive to extremely cold and dry conditions, and so makes use of the buffering effect of alpine vegetation (McEvey 1952). Constructing nests in the milder microclimate of alpine shrubs would prevent environmental damage to nestlings and mature birds during the nesting season.

The shelter provided by alpine shrubs influences general floral ecology and the surrounding community structure. Shrubs have been proven to facilitate seedling establishment in harsh environments by providing milder microhabitats (Jankju 2013). Milder shrub microclimates may affect soil factors, including microbes, composition and quality, leading to widespread, long-term impacts on alpine ecology (Prieto *et al.* 2011). Further research could combine this concept with themes from the following two studies: the first by Sohlenius and Boström (1999), which found a strong influence of vegetation on nematode ecology; and the second by Eskelinen *et al.* (2009), which found that soils below shrubs tend to have high carbon and low nitrogen concentrations.

Do shrub microclimate variables differ with orientation?

The difference between shrub microclimate conditions and ambient conditions ($C_x - C_a$) allows for the comparison between geographic orientations and therefore microclimates within a shrub. It was seen that when comparing these $C_x - C_a$ values, shrub microclimate temperature, wind chill, wind speed and relative humidity are all independent of geographic orientation, but light intensity is dependent, with the brightest orientation being north. This is due to the study site being in the southern hemisphere, with north-facing surfaces receiving more sunlight than those with other aspects. Based on this alone it would appear that orientation is of little importance for nest site position, provided the nest is located within the general shrub microclimate. However, it is likely that it is not standard conditions, but extreme conditions that would determine nest site orientation, as discussed by Norment and Green (2004). The study conducted by Norment and Green (2004) suggested that Australian cold fronts placed a selective pressure on Australian pipits, with nests facing away from incoming cold fronts being more successful. Therefore, by incorporating data from days with extreme temperatures, wind speed and relative humidity we may see more meaning given to the results of this experiment.

Does the importance of orientation vary with ambient conditions?

It was found that the difference in temperature between the shrub microclimate and ambient conditions did not change with ambient conditions and orientation, and that the relationship between the difference in temperature between the shrub microclimate and ambient conditions and ambient temperature is independent of nest orientation. This means that shrub microclimates vary regardless of ambient temperatures and their aspect, and that an increase in ambient temperature is not correlated with a linear increase in microclimate temperature at any of the orientations.

We found that ambient relative humidity has an impact on the difference in relative humidity between the shrub microclimate and ambient relative humidity. This means that generally at low ambient relative humidity, shrub microclimates will be more humid than ambient conditions. Our ANCOVA tests also showed that the relationship between ambient relative humidity and the difference in relative humidity between the shrub microclimate and ambient conditions is significantly affected by orientation. Linear modelling revealed that this is true only at orientations 30° and 120°, with the other two orientations not having a significant correlation between ambient relative humidity and the difference in relative humidity between the shrub microclimate and ambient conditions. These results indicate that shrub microclimates at 30° and 120° are most suitable for organisms aiming to avoid dry conditions.

Nest humidity is of vital importance as it determines levels of water vapour conductance from eggs and nestlings (Portugal *et al.* 2014). Low levels of vapour conductance, specific to each species, are beneficial for avian embryo development, but high levels may lead to desiccation (Walsberg 1980). For this reason, selection of nest sites in microhabitats with the appropriate humidity level is beneficial; this is especially true for avian species in extreme climates.

Future studies

Research in this area would benefit from year-long studies, as shrub microclimate conditions are likely to change with the season. Ideally, the study should incorporate data that represent the extremes of the alpine environment: below-freezing temperatures, extremely high temperatures, strong winds and variable light intensity. As the negative adjusted R^2 values indicated, this study's sample sizes were too small. A larger sample covering a greater area would be beneficial. Also, examining the relationship between $C_x - C_a$ and C_a at different orientations for factors other than temperature and humidity may return interesting results.

This experiment was inspired by a study by Norment and Green (2004), which specifically looked at Australian pipit nests and their orientation. Future experiments should consider implementing their methods for locating Australian pipit nests, as sites chosen by the pipit may have additional factors that have not been yet considered.

Further studies may find the relationship between alpine microclimates and topography, aspect and community structure to be of note. The impact of microclimates on soil conditions and adjacent vegetation cumulatively impacts the environment of an entire system (Young *et al*. 2012). Teasing apart these relationships would allow for improved scientific understanding of the link between small-scale and landscape ecology.

Conclusions

Shrubs provide shelter from extreme alpine conditions, with their microclimates being warmer, less windy and more humid than ambient conditions. The difference between ambient conditions and shrub microclimate conditions are generally independent of orientation. However, orientation has a significant effect on relative humidity at certain orientations, indicating that ambient conditions can have a measureable linear relationship with microclimate conditions. The microclimate in shrubs between a geographic orientation of 22° and 125° is milder when considering relative humidity alone.

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Appendices

In the following code, notes are in green, inputs are in blue, and outputs are in black.

Appendix 1

Code and results for *T*-tests described in Statistical analysis, Methods.

```
#The following code performs a t-test to compare shrub microclimate temperature (Tx)
 with ambient temperature (Ta).
t.test(pipit_data$Tx-Ta)
data:  pipit_data$TxTa
t = 4.4156, df = 147, p-value = 1.94e-05
alternative hypothesis: true mean is not equal to 0 #A difference exists
95 percent confidence interval:
  0.3478914 0.9115680
sample estimates:
  mean of x
0.6297297 #The difference is not significant

t.test(pipit_data$WCx-WCa)
data:  pipit_data$WCx-WCa
t = 6.3699, df = 147, p-value = 2.287e-09
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
  0.6412873 1.2181721
sample estimates:
  mean of x
0.9297297

t.test(pipit_data$WSx-WSa)
data:  pipit_data$WSx-WSa
t = -12.765, df = 147, p-value < 2.2e-16
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
  -4.421085 -3.235672
sample estimates:
  mean of x
-3.828378

t.test(pipit_data$RHx-RHa)
data:  pipit_data$RHx-RHa
t = 9.9693, df = 147, p-value < 2.2e-16
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
  4.739645 7.083328
```

```
sample estimates:
  mean of x
5.911486
```

```
t.test(pipit_data$LIx-LIa)
data:  pipit_data$LIx-LIa
t = -16.2, df = 139, p-value < 2.2e-16
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
  -62.79447 -49.13353
sample estimates:
  mean of x
-55.964
```

Appendix 2

Code and results for Blocked ANOVA tests described in Statistical analysis, Methods.

```
#First, change the labels from numbers to factors
pipit_data$BushF<-factor(pipit_data$Bush)
pipit_data$NestF<-factor(pipit_data$Nest.Direction)

#The following code runs an ANOVA, testing the temperature differences between the
 four orientations
Taov<-aov(Tx.Ta~NestF,data=pipit_data)
summary(Taov)
             Df Sum Sq Mean Sq F value Pr(>F)
NestF         3    5.9   1.983   0.654  0.582
Residuals   144  436.5   3.031
TukeyHSD(Taov) #We used a Tukey test to determine what factors create the
 significance, if any
              diff        lwr       upr     p adj
120-30  -0.3945946 -1.4467963 0.6576071 0.7640146
210-30  -0.5378378 -1.5900395 0.5143638 0.5462809
300-30  -0.2243243 -1.2765260 0.8278773 0.9452873
210-120 -0.1432432 -1.1954449 0.9089584 0.9847475
300-120  0.1702703 -0.8819314 1.2224719 0.9748780
300-210  0.3135135 -0.7386881 1.3657152 0.8658777
#No significant findings

#Wind chill
WCaov<-aov(WCx.WCa~NestF,data=pipit_data)
summary(WCaov)
```

```
              Df Sum Sq Mean Sq F value Pr(>F)
NestF          3    4.4   1.468   0.461   0.71
Residuals    144  459.1   3.188
TukeyHSD(WCaov)
               diff        lwr       upr     p adj
120-30   -0.3297297 -1.4087347 0.7492753 0.8569323
210-30   -0.4729730 -1.5519780 0.6060320 0.6658289
300-30   -0.2243243 -1.3033293 0.8546807 0.9489785
210-120 -0.1432432 -1.2222483 0.9357618 0.9858232
300-120  0.1054054 -0.9735996 1.1844104 0.9942330
300-210  0.2486486 -0.8303564 1.3276537 0.9322200

#Wind speed
WSaov<-aov(WSx.WSa~NestF,data=pipit_data)
summary(WSaov)
              Df Sum Sq Mean Sq F value Pr(>F)
NestF          3      3   1.015   0.075  0.973
Residuals    144   1954  13.569
TukeyHSD(WSaov)
                 diff       lwr      upr     p adj
120-30   -0.281081081 -2.507155 1.944993 0.9877490
210-30   -0.272972973 -2.499047 1.953101 0.9887528
300-30    0.018918919 -2.207155 2.244993 0.9999961
210-120  0.008108108 -2.217966 2.234182 0.9999997
300-120  0.300000000 -1.926074 2.526074 0.9851894
300-210  0.291891892 -1.934182 2.517966 0.9863242

#Relative humidity
RHaov<-aov(RHx.RHa~NestF,data=pipit_data)
summary(RHaov)
              Df Sum Sq Mean Sq F value Pr(>F)
NestF          3     94   31.27   0.596  0.619
Residuals    144   7556   52.47
TukeyHSD(RHaov)
               diff       lwr      upr     p adj
120-30    0.6324324 -3.745082 5.009947 0.9818791
210-30   -0.6756757 -5.053191 3.701839 0.9780679
300-30   -1.5108108 -5.888326 2.866704 0.8063580
210-120 -1.3081081 -5.685623 3.069407 0.8648774
300-120 -2.1432432 -6.520758 2.234272 0.5818192
300-210 -0.8351351 -5.212650 3.542380 0.9598887

#Light intensity
LIaov<-aov(LIx.LIa~NestF,data=pipit_data)
summary(LIaov)
```

```
             Df Sum Sq Mean Sq F value Pr(>F)
NestF          3  10809    3603   2.213 0.0895 .
Residuals    136 221440    1628
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
8 observations deleted due to missingness #This is because I had 138 data for this
 variable, and 147 for all the other variables

TukeyHSD(LIaov)
diff        lwr       upr      p adj
120-30  -11.417943 -36.50749 13.67161 0.6381539
210-30  -23.837971 -48.92752  1.25158 0.0690594
300-30  -17.470371 -42.55992  7.61918 0.2725490
210-120 -12.420029 -37.50958 12.66952 0.5723843
300-120  -6.052429 -31.14198 19.03712 0.9230747
300-210   6.367600 -18.72195 31.45715 0.9118014

#The following code runs a test that is like the paired t-test of ANOVA; it
 incorporates blocking, in this case, bush is used as a block
Tfit<-aov(T~NestF+Error(BushF/NestF),data=pipit_data)
summary(Tfit)
Error: BushF
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals 36   2024   56.23
Error: BushF:NestF
           Df Sum Sq Mean Sq F value Pr(>F)
NestF       3   5.95   1.983    1.24   0.299 #No significant findings
Residuals 108 172.64   1.599

WCfit<-aov(WC~NestF+Error(BushF/NestF),data=pipit_data)
summary(WCfit)
Error: BushF
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals 36   1993   55.37
Error: BushF:NestF
           Df Sum Sq Mean Sq F value Pr(>F)
NestF       3    4.4   1.468     0.9  0.444
Residuals 108  176.1   1.631

WSfit<-aov(WS~NestF+Error(BushF/NestF),data=pipit_data)
summary(WSfit)
Error: BushF
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals 36  89.14   2.476
Error: BushF:NestF
           Df Sum Sq Mean Sq F value Pr(>F)
```

```
NestF       3   3.04   1.015   0.782  0.507
Residuals 108 140.25   1.299

RHfit<-aov(RH~NestF+Error(BushF/NestF),data=pipit_data)
summary(RHfit)
Error: BushF
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals 36  11486   319.1
Error: BushF:NestF
           Df Sum Sq Mean Sq F value Pr(>F)
NestF       3     94   31.27   1.005  0.394
Residuals 108   3361   31.12

LIfit<-aov(LI~NestF+Error(BushF/NestF),data=pipit_data)
summary(LIfit)
Error: BushF
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals 34  45534    1339
Error: BushF:NestF
           Df Sum Sq Mean Sq F value  Pr(>F)
NestF       3  10809    3603   4.482 0.00535 ** #Significant
Residuals 102  81990     804
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Appendix 3a

Code and results for the temperature ANCOVA test, its corresponding ANOVA test, and linear modelling of $T_x - T_a$, T_a, and orientation as described in Methods, Statistical analysis.

```
#Set up
Temp<-read.csv("Temperature ANCOVA data for R.csv")
library(lattice)
library(ggplot2)

Temp$Nest = as.factor(Temp$Nest)
Temp$Nest<-relevel(Temp$Nest, ref="120")

#The following code runs an ANCOVA to test whether ambient temperature has an impact
 on the difference between shrub temperature and ambient temperature; TANCOVA =
 T(emperature) ANCOVA
TANCOVA<-lm(Tx.Ta ~ Ta + Nest + Ta:Nest, data=Temp)
summary(TANCOVA)
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
```

```
(Intercept)  2.06487    1.59583   1.294   0.1978
Ta          -0.08365    0.08527  -0.981   0.3283
Nest30      -1.13825    2.25684  -0.504   0.6148
Nest210     -2.31498    2.25684  -1.026   0.3068
Nest300     -4.99149    2.25684  -2.212   0.0286 *
Ta:Nest30    0.08323    0.12059   0.690   0.4912
Ta:Nest210   0.11793    0.12059   0.978   0.3298
Ta:Nest300   0.28028    0.12059   2.324   0.0216 *

#The following code runs an ANOVA of the ANCOVA to explain results; no significant
 results
anova(TANCOVA)
Response: Tx.Ta
Df Sum Sq Mean Sq F value Pr(>F)
Ta          1   2.21  2.2098  0.7413 0.3907
Nest        3   5.95  1.9829  0.6652 0.5748
Ta:Nest     3  16.99  5.6639  1.9000 0.1324
Residuals 140 417.34  2.9810

#The following code runs a linear model with Ta as the independent variable and Tx.Ta
 as the dependant one
Temp30<-read.csv("T30 data for R.csv")
summary(lm(Tx.Ta~Ta, data=Temp30))
Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.926616   1.267089   0.731    0.469
Ta          -0.000418   0.067706  -0.006    0.995
Residual standard error: 1.371 on 35 degrees of freedom
Multiple R-squared:  1.089e-06,    Adjusted R-squared:  -0.02857
F-statistic: 3.811e-05 on 1 and 35 DF,  p-value: 0.9951

#The following creates a plot of the above findings
Tp1 = ggplot(Temp30, aes(x=Ta,y=Tx.Ta)) + geom_point(shape=1) +
 geom_smooth(method=lm) + ylab("Tx-Ta (%)") + xlab("Ta (%)") + ylim(-10,30)

Temp120<-read.csv("T120 data for R.csv")
summary(lm(Tx.Ta~Ta, data=Temp120))
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  2.06487    1.56933   1.316    0.197
Ta          -0.08365    0.08386  -0.998    0.325
Residual standard error: 1.698 on 35 degrees of freedom
Multiple R-squared:  0.02765,      Adjusted R-squared:  -0.0001354
F-statistic: 0.9951 on 1 and 35 DF,  p-value: 0.3253
```

```
Tp2 = ggplot(Temp120, aes(x=Ta, y=Tx.Ta)) + geom_point(shape=1) +
 geom_smooth(method=lm) + ylab("Tx-Ta (°C)")+ xlab("Ta (°C)")+ ylim(-10,30)

Temp210<-read.csv("T210 data for R.csv")
summary(lm(Tx.Ta~Ta, data=Temp210))
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.25012    1.16700  -0.214    0.832
Ta           0.03427    0.06236   0.550    0.586
Residual standard error: 1.263 on 35 degrees of freedom
Multiple R-squared:  0.008558,     Adjusted R-squared:  -0.01977
F-statistic: 0.3021 on 1 and 35 DF,  p-value: 0.5861

Tp3 = ggplot(Temp210, aes(x=Ta, y=Tx.Ta)) + geom_point(shape=1) +
 geom_smooth(method=lm) + ylab("Tx-Ta (°C)")+ xlab("Ta (°C)")+ ylim(-10,30)

Temp300<-read.csv("T300 data for R.csv")
summary(lm(Tx.Ta~Ta, data=Temp300)) #significant
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  -2.9266     2.1809  -1.342    0.188
Ta            0.1966     0.1165   1.687    0.100
Residual standard error: 2.36 on 35 degrees of freedom
Multiple R-squared:  0.07522,      Adjusted R-squared:  0.0488
F-statistic: 2.847 on 1 and 35 DF,  p-value: 0.1004

Tp4 = ggplot(Temp300, aes(x=Ta, y=Tx.Ta)) + geom_point(shape=1) +
  geom_smooth(method=lm) + ylab("Tx-Ta (°C)")+ xlab("Ta (°C)")+ ylim(-10,30)

#The following code allows you to plot graphs side-by-side
multiplot <- function(..., plotlist = NULL, file, cols = 1, layout = NULL)
{require(grid)plots <- c(list(...), plotlist)numPlots = length(plots)if (is.null(layout))
{layout <- matrix(seq(1, cols * ceiling(numPlots/cols)),ncol = cols, nrow =
ceiling(numPlots/cols))}if (numPlots == 1) {print(plots[[1]])} else
{grid.newpage()pushViewport(viewport(layout = grid.layout(nrow(layout), ncol(layout))))
for (i in 1:numPlots) {matchidx <- as.data.frame(which(layout == i, arr.ind = TRUE))
print(plots[[i]], vp = viewport(layout.pos.row = matchidx$row, layout.pos.col =
matchidx$col))}}}

#The following code combines the four above plots in a grid
multiplot(RHp1, RHp2, RHp3, RHp4, cols=2)

#The following code allows you to combine multiple plots into one
visuals = rbind (Temp30, Temp120, Temp210, Temp300)
visuals$vis=c(rep("30°",37),rep("120°",37),rep("210°",37),rep("300°",37))

#Plotting the graphs and configuring format
ggplot(visuals, aes(Ta,Tx.Ta,group=vis,col=vis)) +
```

```
  geom_smooth(method='lm',
              formula=y~x,aes(fill = vis)) +
  geom_point()+
  theme_light() +
  theme(legend.position="bottom",
        legend.direction="horizontal",
        legend.title = element_blank(),
        legend.text = element_text(size=10)) +
  coord_cartesian(y=c(-1.5, 5))+
  scale_color_hue() +
  labs(y=expression("Tx - Ta (Â°C)"),
       x=expression("Ta (Â°C)")) +
  theme(axis.text=element_text(colour="black", size = 10),
        legend.key=element_rect(fill="white", colour="white"),
        axis.title = element_text(colour="black", size = 14))
ggsave("Tempplot.jpg", width = 180, height = 150, dpi = 300, unit=c("mm"))
#The output is Figure 1
```

Appendix 3b

Code and results for the temperature ANCOVA test, its corresponding ANOVA test, and linear modelling of $RH_x - RH_a$, RH_a, and orientation as described in Methods, Statistical nalysis.

```
#Set up
Relhum<-read.csv("RH ANCOVA data for R.csv")
library(lattice)
library(ggplot2)

Relhum$Nest = as.factor(Relhum$Nest)
Relhum$Nest<-relevel(Relhum$Nest, ref="120")
Relhum$Nest<-relevel(Relhum$Nest, ref="120")

RHANCOVA<-lm(RHx.RHa ~ RHa + Nest + RHa:Nest, data=Relhum)
summary(RHANCOVA)
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  22.8081     4.7386   4.813  3.8e-06 ***
RHx          -0.3984     0.1156  -3.446  0.00075 ***
Nest30       -0.1542     6.7014  -0.023  0.98168
Nest210     -13.5407     6.7014  -2.021  0.04523 *
Nest300     -16.6325     6.7014  -2.482  0.01425 *
RHx:Nest30   -0.0120     0.1635  -0.073  0.94158
RHx:Nest210   0.3070     0.1635   1.878  0.06249
RHx:Nest300   0.3636     0.1635   2.224  0.02774 *
```

```
#Lots of significant results, but we need to run the ANOVA to learn more

anova(RHANCOVA)
Analysis of Variance Table
Response: RHx.Rha
           Df Sum Sq Mean Sq F value    Pr(>F)
RHx         1  748.1  748.11 16.3566 8.631e-05 *** #Sig. Relation: RHx.RHa and RHx
Nest        3   93.8   31.27  0.6836   0.56347
RHx:Nest    3  404.4  134.81  2.9475   0.03503 * #Non-sig. Relation: RHx.RHa and RHx
Residuals 140 6403.3   45.74

Relhum30<-read.csv("RH30 data for R.csv")
summary(lm(RHx.RHa~RHa, data=Relhum30))
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  22.6539     4.4830   5.053 1.37e-05 *** #Significant
RHa          -0.4104     0.1094  -3.753 0.000634 *** #Significant
Residual standard error: 6.398 on 35 degrees of freedom
Multiple R-squared:  0.2869,Adjusted R-squared:  0.2666
F-statistic: 14.08 on 1 and 35 DF,  p-value: 0.0006342

RHp1 = ggplot(Relhum30, aes(x=RHa,y=RHx.RHa)) + geom_point(shape=1) +
 geom_smooth(method=lm) + ylab("RHx-RHa (%)") + xlab("RHa (%)") + ylim(-10,30)

Relhum120<-read.csv("RH120 data for R.csv")
summary(lm(RHx.RHa~RHa, data=Relhum120))
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  22.8081     5.3476   4.265 0.000144 ***
RHa          -0.3984     0.1305  -3.054 0.004298 **
Residual standard error: 7.632 on 35 degrees of freedom
Multiple R-squared:  0.2104,Adjusted R-squared:  0.1878
F-statistic: 9.327 on 1 and 35 DF,  p-value: 0.004298

RHp2 = ggplot(Relhum120, aes(x=RHa,y=RHx.RHa)) + geom_point(shape=1) +
 geom_smooth(method=lm) + ylab("RHx-RHa (%)") + xlab("RHa (%)") + ylim(-10,30)

Relhum210<-read.csv("RH210 data for R.csv")
summary(lm(RHx.RHa~RHa, data=Relhum210))
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  9.26734    4.85003   1.911   0.0643 .
RHa         -0.09142    0.11831  -0.773   0.4449
Residual standard error: 6.922 on 35 degrees of freedom
Multiple R-squared:  0.01677,      Adjusted R-squared:  -0.01132
```

```
F-statistic: 0.5971 on 1 and 35 DF,  p-value: 0.4449

RHp3 = ggplot(Relhum210, aes(x=RHa,y=RHx.RHa)) + geom_point(shape=1) +
 geom_smooth(method=lm) + ylab("RHx-RHa (%)") + xlab("RHa (%)") + ylim(-10,30)

Relhum300<-read.csv("RH300 data for R.csv")
summary(lm(RHx.RHa~RHa, data=Relhum300))
Coefficients:
           Estimate Std. Error t value Pr(>|t|)
(Intercept)  6.17563    4.19530   1.472    0.150
RHa         -0.03479    0.10234  -0.340    0.736
Residual standard error: 5.988 on 35 degrees of freedom
Multiple R-squared:  0.003291,     Adjusted R-squared:  -0.02519
F-statistic: 0.1156 on 1 and 35 DF,  p-value: 0.7359

RHp4 = ggplot(Relhum300, aes(x=RHa, y=RHx.RHa)) + geom_point(shape=1) +
 geom_smooth(method=lm) + ylab("RHx-RHa (%)") + xlab("RHa (%)") + ylim(-10,30)

#Multiplot set up, as before
multiplot <- function(...) #Same as above
multiplot(RHp1, RHp2, RHp3, RHp4, cols=2)

#Setting up the visuals
visuals = rbind (Relhum30, Relhum120, Relhum210, Relhum300)
visuals$vis=c(rep("30°",37),rep("120°",37),rep("210°",37),rep("300°",37))

#Plotting the graphs and configuring format
ggplot(visuals, aes(RHa,RHx.RHa,group=vis,col=vis)) +
  geom_smooth(method='lm',
              formula=y~x,aes(fill = vis)) +
  geom_point()+
  theme_light() +
  theme(legend.position="bottom",
        legend.direction="horizontal",
        legend.title = element_blank(),
        legend.text = element_text(size=10)) +
  scale_color_hue() +
  coord_cartesian(y=c(-9.5, 20.5))+
  labs(y=expression("RHx - RHa(%)"),
       x=expression("RHa(%)")) +
  theme(axis.text=element_text(colour="black", size = 10),
        legend.key=element_rect(fill="white", colour="white"),
        axis.title = element_text(colour="black", size = 14))
ggsave("RHplot.jpg", width = 180, height = 150, dpi = 300, unit=c("mm"))
#The output is Figure 2
```