Faculty of Science and Engineering School of Molecular and Life Sciences

The Patterns and Processes of Insect Pollinator Re-assembly across a Post-mining Restoration Landscape

Emily Paige Tudor
0000-0002-2628-3999

This thesis is presented for the Degree of Master of Research (Environmental Science) of Curtin University

January 2021
Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Invertebrate collections conducted for the purposes of this thesis were made under Fauna collection (scientific or other purposes) licences FO25000073 and FO25000230 provided by the Department of Biodiversity, Conservation and Attractions (Regulation 25; Biodiversity Conservation Regulations 2018).

Additional funding was generously provided by Alcoa of Australia Ltd under the student placement agreement CW2270400 and in-kind support was generously provided by Kings Park Science.

Signature:

Date: 18th January 2020
General Abstract

Restoration ecology is rapidly evolving and growing in global significance for recovering habitat that has been damaged, degraded or destroyed. However, fauna remain an undervalued component of restoration as it is often assumed that fauna will return to restored landscapes following the reestablishment of vegetation. Insect pollinators are among the most biologically diverse and functionally important terrestrial taxa and serve to pollinate approximately 87.5% of all flowering plants. Therefore, insect pollinators play critical roles in the subsequent recruitment and community establishment of vegetation following restoration. However, the composition, structure, and function of insect pollinators in response to restoration has been largely overlooked within the Northern Jarrah Forest (NJF), where the impacts of restoration have otherwise been conspicuously well documented.

This thesis presents an integration of in situ field and ex situ laboratory studies to provide a deeper insight into the patterns and processes underpinning the insect pollinator community reassembly in post-mining Jarrah Forest restoration. Insect pollinators were collected using vane trapping and trap nests across a sequence of restored sites to explore the community level responses of insect pollinators to the vegetation community and habitat structure. The insect community transitioned from bee-dominated to fly- and beetle-dominated assemblages along the restoration age gradient. Additionally, these communities were more responsive to changes in vegetation structure (i.e., density) than the abundance, richness, diversity, or composition of the vegetation community itself.

Vegetation density was a strong correlate of thermal conditions in restoration sites in the NJF, which in turn had a significant effect on the reproductive responses of a key group of insect pollinators, the cavity-nesting Hymenoptera. Cavity-nesting bees responded strongly and positively to temperature increases associated with open, early successional habitat, while temperature had no significant effect on cavity-nesting wasps. The bees, in particular, had strong preferences for highly exposed, early-stage restoration sites, which were warmer than the rest of the chronosequence. These findings raised the question of what ecophysiological mechanisms were driving the preferences of the bee community, and the value of early successional patches for pollinators.

Given the strong association between high environmental temperatures and nest site-selection, I quantified the thermal performance of both adult and larval cavity nesting bees as a potential
mechanism driving this pattern, measuring metabolic rate as a proxy for developmental rates and energetic requirements. The cavity-nesting bee larvae possessed a ‘generalist’ thermal performance with a higher thermal tolerance and greater breadth compared to the cavity-nesting wasp larvae, a ‘thermal specialist’ with a reduced tolerance and narrower performance breadth. Moreover, comparisons between adult and larval *Megachile aurifrons* Latreille, 1802 demonstrated that cavity-nesting bee larvae had a lower thermal optimum and peak metabolic rate but greater performance breadth than adult conspecifics. Early successional restoration appears to optimise the thermal performance of *M. aurifrons* across both life-stages. In an ecological context, it is likely that the dominant cavity-nesters use a number of physiological and social behavioural adaptations for their habitat selection to stay within habitats that support their basic metabolic physiology and energetic requirements while minimising likelihood of parasitism. However, the precise mechanisms underpinning the responses of parasitoid wasps to vegetation structure remain unclear.

Overall, the research in this thesis highlighted that that early successional restoration provides valuable habitat for the insect pollinator community primarily as a source of thermal refuge from the suboptimal conditions of the closed-canopy forest. These findings are highly relevant for future restoration efforts and suggest that density-reduction treatments may increase microclimate heterogeneity within dense, mature Jarrah Forest, and increase the suitability of these habitats to the pollinator community. However future research should explore the effects of forest thinning prior to broad-scale implementation to empirically inform adaptive management actions and maximise the biodiversity value of restored habitats.
Acknowledgements

Science is a collaboration, between many people, passions and professions and this thesis would not have been achieved without those who have contributed their time and support so generously.

First and foremost, I wish to thank my supervisors, Dr Sean Tomlinson, Dr Adam Cross, and chairperson Bill Bateman, who provided unfailing assistance, encouragement, and patience every step of the way. I consider them all excellent academics in their own rights, effective mentors and genuine friends who have played critical roles in fostering my research journey and nurturing my passion for physiology, restoration, and ecology. A special thank you also goes to Wolfgang Lewandrowski, for his expert physiological knowledge, statistical wizardry, reassurance, and enough caffeine to get me though. For that and so much more, I am incredibly grateful to all of you.

I extend my sincerest gratitude to Alcoa of Australia Ltd for providing additional funding for this research. To Andrew Grigg, for agreeing that the curiosities of the insect pollinator community was a worthwhile research investment. To Matthew Daws for his invaluable knowledge of the Jarrah Forest and providing perspective and opportunities that I would not have received otherwise and to Cameron Richardson for helping me wrangle the LiDAR data. A huge thank you also goes to Cameron Blackburn, for his outstanding plant identification skills, for saving any six-legged (or more) beasties for me to nerd out over and going above and beyond to make every site visit an enjoyable one.

Thanks to all the staff and students in Kings Park Science, for accepting me in as one of their own and providing me access to the research and laboratory facilities: Thank you for the microscopes necessary to spends hours on end cleaning, sorting, and identifying insects, the incubators that kept bee and wasp brood cosy throughout development, X-rays to peer into nesting tubes and the Q2 scanner to deep dive into the respirometry trials. I am also incredible grateful to Emma Dalziell, for helping me learn how to swim after being thrown in the deep end with the Q2.

To my field assistants, Luisa Ducki and Kieran Love, thank you for soaking up the sun with me on the gruelling summer scorchers or getting soaking wet from the dense thickets of water-bush. A special thank you must be extended to Sean again, for making himself available for
almost every field day. Putting up with me in the field for 10-12-hour straight listening to endless rambling and Bon Jovi on repeat is no easy feat.

To my botanical besties, Nate, Eloise, Luisa, thank you for embracing me as your crazy bug lady friend and sharing this journey with me. I am so grateful for your friendship, support and encouragement and every attempt made to convert me to the ‘plant-side’.

Words cannot express how grateful I am to my parents. Mum and Dad, you two will always be the most important people in my life, and my entire academic journey is owed all to you. Thank you for your patience, support, endless love and for always accepting “but I’m writing my thesis” as a reasonable excuse to get out of other responsibilities. Everything that I do is an attempt to make you both proud and though neither of you will ever read this thesis in its entirety, it is dedicated to the both of you.

Last, but not least, I also acknowledge the contribution of the thousands of insects that have formed a part of this study and fuelled my ever-growing obsession for anything creepy-crawly. Without you, the world as we know it would not exist.
Statement of Contribution by Others

Chapter Two: Integrating animal physiology and thermal biology into a descriptive and predictive restoration science

I, Emily Paige Tudor, collected and analysed the data and led the writing of the manuscript; all authors contributed to revisions for the following manuscript intended for publication to the peer-reviewed literature:


Signature: Date: 15th January 2021

I, as a co-author endorse that this level of contribution indicated by the candidate above is appropriate.

Dr Wolfgang Lewandrowski

Signature: Date: 15 January 2021

Dr Sean Tomlinson

Signature: Date: 15 January 2021
Chapter Three: Build it and they will come: Insect pollinator community reassembly in spatiotemporally heterogenous forest restoration

I, Emily Paige Tudor, contributed to the conceptualisation and design of the study, implementation of field experiments and the collection, curation, and formal analysis of data with guidance from ATC and ST; I led the writing of the manuscript and all authors contributed to revisions for the following manuscript intended for publication to the peer-reviewed literature:

Tudor, E.P., Cross, A.T and Tomlinson S. (2021). Build it and they will come: Insect pollinator community reassembly in spatiotemporally heterogenous forest restoration. In preparation for submission

Signature:  
Date: 15th January 2021

I, as a co-author endorse that this level of contribution indicated by the candidate above is appropriate.

Dr Adam T. Cross

Signature:  
Date: 16th January 2021

Dr Sean Tomlinson

Signature:  
Date: 15th January 2021
Chapter Four: Reproductive responses of cavity-nesting pollinators, predators and parasitoids to vegetation structure and microclimate associated with post-mining restoration

I, Emily Paige Tudor, contributed to the conceptualisation and design of the study, implementation of field experiments and the collection, curation, and formal analysis of data with guidance from ATC and ST; I led the writing of the manuscript and all authors contributed to revisions for the following manuscript intended for publication to the peer-reviewed literature:


Signature: Date: 15th January 2021

I, as a co-author endorse that this level of contribution indicated by the candidate above is appropriate.

Dr Adam T. Cross

Signature: Date: 16th January 2021

Dr Sean Tomlinson

Signature: Date: 15th January 2021
Chapter Five: Interspecific variation in the thermal tolerance and performance of solitary bees and their parasitoid associate

I, Emily Paige Tudor, contributed to the conceptualisation and design of the study with ST; I implemented laboratory experiments; I collected and curated the data; I formally analysed the data with guidance from WL and ST; I led the writing of the manuscript and all authors contributed to revisions for the following manuscript intended for publication to the peer-reviewed literature:


Signature: Date: 15th January 2021

I, as a co-author endorse that this level of contribution indicated by the candidate above is appropriate.

Dr Wolfgang Lewandrowski

Signature: Date: 15th January 2021

Dr Sean Tomlinson

Signature: Date: 15th January 2021
Chapter Six: Ontogenetic variation of the physiological performance and thermal tolerance within *Megachile aurifrons* (Hymenoptera: Megachilidae).

I, Emily Paige Tudor, contributed to the conceptualisation and design of the study with ST; I implemented laboratory experiments; I collected and curated the data; I formally analysed the data with guidance from WL and ST; I led the writing of the manuscript and all authors contributed to revisions for the following manuscript intended for publication to the peer-reviewed literature:


Signature: Date: 15th January 2021

I, as a co-author endorse that this level of contribution indicated by the candidate above is appropriate.

Dr Wolfgang Lewandrowski

Signature: Date: 15th January 2021

Dr Sean Tomlinson

Signature: Date: 15th January 2021
Acknowledgement of Country

We acknowledge that Curtin University works across hundreds of traditional lands and custodial groups in Australia, and with First Nations people around the globe. We wish to pay our deepest respects to their ancestors and members of their communities, past, present, and to their emerging leaders. Our passion and commitment to work with all Australians and peoples from across the world, including our First Nations peoples are at the core of the work we do, reflective of our institutions’ values and commitment to our role as leaders in the Reconciliation space in Australia.
Preface

This thesis is presented in the form of a ‘traditional thesis’ according to Curtin University Guidelines for Thesis Preparation. Chapters 2 to 6 have been prepared as manuscripts for peer-reviewed publication in the scientific literature with the exception of formatting consistent with the thesis.
Table of Contents

Declaration.............................................................................................................i
General Abstract ..................................................................................................ii
Acknowledgements..............................................................................................iv
Statement of Contribution by Others .................................................................vi
Acknowledgement of Country............................................................................xi
Preface..................................................................................................................xii
Table of Contents .................................................................................................xiii
List of Figures .........................................................................................................xviii
List of Tables ..........................................................................................................xxiii

CHAPTER ONE: General introduction and thesis overview ...................................1
  1.1 Restoration of insect communities..............................................................1
  1.2 Region and Study System............................................................................2
  1.3 Thesis Overview ........................................................................................4
  1.4 References ...................................................................................................7

CHAPTER TWO: Restoration ecophysiology: Integrating animal physiology and thermal biology into a descriptive and predictive restoration science ..................14
  2.1 Introduction ................................................................................................14
  2.2 The place of fauna, and habitat selection in ecological restoration ............15
  2.3 The integration of physiology and restoration is still overdue ......................17
  2.4 Using temperature as a driving mechanism within restoration science ........20
  2.5 Towards the development of ‘Restoration Ecophysiology’ ...........................21
  2.6 Conclusions .................................................................................................22
  2.7 References ...................................................................................................23

CHAPTER THREE: Build it and they will come: Insect pollinator community reassembly in spatiotemporally heterogenous forest restoration ..........................31
<table>
<thead>
<tr>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4.1</td>
</tr>
<tr>
<td>4.4.2</td>
</tr>
<tr>
<td>4.4.3</td>
</tr>
<tr>
<td>4.4.4</td>
</tr>
<tr>
<td>4.4.5</td>
</tr>
<tr>
<td>4.5</td>
</tr>
<tr>
<td>4.5.1</td>
</tr>
<tr>
<td>4.5.2</td>
</tr>
<tr>
<td>4.5.3</td>
</tr>
<tr>
<td>4.5.4</td>
</tr>
<tr>
<td>4.6</td>
</tr>
</tbody>
</table>

**CHAPTER FIVE: Interspecific variation in the thermal tolerance and performance of solitary bees and their parasitoid associate.**

<table>
<thead>
<tr>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
</tr>
<tr>
<td>5.2</td>
</tr>
<tr>
<td>5.3</td>
</tr>
<tr>
<td>5.3.1</td>
</tr>
<tr>
<td>5.3.2</td>
</tr>
<tr>
<td>5.3.3</td>
</tr>
<tr>
<td>5.3.4</td>
</tr>
<tr>
<td>5.4</td>
</tr>
<tr>
<td>5.4.1</td>
</tr>
<tr>
<td>5.4.2</td>
</tr>
<tr>
<td>5.5</td>
</tr>
<tr>
<td>5.5.1</td>
</tr>
<tr>
<td>5.5.2</td>
</tr>
<tr>
<td>5.5.3</td>
</tr>
</tbody>
</table>
5.5.4 Implications for restoration and landscape management.................110
5.5.5 Limitations to interpretation..................................................110
5.5.6 Conclusions........................................................................111
5.6 References.............................................................................112

CHAPTER SIX: Ontogenetic variation of the physiological performance and thermal
tolerance within *Megachile aurifrons* (Hymenoptera: Megachilidae)...............122

6.1 Abstract.................................................................................122
6.2 Introduction..........................................................................123
6.3 Methods.................................................................................125
6.3.1 Species collection and rearing..............................................125
6.3.2 Respirometry measurements...............................................125
6.3.3 Statistical analysis.................................................................126
6.4 Results..................................................................................129
6.4.1 Body mass and allometric comparisons...............................129
6.4.2 Thermal performance and tolerance comparisons...............129
6.5 Discussion..............................................................................133
6.5.1 Thermal performance comparisons......................................133
6.5.2 Thermal tolerance comparisons..........................................134
6.5.3 Implications for landscape management..............................135
6.5.4 Future research directions....................................................136
6.5.5 Conclusions.........................................................................137
6.6 References.............................................................................139

CHAPTER SEVEN: General discussion and concluding remarks.........................149

7.1 Overview.................................................................................149
7.2 Summary of findings...............................................................151
7.2.1 Chapter Two.......................................................................151
7.2.2 Chapter Three ....................................................................151
7.2.3  Chapter Four ................................................................. 153
7.2.4  Chapter Five ............................................................... 154
7.2.5  Chapter Six ................................................................. 155
7.3  Priorities for future research and action ........................................... 156
  7.3.1  Taxonomic perspectives ..................................................... 156
  7.3.2  Physiological perspectives .................................................. 156
  7.3.3  Methodological perspectives ............................................... 157
  7.3.4  Management perspectives ................................................. 158
  7.3.5  Opportunities for adaptive management in Jarrah Forest restoration .... 162
7.4  Concluding remarks ............................................................ 162
7.5  References ............................................................................ 164
List of Figures

**Figure 2.1:** Five-tiered literature search demonstrating the a) development of the restoration ecology literature; b) the representativeness of fauna within restoration ecology; c) the consideration into fauna habitat preference, selection and suitability; d) the application of ecophysiological approaches and mechanisms in explaining habitat selection and; e) the assessment of temperature as a driving mechanism for faunal responses to restoration .......19

**Figure 3.1:** Locality (a) of the Northern Jarrah Forest (shaded) in the southwest corner of Western Australia (b) and location of sites sampled across a landscape matrix of post-mining restoration (c). The polygons represent patches of restoration in a b in a broader landscape of unmined jarrah forest last disturbed by logging approximately 80 years prior to our study. The shading gradient indicates restoration age, where the darkest shading indicates the oldest restoration. Sites in restoration plots are represented by black circles, and reference sites in unmined forest are represented by black triangles. The numbers next to each restoration site indicate the year that restoration was completed while the numbers next to reference sites are site identifiers. ..................................................................................................................................................36

**Figure 3.2:** The effect of restoration age class (years) on vegetation (▲) and insect (●) a) species abundance; b) species richness and; c) Simpson’s diversity, as well as d) vegetation density (filled voxels; FV) and e) canopy height (m) in restored Jarrah Forest of different ages in southwestern Australia. Reference values from unmined forest are indicated by MRF. ....39

**Figure 3.3:** a) Unconstrained non-metric multidimensional scaling (NMDS; Stress 0.22) showing the relative differences in vegetation composition and indicator vegetation species (a < 0.002), and b) canonical correspondence analysis (CCA) showing the insect community composition and indicator insect families (a < 0.05) across 41-years of post-mining restoration in the Northern Jarrah Forest. The environmental variables that significantly influenced the insect community (represented through vector overlays) were vegetation density (% of filled voxels; VDe), vegetation species richness (VSpRi) and vegetation species abundance (VSpAb); Points represent restoration age class centroids and standard errors; 1-5 year (■), 5-15 (▼), 15-20 (▲), 20-30 (●), 30-45 (●) and unmined remnant forest (MRF; ●)...............41
**Figure 3.4:** Species richness (a) and abundance (b) of the insect community (comprised of Coleoptera, Diptera, Lepidoptera and Hymenoptera) across a gradient of increasing vegetation density (% of filled voxels; FV) in restored and unmined Jarrah Forest of south-western Australia. Both species richness and abundance decline as forest habitats become increasingly dense, a characteristic of aging restoration. Shaded regions around marginal effect regression lines represent 95% confidence interval .................................................................43

**Figure 4.1:** Locality (a) of the Northern Jarrah Forest (shaded) in the southwest corner of Western Australia (b) and location of sites sampled across a landscape matrix of post-mining restoration (c). The polygons represent patches of restoration in a broader landscape of unmined jarrah forest last disturbed by logging approximately 80 years prior to our study. The shading gradient indicates restoration age, where the darkest shading indicates the oldest restoration. Sites in restoration plots are represented by black circles, and reference sites in unmined forest are represented by black triangles. The numbers next to each restoration site indicate the year that restoration was initiated.................................................................66

**Figure 4.2:** Schematic illustration of fully constructed trap-nest with individual nesting block with 10 mm diameter removable paper straw nesting cavities with examples of capped nests; X-ray imagery of constructed nests and corresponding emergent adult cavity-nesting Hymenoptera. *Parasitoid of Megachile, †Spider predator. .................................................................68

**Figure 4.3:** Three-dimensional fitness landscape for the three most dominant species, a) *Megachile aurifrons*; b) *M. canifrons* and; c) *Gasteruption breviscutum*. Abundance was measured as the total number of offspring produced per month and shown on the z-axis. The height and colour of the landscape display the fitness gain resulting from various combinations of breeding month and forest age........................................................................................................72

**Figure 4.4:** General mean ± standard error of the mean for the a) community level reproductive responses for CNH nest production (▲) and brood abundance (●); b) taxa-specific patterns of brood abundance for wasps (▲) and bees (●); c) structural densities for overstorey (▲), midstorey (●) and understorey (■) vegetation and; d) mean daily temperature (°C; ●); e) temperature range (°C; ●) and; f) relative humidity (RH%; ●), across early (ESR), mid (MSR)..............
and late-successional (LSR) stages of restoration compared to those in unmined reference forests (MRF). ........................................................................................................... 73

Figure 4.5: Relationship between the most significant predictive variables (x-axes) for each reproductive response as determined by generalised linear mixed effect models: a) total nest construction; b) total brood abundance; c) bee brood abundance and d) wasp brood abundance. ........................................................................................................................................ 77

Figure 4.6: Canonical correspondence analysis biplot visualising the effect microclimatic and structural variables on the cavity-nesting hymenopteran community sampled within early-successional (■), mid-successional (▲), late-successional (♦) and unmined forest (○). Response variables included within CCA model: mean daily temperature (D.Ta), Daily temperature range (R.Ta), understorey vegetation density (% filled voxels; V.Us), overstorey vegetation density (% filled voxels; V.Os). The explanatory effect was only significant for daily temperature and overstorey density (permutation test, P < 0.01) ........................................................................................................... 78

Figure 5.1: Diagram of the Q2 fluorescence respirometer and experimental layout afforded by the machine’s architecture. A single 500 μL vial served as a closed respirometry chamber for an individual cavity-nesting hymenopteran larva (a) that was placed within a 48-well measurement plate (b). Each row (1-3) was assigned a species while each column (A-F) represents a potential replicate measurement for each respective species. Following Tomlinson et al. (2018a), the fourth row was filled with ‘blank vials’ (B) to capture the effects of changing atmospheric pressures between repeated measures. Two vials left of plate indicate 0% oxygen (x) and atmospheric oxygen (o) that were used as calibration readings prior to measurement. The dashed line represents the recording sequence followed by the fluorometer. Plates were assigned one of four available temperatures (c). These were set at nominal intervals of ~2.5 °C between 20–48°C. ........................................................................................................................................ 100

Figure 5.2: Thermal performance curves modeled from the beta-distribution functions of allometrically corrected resting metabolic rates for three interacting species of a host-parasitoid system: *Megachile aurifrons* (solid line; ○), *M. canifrons* (dashed line; ▲) and *Gasteruption breviscutum* (dotted line; ■) ........................................................................................................................................ 105
Figure 6.1: Relationship between log_{10} scaled mass (g) and metabolic rate (μW) Q_{10} corrected to 25°C for a) adult *Megachile aurifrons* females (●) plotted against 391 other insect taxa (○) compiled from Chown *et al.* (2007) and; b) *M. aurifrons* larvae (♦) plotted against metabolic data across 25 other species during larval development (○) assimilated from Maino and Kearney (2014). Both developmental stages scaled as expected against their respective interspecific allometries though larvae had significantly lower mass-specific metabolic rates compared to adults. Solid lines represent the interspecific allometries for insect adults (a) and larvae (b), and dashed lines represent 95% confidence intervals. .................................................. 130

Figure 6.2: Thermal performance curves modeled from the beta-distribution function (Eq 3 in Table 6.1) of allometrically corrected resting metabolic rates for female adult (dotted line; ▲) and larval (solid line; ○) *Megachile aurifrons*. Note the two different vertical scales pertaining to adults and larvae respectively. ................................................................. 132

Figure 7.1: A summary of the findings from the experimental chapters of this thesis highlighting an interaction between the thermal gradient (a; blue reflects cooler conditions while red represents warmer conditions), where late successional restoration is generally cooler than early stages, and vegetation density, represented by the green cells, is generally increased within late-successional restoration (b). Species abundance (c) and species richness (d) both increase within early successional restoration in response to reduced vegetation density (x-axes reflect vegetation structural gradient). The reproductive output (brood abundance) of cavity-nesting Hymenoptera increased with increasing temperatures (e; x-axes reflect thermal gradient) and was highest within early-successional restoration, and peak metabolic performances of cavity-nesting Hymenoptera aligned closely with the thermal profiles of early-successional restoration (f). ................................................................. 150

Figure 7.2: Schematic overview representing how organism physiology can be used across an adaptive management cycle to describe, predict, and explain organism responses to ecological restoration, contribute to the evaluation of restoration trajectories and inform management actions to feed back into future restoration planning and implementation in a flexible, iterative process of decision making and knowledge acquisition. ................................................................. 161
Figure A2.1 The patterns of reproductive activity in terms of brood abundance (yellow) and nest construction (blue) for cavity-nesting Hymenoptera plotted against the average daily temperature per month for each site per monitoring round (month; 1) December; 2) January; 3) February; and 4) March). The daily temperature was calculated for times between 6:00am and 6:00pm.

Figure A3.1: Cumulative mean time across early (ESF), mid (MSF) and late-successional (LSF) stages of forest restoration and unmined reference forests (MRF) sites: a) within the optimal preferred conditions (defined as temperatures between thermal preference ($T_{\text{pref}}$) and thermal optima ($T_{\text{opt}}$) of *Megachile aurifrons* (■) and *Gasteruption breviscutum* (●); b) exceeding the critical thermal maximum for *M. aurifrons* (■) and *G. breviscutum* (●); and c) above 38.5°C (■), representing thermal conditions that allow *M. aurifrons* (host) to outperform *G. breviscutum* (parasitoid).

Figure A4.1: Cumulative mean time a) within the optimal preferred conditions (defined as temperatures between thermal preference ($T_{\text{pref}}$) and thermal optima ($T_{\text{opt}}$) of *Megachile aurifrons* larvae (■) and adults (●) across early (ESF), mid (MSF) and late-successional (LSF) stages of forest restoration and unmined reference forests (MRF) sites.
List of Tables

Table 4.1: Parameter estimates and AICc and Log-Likelihood comparisons between full and reduced Generalized Mixed Linear Models for each response variable of: a) total larvae abundance, b), total nest production, c), taxa specific response of bee species (Megachile aurifons, M. canifrons and M. erythropyga, Hylaeus alycioneus ) and, d) taxon specific response of wasp species (Gasteruption breviscutum and Turneromyia sp.) against explanatory variables; Average daily temperature (°C; DTa), Temperature range (RTa), Overstorey density (VOS) and, Midstorey density (VMS), Understorey Density (VUS). .................................................................70

Table 4.2: Linear relationships between the microclimatic variables of mean daily temperature (°C), daily temperature range, and mean relative humidity (%) and structural densities (% filled voxels) for overstorey (VOS), midstorey (VMS) and understorey (VUS) vegetation. Significant results are bolded. .........................................................................................................................................................75

Table 4.3: Parameter estimates (PE) and standard errors (SE) for each response variable included in the selected Generalised Mixed Linear Models; a) total larvae abundance, b), total nest production, c), taxa specific response of bee species (Megachile aurifons, M. canifrons, M. erythropyga and Hylaeus alycioneus) and, d) taxa specific response of wasp species (Gasteruption breviscutum and Turneromyia sp.) against explanatory variables; Average daily temperature (°C; DTa), Temperature range (TR), Overstorey density (VOS) and, Midstorey density (VMS), Understorey Density (VUS) and; the z-statistic. Asterisks indicate significance level as follows: ‘*’ P < 0.05; ‘**’ P < 0.01; ‘***’ P < 0.001......................................................................................................................76

Table 5.1: Candidate model equations for the characterisation the thermal performance of cavity-nesting Hymenoptera. The beta-distribution function (Yan and Hunt, 1999) resolved the best fit for both global models and all other candidate models deviated more than six AIC units from this model. .................................................................................................................................................................103

Table 5.2: Mass and parameter estimates for the thermal performance and tolerance of cavity-nesting Hymenoptera in a host-parasitoid system. Rmax (upper performance asymptote), T opt
(thermal optimum) and CT_{max} (critical thermal maximum), were quantified by fitting a beta-
distributional model (Yan and Hunt, 1999), while T_{pref} (thermal preference) and performance
breadth were calculated following Tomlinson (2019), and performance breadth was calculated
as the difference between T_{pref} and T_{opt}.

Table 5.3: Pairwise comparisons of parameters estimated from the three-parameter beta-
distribution model (Eq 3; Table 5.1) for metabolic rate of cavity-nesting Hymenoptera hosts
(Megachile aurifrons and Megachile canifrons) and parasitoid (Gasteruption breviscutum) at
various constant temperatures. Asterisks indicate significance level as follows: ‘*’ P < 0.05;
‘**’ P < 0.01; ‘***’ P < 0.001.

Table 6.1: Candidate model equations examined for use in the characterisation the thermal
performance of adult (A) and larvae (L) Megachile aurifrons. The beta-distribution function
(Yan and Hunt, 1999) emerged as the best fit for both global models and was selected for
further analysis.

Table 6.2: Mass and thermal performance estimates of Megachile aurifrons adults and larvae.
Parameter estimates R_{max} (upper performance asymptote), T_{opt} (thermal optimum) and CT_{max}
(critical thermal maximum), were estimated by fitting a beta-distributional model (Yan and
Hunt, 1999), while T_{pref} (thermal preference) was calculated following Tomlinson (2019)
equation 12.2, and performance breadth was calculated as the difference between T_{pref} and T_{opt}.

Table 6.3: Pairwise comparisons of parameters estimated from the three-parameter beta-
distribution model (Eq 3 in Table 6.1) for metabolic rate of Megachile aurifrons at various
constant temperatures. Asterisks indicate significance level as follows: ‘*’ P < 0.05; ‘**’ P <
0.01; ‘***’ P < 0.001.

Table A1.1: Results of an indicator species analysis for the vegetation community across
sampled restoration sites age range (in years) and unmined forest (MRF) in the Northern Jarrah
Forest. The age class columns (1-5, 5-15… MRF) indicate (with ones) which restoration site
ages were preferred by the species. The final two columns represent the associate statistic (Stat) and p-value (P) of permutational test conducted via multilevel pattern analysis using the multipatt function in the indispecies statistical package for R (Cáceres & Legendre 2009).

**Table A1.2**: Results of an indicator species (morphospecies reported at family level resolution) analysis reported at family level for in the insect pollinator community across sampled restoration sites (age range in years) and unmined forest (MRF) sampled in the Northern Jarrah Forest. The age class columns (1-5, 5-15… MRF) indicate (with ones) which restoration site ages were preferred by the species. The final two columns represent the associate statistic (Stat) and p-value (P) of permutational test conducted via multilevel pattern analysis using the multipatt function in the indispecies statistical package for R (Cáceres & Legendre 2009). NAs in the P value column correspond to those species which occur in all restoration age classes.

**Table A1.3**: Post-hoc pairwise tests for one-way permutational multivariate analysis of variance on ranked Bray-Curtis dissimilarity matrix for (log10 + 1) transformed vegetation community data across corresponding survey site restoration years. Non-significant comparisons (bolded) indicate that compositions from each site are statistically indistinguishable.

**Table A1.4**: Post-hoc pairwise tests for one-way permutational multivariate analysis of variance on ranked Bray-Curtis dissimilarity matrix for (log10 + 1) transformed insect pollinator community data across sampled sites defined by their year of restoration. Significant comparisons (bolded) indicate that compositions from each site are statistically different.
CHAPTER ONE

General introduction and thesis overview

1.1 Restoration of insect communities

The fragmentation, degradation and destruction of our ecosystems are significant drivers of biodiversity declines. The evolving science and practice of restoration ecology provides a promising approach for addressing and mitigating ecosystem degradation (Menz, Dixon & Hobbs 2013; Suding et al. 2015), but its practical implementation is complex and often a very long-term enterprise (Miller et al. 2017). Furthermore, fauna continue to be an undervalued component of applied restoration (Cross et al. 2019), despite playing critical roles in plant reproduction (e.g. pollination and seed dispersal) and subsequent recruitment and community structure (Catterall 2018). Rather, fauna are often assumed to return to restored landscapes unaided following the reestablishment of vegetation, a process anecdotally referred to as the ‘Field of Dreams’ Hypothesis (Palmer, Ambrose & Poff 1997). Such biases towards the restoration of floral community overlooks key components of a functioning ecosystem, as restoring vegetation does not always support the return of fauna, nor the associated services they provide (Jones & Davidson 2016).

While global declines of vertebrate communities are well-documented (Ceballos, Ehrlich & Dirzo 2017), comparatively less attention has been directed to the concomitant decline of invertebrates (Saunders 2019). The consequences of this oversight are now becoming apparent as a growing number of studies report threats of cascading extinctions associated with insect declines (Potts et al. 2010; Hallmann et al. 2017; Sánchez-Bayo & Wyckhuys 2019). Insects play critical roles in ecosystem function and the provision of ecosystem services (Noriega et al. 2018). Insect pollination alone is relied upon by approximately 308,000 (87.5%) angiosperms and estimated to have a global economic value of approximately $577 billion (Ollerton, Winfree & Tarrant 2011; IPBES 2016). However, a recent review by Sánchez-Bayo & Wyckhuys (2019) concluded that ‘business as usual’ could lead to the extinction of 40% of invertebrate species globally in coming decades. These biodiversity losses are expected to have severe consequences both for the ecological functioning of ecosystems and the ecosystem services that humans rely upon (Díaz et al. 2006).
Insects are perhaps one of the most critically important taxa due to a large number of plant species that are directly and indirectly dependent upon insect pollinators (Ollerton, Winfree & Tarrant 2011; Catterall 2018). Furthermore, insects have the tendency to support pollination of specialist species that are expected to have increased vulnerability to disturbance and fragmentation (Fründ, Linsenmair & Blüthgen 2010; Blüthgen & Klein 2011; Weiner et al. 2014). On the occasion that practitioners consider fauna, they often use ‘indicator’ species that lend themselves to easy censuses (González et al. 2014). Adapting the narrow scope of indicator species towards functional approaches (e.g., survey of insect pollinator assemblages) combined with analysis of the drivers of change (e.g., habitat characteristics) is also recommended to deliver effective, efficient, and encompassing measures of ecosystem dynamics (Diffendorfer et al. 2007; Aubin et al. 2013; Gatica-Saavedra, Echeverría & Nelson 2017). The restoration of degraded ecosystems has been promoted to tackle biodiversity and habitat loss at increasingly large scales (Menz, Dixon & Hobbs 2013; Perring et al. 2015; Murcia et al. 2016), and the monitoring of large-scale restoration efforts could also benefit from the adoption of a broader frame of reference regarding key animal groups.

The inherent characteristics of insect pollinators, including their taxonomic richness, niche occupancy, environmental sensitivity, and phenological, physiological and morphological diversity, make them ideal functional bioindicators for ecological restoration (Buchori et al. 2018). Insect pollinators can be surveyed through feasible, scalable, and cost-effective methodologies (e.g., vane trapping) at the individual, population, and community level, with each scale having the capacity to provide a unique insight into the functioning of an ecosystem (Martins & Antonini 2016; Buchori et al. 2018). While these approaches are sometimes labour-intensive, the potential ecological insights they yield are substantial. Moreover, if measures are not taken to understand, promote and maintain functional insect pollinator communities in ecological restoration, these efforts will likely fall short of success; failing to meet the requirement of reinstating sustainable, resilient and functional ecosystems and increasing the potential for local collapses in these ecologically significant communities (Menz et al. 2011).

1.2 Region and Study System

This research is focussed within the Northern Jarrah Forest (NJF) in the biodiversity hotspot of Southwest Western Australia (Myers et al. 2000). The NJF is a dry-sclerophyllous
Chapter 1: General Introduction

forest on ancient, nutrient-poor, lateritic soils (Churchward & Dimmock 1989; Wardell-Johnson & Horwitz 1996). The NJF experiences a Mediterranean climate (Gentilli 1989), with warm, dry summers, reaching an average of 29.7°C in January, and cool, wet winters averaging a minimum of 5.5°C in July (BOM 2020). The average rainfall for the region accumulates to an average of 1225.9 mm annually (BOM 2020). The overstorey is dominated by Jarrah (*Eucalyptus marginata* Sm.) and Marri (*Corymbia calophylla* (Lindl.) K.D.Hill & L.A.S.Johnson) interspersed with a midstorey of mainly *Banksia grandis* Willd., * Allocasuarina fraseriana* (Miq.) L.A.S. Johnson, *Persoonia longifolia* R.Br., and an understorey characterised by *Macrozamia riedlei* (Guich.) C.A. Gardner and *Xanthorrhoea preissii* Endl. along with small shrubs predominantly of the families Asteraceae Bercht. & J. Presl, Proteaceae Juss., Restionaceae R.Br. and Fabaceae Lindl. The region is estimated to contain at least 784 flora species across 95 families (Bell & Heddle 1989).

The study region has been mined for bauxite by Alcoa of Australia Ltd (Alcoa) since 1963 and approximately 550–600 hectares of native vegetation cleared, mined and restored each year (Koch 2007a). Alcoa’s goals, targets, and objectives for restoration of mined land have changed over the years. Some of the earliest post-mining treatments in 1966 comprised of exotic pine plantations with the objective to establish a post-mining landscape for timber production (Tacey 1979; Koch 2007b). Following this, revegetation comprised largely of species that are resistant to Jarrah dieback (Shearer & Tippett 1989), however, post-1988 practice shifted towards returning the locally native overstorey species. The contemporary objective of Alcoa’s restoration initiatives is “to restore a self-sustaining Jarrah Forest ecosystem, planned to enhance or maintain water, timber, recreation and conservation values” (Gardner 2001; Koch 2007a). Alcoa’s restoration practice is based on an adaptive management paradigm, that lends itself to a high degree of integration between research and practice (Grant & Koch 2007).

Knowledge of invertebrate recolonisation is considered essential to facilitating the restoration efforts to meet the objective of reinstating a self-sustaining ecosystem as invertebrates are drivers of ecosystem processes such as pollination, seed dispersal and decomposition. As such, Alcoa has directed integrated research efforts towards invertebrate taxa. Specifically, there have been previous studies directed towards taxa such as Acari (Cuccovia & Kinnear 1999), Araneae (Brennan 2003; Majer, Brennan & Koch 2003; Brennan, Moir & Majer 2004), Collembola (Greenslade & Majer 1993), Formicidae (Majer 1976; Andersen & Majer 2004; Majer *et al.* 2013), Hemiptera (Moir *et al.* 2005; Moir & Brennan
2007; Moir et al. 2010; Orabi, Moir & Majer 2010), Isoptera (Nichols & Bunn 1980; Bunn 1983) among other ground-dwelling and predatory invertebrates (Abbott 1985; Nichols & Burrows 1985; Collett 2003). To date, one of the most noteworthy exceptions to the Alcoa’s involvement with invertebrate research is insect pollinators (Majer, Brennan & Moir 2007). While the establishment of vegetation following restoration treatment would suggest that adequate pollination is occurring, the structure, composition, and function of insect pollinator communities in response to post-mining restoration is largely unknown within the NJF and warrants further research.

1.3 Thesis Overview

The rationale to this thesis, therefore, is to examine the patterns and processes underpinning insect reassembly and habitat selection across a post-mining restoration chronosequence in the NJF where approximately 550–600 hectares cleared, mined and restored annually (Koch 2007a). Particular attention was paid to four insect orders (Coleoptera, Diptera, Hymenoptera and Lepidoptera) that are considered the most common insect pollinators (Ollerton 2017).

Specifically, this thesis aims to:

1. Characterise the extent to which animal ecophysiology has been used in ecological restoration and identify ways that physiological mechanisms may be used to inform restoration practice and management through a review of the literature;
2. Assess the effects of vegetation community composition and structure on insect species richness, diversity and abundance across various stages of ecological restoration;
3. Identify the role of habitat structure and microclimate in driving habitat selection of native cavity-nesting Hymenoptera;
4. Develop a mechanistic understanding of how inter- and intraspecific physiological variation and thermal tolerance among key hymenopteran pollinators may contribute to their habitat selection.

Chapter two re-examines the place of fauna within terrestrial restoration ecology and explores the biases that still exist within the scientific literature pertaining to the patterns and processes underpinning faunal recovery within restoration. To quantify this bias, we conducted a literature review of studies that examined physiological mechanisms underpinning habitat
suitability and selection for fauna in restored landscapes. This chapter highlights the benefits of drawing synergistic links between patterns and their motivating processes, especially when tied to demographic responses or ecological service provision.

In Chapter three, I examine how vegetation and insect communities varied across a post-mining restoration chronosequence within the NJF and determined whether vegetation structure or composition was key to structuring the pollinating insect community following initial restoration.

Chapter four unravels the responses of the holometabolous cavity-nesting Hymenoptera to ecological restoration, as this pollination guild has unique habitat requirements and was underrepresented within the survey effort for Chapter two. This chapter investigates whether habitat structure or microclimate best explained the associations between nesting activity and reproductive output of cavity-nesting Hymenoptera, and whether the drivers of variation were consistent between component taxa (e.g., bees and wasps).

In Chapter five, I evaluated the physiological performance and thermal tolerance (e.g., upper and lower thermal tolerance thresholds and thermal optima) of a model cavity-nesting host-parasitoid system. This chapter aimed to quantify interspecific differences in thermal performance between the larvae of two dominant Megachile Latreille, 1802 (Hymenoptera: Megachilidae) bee species and a parasitoid wasp, Gasteruption Latreille, 1796 (Hymenoptera: Gasteruptiidae).

Chapter six explores the ontogenetic physiological variation within Megachile aurifrons Smith, 1853 to develop a mechanistic understanding into whether the pattern of nest site selection is driven primarily by maternal adult thermal requirements, or those of the offspring. Thermal biology and metabolic rate form fundamental theories within ecology and characterising the variation that exists within and between species is essential to understand the physiological processes driving habitat selection and therefore, their return to restored landscapes. Dissecting the thermal requirements of both interacting species and various developmental stages can rapidly elucidate the direct and indirect impacts of changing thermal landscapes.

I conclude this thesis by populating an adaptive management paradigm, identifying ways to incorporate novel measurements into an established planning and monitoring structure to gain new insights into ecological restoration. In doing so, I effectively link physiology, behaviour, fitness, and function and highlight how such integrated approach can promote the
co-development of the practical knowledge necessary to inform case-by-case restoration initiatives while enhancing our ecological knowledge of restored ecosystems.
Chapter 1: General Introduction

1.4 References

Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.


BOM (2020) Bureau of Meterology, Australia. Station Name; Dwellingup (009538).


Chapter 1: General Introduction


Chapter 1: General Introduction


Shearer, B.L. & Tippett, J.T. (1989) *Jarrah dieback: the dynamics and management of Phytophthora cinnamomi in the jarrah (Eucalyptus marginata) forest of south-western Australia*. Department of Conservation and Land Management, Perth, WA.


CHAPTER TWO

Restoration ecophysiology: Integrating animal physiology and thermal biology into a descriptive and predictive restoration science

2.1 Introduction

Intensifying anthropogenic pressures have led to global-scale ecological changes (Crutzen 2006). Land-use change has been heavily scrutinised for its role in biodiversity losses, with up to half of the earth’s natural ecosystems having been encroached upon by agricultural, industrial, or urban development at the turn of the last century (Kennedy et al. 2013; Maxwell et al. 2016; Sánchez-Bayo & Wyckhuys 2019). Ecological restoration can work to ameliorate, or even reverse, biodiversity and habitat loss in the face of these anthropogenic pressures (Suding et al. 2015). The core objectives of ecological restoration lie in assisting in the recovery of damaged, degraded or destroyed habitat to a self-sustaining, functioning and resilient ecosystem (Miller et al. 2017). However, it continues to be an evolving field, where coupling expanding knowledge with practical implementation is complex and often a very long-term enterprise (Miller et al. 2017; Bertuol-Garcia et al. 2018).

Many of the long-term questions regarding restoration success centre on understanding how functional groups respond to ecological restoration (Perring et al. 2015; Miller et al. 2017). However, much of the contemporary restoration science and practice is largely descriptive, such that patterns are identified (e.g., taxonomic composition, presence or absence of key taxa, and diversity indices) and associated with different ecological states or management practices (Miller et al. 2017). While these approaches provide insight into the spatiotemporal variation of species and community assemblages, such organism-environment associations are limited in their capacity to support causal and mechanistic interpretation (Weiner 1995; Lawton 1999). Understanding causality should form the foundation for decision-making and monitoring protocol (Kearney, Shine & Porter 2009), but the mechanisms underpinning patterns in ecological restoration are often unexplored (Suding 2011; Brudvig 2017). There is no doubt that focus should be directed towards the ecology and behaviour of animals in response to restoration (Cross et al. 2019), we suggest that understanding the responses of animals to
restoration may be best achieved by understanding the abiotic and biotic requirements that structure their populations, and the physiological tolerances that constrain them.

How to effectively manage the development of functional and sustainable restoration is critical to both ecologists and land managers alike (Forup & Memmott 2005; Forup et al. 2008). A mechanistic approach to understanding how fauna respond to restoration efforts at local and landscape scales provides insight into the processes underpinning the patterns that emerge throughout restoration, potentially allowing the prediction of desired or undesired restoration trajectories. The value of understanding physiological mechanisms underpinning animal responses to environmental change has been articulated previously, (Cooke & Suski 2008; Tomlinson et al. 2014; Jones & Davidson 2016; Tuff, Tuff & Davies 2016), though the integration of such recommendations into practice remains unclear. Therefore, our aim was to deliver an updated perspective on the integration of animal ecophysiology and restoration ecology and identify ways in which physiological data and theory may be used to inform restoration practice and management.

2.2 The place of fauna, and habitat selection in ecological restoration

Several independent reviews have consistently demonstrated that animals are poorly considered in the restoration ecology literature (Majer 2009; Cristescu, Frère & Banks 2012; Cross et al. 2019), and that critical ecophysiological constraints, such as thermal biology, are rarely considered (Tuff, Tuff & Davies 2016). To quantify this bias, we conducted a literature review of studies that examined physiological mechanisms underpinning habitat suitability and selection for fauna in restored landscapes within ISI Web of Science (Core Collection). We refined our search terms across five ‘tiers’ and systematically narrowed our searches from restoration ecology in the broadest sense, to studies exploring thermal biology as a key driver of patterns and processes within ecological restoration (Figure 2.1). We limited our search to studies dealing with terrestrial fauna (i.e., insects, birds, mammals, and reptiles). However, we acknowledge that many aquatic ecosystems also require restoration and believe several concepts discussed here may also translate into current and future freshwater or marine restoration programs.

Over the last fifty years, restoration has established a prominent presence within the ecological literature with 54,661 results returned from a search of the terms ‘restor* or
Chapter 2: Integrating Ecophysiology and Restoration Ecology

*rehabilitat* or *revegetat*’ and ‘*environment* or *ecolog*’. Furthermore, research interest has grown year-on-year (Figure 2.1a; \(F_{1, 49} = 143.2, \ P < 0.001\)) with the last decade alone accounting for 71.3% of publications. However, topographical, vegetative and hydrological factors frequently dominate the restoration literature and monitoring schemes (McAlpine *et al.* 2016). The paucity of studies concerning animals in reclaimed lands was recognised over three decades ago (Butcher, Majer & Unsworth 1989; Majer 1989), and persisted into the first decade of the 21st Century when Majer (2009) highlighted that only 12.4% of papers reporting ecological restoration focused on fauna, with birds typically receiving the most attention. To re-examine the place of fauna within terrestrial restoration ecology we refined our first-tier search by the terms “invertebrate* or insect* or arthropod* or vertebrate* or fauna* or animal* or mammal* or bird* or reptile*”. As with the broader ecological restoration literature, publications dedicated to fauna have increased in the last 30 years (Figure 2.1b; \(F_{1, 41} = 235.55, \ P < 0.001\)). Proportionally, however, representation of animal studies remain relatively unchanged since Majer (2009), representing 13.3% of research reports that we identified. Despite several reports explicitly advocating for greater consideration of fauna in restoration science and practice (Majer 2009; Cross *et al.* 2019), the disparity between animal and plant-based studies remains.

Best practice guides to restoration ecology articulate the central role of reference ecosystems against which to gauge restoration success and tend to suggest that return of vegetation to these representative states should be the main metric of success (McDonald *et al.* 2016; Miller *et al.* 2017). This persists with a long-running assumption that animals return to restoration sites unaided (Palmer, Ambrose & Poff 1997; Ruiz-Jaen & Aide 2005; Palmer, Zedler & Falk 2016). However, recent meta-analyses provide, at best, equivocal evidence that fauna compositions and ecosystem services successfully recover without intervention (Benayas *et al.* 2009; Crouzeilles *et al.* 2016; Shimamoto *et al.* 2018). Animals play critical roles in the delivery of ecosystem services such as nutrient cycling, seed dispersal, and pollination (Kremen *et al.* 2007; Noriega *et al.* 2018). As such, robust, functional and resilient faunal communities lay the foundations for the continuity of plant reproduction and, consequently, the long-term outcomes for restored ecosystems (Montoya, Rogers & Memmott 2012). Overlooking the key faunal communities may limit successful ecological recovery, as restoring vegetation does not always beget the return of fauna, nor the associated services they provide (Jones & Davidson 2016).
The central principal of ecological restoration is the reinstatement of a functional ecosystem following some initial degradation, impairment or destruction (Miller et al. 2017; SERA 2017). Generally, the response of animals in degraded or unsuitable landscapes is to move, adapt or die (Huey et al. 2012), and habitat selection by animals has been mainstay of research attention from ecologists (Arthur et al. 1996; Morris 2003; Mayor et al. 2009). Habitat selection by animals is driven by competing costs and benefits of any given habitat to an organism’s performance and fitness (Mayor et al. 2009). Habitat selection has a profound influence on population dynamics (Pulliam & Danielson 1991), biotic interactions (Martin 2001), and community reassembly (Binckley & Resetarits Jr 2005). As such, it should be considered as a significant, spatially dependent process driving fauna recovery (Hale & Swearer 2017). In the context of ecological restoration, which seeks to ‘fast track’ natural succession, landscapes could be specifically constructed to meet the requirements of animals if the constraints causing them to avoid restoration landscapes were understood (Hale et al. 2019). However, research into the suitability, preferences, and selection of habitats represented less than 2% of the broader restoration ecology literature that we identified (Figure 2.1c), and only 8.5% of that relating to fauna.

2.3 The integration of physiology and restoration is still overdue

Restoration ecology currently depends on the integration of disciplines such as landscape ecology, synecology, and functional ecology (Cairns Jr & Heckman 1996; Jellinek et al. 2019), along with well-recognised ecological theories including succession and dispersal, (Falk, Palmer & Zedler 2006; Palmer, Zedler & Falk 2016). The value of multidisciplinary science is clearly appreciated. However, ecophysiology has received comparatively little attention (Cooke & Suski 2008). Given that preference or avoidance of different habitats is often motivated by physiological constraints of animals, among other pressures (Huey 1991), and that physiological requirements can profoundly alter the interaction between animals and their biotic niche (Nowakowski et al. 2018), this is a substantial oversight. Moreover, the integration of physiology and restoration ecology was reviewed by Cooke and Suski (2008), emphasising the increasingly accessible, convenient and robust physiological tools available to investigate physiological mechanisms structuring ecological restoration. As such, Cooke and Suski (2008) called for the development and validation of physiological models that account for abiotic and biotic dynamics across degraded and restored systems. Moreover, the
incorporation of laboratory and field approaches to evaluate the relationship between habitat quality and organism performance, and the extent that this translates into population dynamics was also advocated (Cooke & Suski 2008). While some studies have recently advanced these efforts by providing theory, model and test in a single restoration context (Tomlinson et al. 2014; Tomlinson et al. 2017a; Tomlinson et al. 2018b), our search for terms “mechanis* or physiolog* or respir* or metabol* or ecophysiol* or biophys*” yielded negligible returns in the field of ecological restoration (Figure 2.1d). Only 44 empirical studies (0.08% of the broader literature) incorporated physiological or biophysical assessments in the context of fauna re-assembly in restoration. While Cooke and Suski (2008) concluded that the integration of fields was overdue more than decade ago, we contend that animal physiology continues to be largely overlooked in the restoration process and is an undervalued approach for predicting restoration outcomes.

Restoration ecology has been predominantly driven by a pattern-oriented paradigm, such that reference sites are selected as targets and monitoring is periodically undertaken to assess whether the restoration is on a trajectory to match the observed structure of the reference community (McDonald et al. 2016; Miller et al. 2017). In many cases, population-level proxies for demography (e.g., abundance and species density) serve as metrics to evaluate restoration trajectory, and more broadly, success (Ruiz-Jaen & Aide 2005; Cortina et al. 2006). Unfortunately, when patterns are used as targets without reference to function, the cause of restoration failure is often unclear, and therefore harder to effectively address. In this instance, ecophysiology can help establish links between pattern and process thereby providing critical insight into the mechanisms underpinning species responses to ecological restoration.
**Figure 2.1:** Five-tiered literature search demonstrating the a) development of the restoration ecology literature; b) the representativeness of fauna within restoration ecology; c) the consideration into fauna habitat preference, selection and suitability; d) the application of ecophysiological approaches and mechanisms in explaining habitat selection and; e) the assessment of temperature as a driving mechanism for faunal responses to restoration
2.4 Using temperature as a driving mechanism within restoration science

The influence exerted by temperature on most physiological and biochemical processes, such as metabolism, make it one of the most pervasive abiotic factors driving much of the biology and ecology of both plant and animal taxa (Angilletta 2006; Gilbert et al. 2014; Buckley, Ehrenberger & Angilletta Jr 2015). Tuff, Tuff and Davies (2016) suggested thermal biology was an obvious first element of ecophysiological measurement to integrate more strongly into ecological restoration. At large scales and coarse resolution, climate change has prioritised research into temperature and temperature-driven contributions to conservation priorities (Tylianakis et al. 2008; Gilman et al. 2010; Lister & Garcia 2018). However, habitat degradation and subsequent restoration can cause localised shifts in the thermal environment (Meyer, Sisk & Covington 2001), and whilst occurring at a smaller scale, the effects can be just as strong, especially when they act synergistically with climate change (Pyke 2004; Hof et al. 2011). Depending on context and stage of ecological restoration some sites can be warmer, more exposed, and more desiccating environments, or they can be cooler and less suitable for native animals to manage their thermal biology (Garcia & Clusella-Trullas 2019). Consequently, temperature is expected to mediate the responses of species to restoration, as the recovery of taxa depends, in part, on the ability of individuals to tolerate novel temperature regimes arising from degraded and restored ecosystems (Meyer & Sisk 2001; Meyer, Sisk & Covington 2001).

Calls to explore the thermal drivers of habitat selection are not new (Huey 1991), and have been reiterated recently (Tuff, Tuff & Davies 2016; Tomlinson et al. 2018b; Garcia & Clusella-Trullas 2019). However, only 12 out of 54,668 studies (0.02% of the restoration ecology literature) incorporated thermal physiology into the patterns of faunal recovery (Figure 2.1e). Furthermore, to our knowledge, thermal performance and tolerance has not been used to explain patterns of population or community reassembly within an ecological restoration context. By examining temperature driven effects through measures of thermal performance, restoration ecologists may be able to better predict potential demographic bottlenecks (e.g., vulnerable life-stages) and forecast which species will persist, decline, or drop out of the modified systems, or those that have the capacity to recover with ongoing restoration management.
2.5 Towards the development of ‘Restoration Ecophysiology’

Where ecophysiology has been applied to answer questions about fauna in changing environments, it has generally proven to be readily incorporated, transparent, and insightful. In restoration ecology, however, thermal biology tends to be applied through the lens of spatial ecology, aiming to identify how some parts of the landscape are more beneficial (or less challenging) for fauna than others. For example, the small-scale distribution of Scarabaeidae (*Phyllococerus* Waterhouse, 1976 and *Colpochila* spp. Erichson, 1843) across a restoration landscape was determined through modelling projected thermal constraints against freely available high-resolution spatial data (Tomlinson 2020). Similarly, the thermal energetics was projected for pollinating bees (*Apis mellifera* Linnaeus, 1758 and *Amegilla* (*Notomegilla*) chlorocyanea Cockerell, 1914) and wasps (*Zaspilothynnus nigripes* Guérin-Méneville, 1842 and *Lissopimpla excelsa* Costa, 1864) in the context of a highly fragmented restoration landscape (Tomlinson et al. 2018b). However, such models are effectively hypotheses without the validation obtained from field-based studies and therefore, need ‘real-world’ data from free-ranging animals pursuing normal biological activity.

The minimal maintenance energetic requirements of an animal can be readily measured in the form of resting metabolic rates (RMR) and standard metabolic rates (SMR) in ectotherms, or basal metabolic rate (BMR) in endotherms (Withers 1992). The relationship between this fundamental currency of ecology (Kleiber 1961), and abiotic conditions such as ambient temperature, pH, and salinity forms the basis of many of the biophysical models that seek to explain how animals interact with their environment in geographical space (Kearney & Porter 2009; Kearney et al. 2010; Kearney et al. 2013). Though rarely explored, when such studies have been undertaken to characterise the cost of living of an animal *in situ* (e.g., through field metabolic rates; FMR), energetic requirements and expenditure can be remarkably similar to the modelled expectations. While some of the recently developed isotopic and telemetric techniques for measuring FMR may not be suitable for some taxa and environments (Cooke et al. 2004; Cooke 2008; Tomlinson et al. 2014), where feasible, such techniques can present important insights into the physiological constraints on organisms under environmentally relevant conditions.

In cases where measurements of true FMR are not feasible, there are several integrated approaches that may elucidate major physiological and energetic processes. For example, Thompson, Halstead and Donnelly (2018) combined simple physiological experiments with
field studies to understand how temperature drives habitat occupancy of two anoles (*Norops humilis* Peters, 1863 and *N. limifrons* Cope, 1862) across forest regeneration stages, linking the thermal constraint of the two species with their avoidance of early stages of restoration. This integrated approach, combining biophysical ecology, behaviour and microclimatic modelling can be implemented with relative ease across a suite of taxa and environments, while providing meaningful, mechanistic insights into how behaviour and physiology can drive habitat selection.

2.6 Conclusions

Determining the mechanisms underpinning the responses of organisms to ecological restoration represents an undervalued step towards assessing restoration success and predicting possible trajectories of recovery. Although ecological restoration is increasingly considering fauna and their habitat preferences, insight into the mechanisms and processes underpinning the patterns emerging in restored ecosystems remains sparse. The evaluation of restored habitats can be enhanced by linking patterns with processes, especially when the return of some animal groups is tied to demographic responses or ecological service provision. Comparative physiologists are well suited to this role and can build upon the existing framework of approaches for describing faunal responses to restoration as well as predicting trajectories and outcomes. Through integrating ecophysiology with thermal biology and other established disciplines such as community and population ecology, restoration ecophysiology may offer exciting new pathways to increase restoration success with a renewed interest in developing mechanistic understandings and predictive restoration science.
2.7 References

*Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.*


CHAPTER THREE

Build it and they will come: Insect pollinator community reassembly in spatiotemporally heterogenous forest restoration

3.1 Abstract

Anthropogenic habitat degradation resulting from agricultural, mining, and urban development is a leading cause of global insect pollinator declines, but the recovery of insect pollinators during ecological restoration is rarely assessed. We quantified the relationships between the insect community, vegetation community and changing forest structure across a 41-year sequence of post-mining ecological restoration in south-western Western Australia. Insect community composition in restored sites resembled that of reference forest communities within approximately a decade of restoration activity, transitioning from bee-dominated to fly- and beetle-dominated assemblages along an ecological restoration age gradient. Although increasing vegetation diversity was related to high insect diversity, insect community composition was most strongly influenced by the emergent properties of vegetation structure (namely, vegetation density). Within the highly-connected landscape matrix examined, insects returned through natural processes of reassembly and dispersion. However, there was no evidence of peak species richness or abundance of insect pollinators in the oldest restoration sites, nor in the reference unmined forest, and ongoing management of these forests for optimal insect biodiversity is complex and requires further research.
3.2 Introduction

Approximately 87.5% of all flowering plants rely upon animal-mediated pollination, and its prevalence in crop species provides a global economic value of approximately $577 billion in agricultural systems (Ollerton 2017). Insect pollination is the most common form of animal-mediated pollination (Ollerton, Winfree & Tarrant 2011), and involves a huge diversity of insect pollinators (Ramos & Schiestl 2019). The most significant pollinator groups are the four most speciose insect orders (Wardhaugh 2015): bees, wasps, ants and sawflies (Hymenoptera), flies (Diptera), butterflies and moths (Lepidoptera), and beetles (Coleoptera). However, pollination networks are threatened globally by declines in insect populations (Carvalheiro et al. 2010; Cameron et al. 2011; Weiner et al. 2014), principally due to agricultural intensification (Kovács-Hostyánszki et al. 2017), deforestation (Newton et al. 2018), urbanisation (Guenat et al. 2019), and mining development (Mir et al. 2017; Seitz & Leonhardt 2019).

To halt and reverse biodiversity declines, there is an increasing need for active ecological recovery (ecological restoration or rehabilitation) of damaged, degraded or destroyed ecosystems (Miller et al. 2017; SERA 2017). However, restoration planning and monitoring is frequently biased towards re-establishing vegetation communities, and often assume that animals will colonise restored ecosystems unaided (Palmer, Zedler & Falk 2016; Cross et al. 2019; Cross, Bateman & Cross 2020). This bias overlooks essential interactive ecosystem functions such as seed dispersal (Li Vigni & Melati 1999), nutrient cycling (Belovsky & Slade 2000), decomposition (Ulyshen, Müller & Seibold 2016), and pollination (Winfree, Bartomeus & Cariveau 2011a), which are foundational processes in the re-establishment of vegetation communities.

Re-establishing pollination networks is critical to successful, sustainable ecological restoration (Dixon 2009). Vegetation community composition and structure in restored ecosystems influence the abundance and composition of flower-visiting invertebrate communities (Potts et al. 2003; Morandin & Kremen 2013a; Morandin & Kremen 2013b). However, while there is some evidence that ecological restoration can return diverse and self-sustaining vegetation communities (Koch & Hobbs 2007; Ritchie & Krauss 2012), it is unclear whether these insect communities recover in restored habitats. Largely, this stems from uncertainty over whether the habitat requirements of insect pollinators or flower-visitors are met (Roulston & Goodell 2011). Directing special attention to reinstating potential pollinators...
may have positive flow-on effects to plant communities through increasing reproductive success and genetic stability (Grass et al. 2018; Kremen, M’Gonigle & Ponisio 2018), potentially increasing long-term restoration success (Menz et al. 2011). However, there is a knowledge gap regarding insect community composition and diversity (Majer, Brennan & Moir 2007), especially in floristically and entomologically diverse ecosystems (Marques et al. 2017); for example, those of the Southwest Australian Floristic Region (SWAFR; Phillips, Hopper & Dixon 2010; Gioia & Hopper 2017). The monitoring of ecological restoration often misses opportunities to examine the effectiveness of current practice and potential for targeted enhancement of pollinator communities and other functional guilds (Burkhalter, Moon & Rossi 2013; Venturini et al. 2017), leaving additional knowledge gaps regarding how the return of insect communities influences the diversity, composition and structure of vegetation communities.

In this study, insect assemblages were examined across a 41-year sequence of post-mining rehabilitation and restoration in the Northern Jarrah Forest (NJF; Thackway & Cresswell 1997) of the SWAFR, and compared to the assemblages of adjacent unmined Jarrah Forest (Figure 3.1). Virtually no pristine old-growth Jarrah Forest remains in the SWAFR (McCaw, Robinson & Williams 2011), and thus unmined areas that were logged nearly a century ago represent the most ecologically intact remaining forest (Heberle 1997; McCaw, Robinson & Williams 2011). The congruence between vegetation communities and insect pollinator communities during ecological recovery was assessed by a) examining how the species richness, abundance and composition of vegetation and insect communities varied across the restoration chronosequence; and b) determining whether vegetation community composition or vegetation structure (canopy height and vegetation stratum density) were significant drivers of variation in insect pollinator assemblages.

3.3 Methods

3.3.1 Study area

The study area has been mined for bauxite by Alcoa of Australia Ltd since 1963, with approximately 550–600 hectares of native vegetation cleared, mined and restored annually (Koch 2007a). Ecological restoration techniques have developed markedly over the nearly 60-year period of operation (Koch & Hobbs 2007). Prior to 1988, post-mining activities were
characterised by overstory plantations of *Eucalyptus* species that were resistant to Jarrah dieback (Shearer & Tippett 1989), but not local to the Jarrah Forest. Since 1988 recovery activities have returned only indigenous species including an overstorey of *Eucalyptus marginata* Sm. and *Corymbia calophylla* (Lindl.) K.D.Hill & L.A.S.Johnson. Alcoa’s current objectives are to develop post-mining landscapes that are reflective of the surrounding unmined Jarrah Forest that integrate with forest management regimes (Gardner & Bell 2007). As rehabilitation and restoration both fall within the restorative continuum of activities (Gann *et al.* 2019), and recognising that the goal of pre-1988 activities was to return functional, resilient, self-sustaining vegetation communities, all post-mining ecological activities are hereafter collectively referred to as restoration.

Eighteen survey sites were laid out approximately 2.24 ± 0.24 km apart (range 4.66–1.34 km) to ensure spatial independence (Zurbuchen *et al.* 2010). Fifteen sites were identified across a successional restoration age gradient from 2–41 years since establishment (Figure 3.1; 1979–2018) while three control sites were also selected within adjacent unmined forest. There was an unavoidable north-south gradient in restoration age resulting from the temporal progression of bauxite extraction and subsequent restoration, however, our study area was only 14.3 km long, and is unlikely to impart climatic correlates that influenced ecology at sampled sites (Hawkins & Felizola Diniz-Filho 2004).

### 3.3.2 Sample collection and processing

Insect sampling ran for a fortnight each month between July and December 2019, using yellow and blue ultraviolet polypropylene vane traps (SpringStar Inc., Woodinville, Washington). Each survey site consisted of a 50 × 50 m quadrat of four traps (two yellow and two blue) suspended approximately 1.2 m above the ground from wooden stakes. The colour of the trap mounted at each of the four corners at each survey site was randomised at the time of establishment by coin flip. Each trap was filled with a preservative solution comprising ethylene glycol (CAS Number: 107-21-1) and denatonium benzoate (CAS Number: 3734-33-6) diluted 2:1 with tap water. All survey sites remained in place for the full extent of the study (166 days), with traps emptied and shrouded with black polyethylene covers between trapping periods to ensure no insect capture occurred during these times and that the stimulus of the traps was removed from the local insect community between surveys.
Trap contents were transported to the Kings Park Science laboratory (Department of Biodiversity, Conservation and Attractions, Kings Park, Western Australia), where specimens were filtered from the ethylene glycol solution using a 355-micron sieve before being washed under a low-pressure water stream to remove excess ethylene glycol and stored in 70% ethanol. Analyses were restricted to insect Orders known to provide significant pollination services, including Diptera, Hymenoptera, Lepidoptera and Coleoptera (Ollerton 2017). Over 70% of Australia’s insect taxa remain undescribed (Austin et al. 2004), as such, analyses were conducted upon morphospecies to reflect species diversity. However, formal identifications were reported at family level as genus and species level identifications are difficult due to the lack of experts available to consistently identify specimens to this resolution. Specimen identifications were confirmed with the aid of open-access galleries and databases of insect taxa in the Atlas of Living Australia (ALA, http://www.ala.org.au), Australian National Insect Collection Database (CSIRO, http://anic.ento.csiro.au/database), and Western Australian Museum (http://museum.wa.gov.au/research/research-areas/terrestrial-zoology), along with interactive insect identification keys (CSIRO, http://anic.ento.csiro.au/insectfamilies), and the references provided therein.

3.3.3 Site characterisation

Vegetation surveys were conducted between August and September 2019, using four replicate 4 × 4 m plots at each site to determine plant species richness (species counts) and abundance (individual counts). While this sampling effort is smaller than previous studies (e.g. Koch & Ward 1994), and may not effectively represent overstorey compositions or flowering phenology, the aim was to develop consistent vegetation correlates against which to measure the response of the insect community. Simpson’s diversity index was calculated for vegetation communities in each plot. Canopy height and vegetation density were characterised using Airborne Light Detection and Ranging (LiDAR) in 2018 via an Optech ALTM 3100 (Teledyne Optech, Toronto, Canada) data supplied by Alcoa of Australia Ltd. Vegetation point clouds were divided into a multi-layer 50 × 50 m raster grid, and the total number of filled voxels (volumetric pixels; FV) was calculated for each metre height band. Canopy height models (CHM) were created by the triangulation of the maximum height points across each site. The number of FV was combined for each meter height band for each site, up to the maximum
canopy height and square-root transformed prior to analysis. This calculation was used as a proxy for vegetation density.

Figure 3.1: Locality (a) of the Northern Jarrah Forest (shaded) in the southwest corner of Western Australia (b) and location of sites sampled across a landscape matrix of post-mining restoration (c). The polygons represent patches of restoration in a b in a broader landscape of unmined jarrah forest last disturbed by logging approximately 80 years prior to our study. The shading gradient indicates restoration age, where the darkest shading indicates the oldest restoration. Sites in restoration plots are represented by black circles, and reference sites in unmined forest are represented by black triangles. The numbers next to each restoration site indicate the year that restoration was completed while the numbers next to reference sites are site identifiers.
3.3.4 Statistical analysis

All statistical analyses and ordinations were performed in the R statistical environment (version 3.6.2) using RStudio Version 1.2.5001 (R Development Core Team 2019). Summary statistics are presented as means ± standard error for restoration age classes. The main effect of restoration age (in years) on (1) plant and insect species richness, (2) species abundance and (3) Simpson’s diversity was examined using one-way analysis of variance (ANOVA; Kaufmann & Schering 2014).

Generalised linear mixed-effect models (GLMM; McCulloch & Neuhaus 2005) assuming a Poisson error distribution were used to examine the effects of the vegetation community and structure on insect abundance and richness. Models were fit using the `glmer` function in the R statistical package ‘lme4’ (Bates 2007). Prior to GLMM analysis, Pearson correlation coefficients and variance inflation factors (VIF) were assessed using the packages ‘PerformanceAnalytics’ and ‘Car’ setting thresholds for variable exclusion set at <0.7 and <3, respectively (Fox et al. 2012; Peterson et al. 2018). Vegetation Simpson’s diversity and canopy height exceeded these thresholds. Therefore, the ‘full’ model explaining insect community patterns included vegetation structural density, vegetation abundance and vegetation species richness as explanatory variables with sampling round and site included as random effects to account for repeated measurements at each site. Model reduction using the ‘MuMIn’ package (Bartoń 2014) was used to identify the most parsimonious model by Akaike’s Information Criterion for small sample sizes (AICc; Burnham & Anderson 2002). However, model reductions did not substantially increase model parsimony and full models were maintained for final analysis. Marginal effect regression lines were generated within the R package “ggeffects” with the `ggpredict` function (Lüdecke 2018).

A Bray-Curtis dissimilarity matrix was used for a subsequent, non-parametric permutational multivariate analysis of variance (PERMANOVA; Anderson 2005) to determine the effect of restoration age and sampling period on insect and vegetation community assemblage. Post hoc pair-wise comparisons between variables were conducted to determine site-level differences using the `pairwise.perm.manova` function in the ‘RVAideMemoire’ package (Anderson 2014; Hervé & Hervé 2020). An indicator species analysis was performed using the `multipatt` function in the ‘indispecies’ package (Cáceres & Legendre 2009) to identify insect and plant species that can be considered indicators of restoration and unmined sites. Non-metric Multidimensional Scaling (NMDS; Agarwal et al. 2007) was used to construct an
unconstrained ordination of the vegetation community. Canonical correspondence analysis (CCA) was used to construct constrained ordination of the insect community, and characterise the strength and direction of environmental variable effects, excluding canopy height due to collinearity violations (ter Braak 1986). Vectors were superimposed onto CCA biplots with significance established through 999 permutations using the *envfit* function in the ‘vegan’ statistical package (Oksanen et al. 2007).

3.4 Results

3.4.1 Vegetation Community

A total of 5,569 individuals from 145 plant species across 39 families were identified from sample sites (Table A1.1). Indicator species analysis revealed that younger restoration (1–5-year-old) was characterised by native Fabaceae such as *Acacia lateritica*ola and *A. drummondii* (Figure 3.3a; Table A1.1), while older restoration sites and unmined forest were dominated by native overstorey trees including *Eucalyptus marginata*, *Corymbia calophylla* (both Myrtaceae) and midstorey shrubs including *Bossiaea aquifolium* Benth (Fabaceae; Figure 3.3a). However, 20 species were unique to unmined forest sites, such as *Xanthorrhoea preissii* Endl. and *X. gracilis* Endl. (Xanthorrhoeaceae) and *Pteridium esculentum* (G.Forst.) Cockayne (Dennstaedtiaceae). The oldest restoration sites (>30 years old) were dominated by the introduced non-native overstorey species *Eucalyptus resinifera* Sm. (Figure 3.3a).

Vegetation abundance increased from 1–5-year-old restoration (155 ± 24.3 plants/plot) to 5–15-year-old (468 ± 120.7 plants/plot) and 15–20-year-old sites (464 ± 1.5 plants/plot), then decreased markedly along the restoration gradient to 30–45-year-old sites (160 ± 31.5 plants/plot; \(F_{1,15} = 0.944, P = 0.347\); Figure 3.2a). However, species richness and Simpson’s diversity increased along the same age gradient, being highest in 30–45-year-old sites (richness: 36 ± 1.5 species/plot; diversity: 0.9 ± 0.01 species/plot) where it was comparable with both the richness (44.7 ± 3.2 species/plot; \(F_{1,15} = 5.46, P = 0.033\)) and diversity (0.9 ± 0.01 species/plot; \(F_{1,15} = 6.607, P = 0.021\)) of unmined forest (Figures 3.2b and 3.2c).


Figure 3.2: The effect of restoration age class (years) on vegetation (▲) and insect (●) a) species abundance; b) species richness and; c) Simpson’s diversity, as well as d) vegetation density (filled voxels; FV) and e) canopy height (m) in restored Jarrah Forest of different ages in southwestern Australia. Reference values from unmined forest are indicated by MRF.
Restoration age had a significant effect on both vegetation density (Figure 3.2d; \( F_{1,15} = 6.456, \ P < 0.022 \)) and canopy height (Figure 3.2e; \( F_{1,15} = 10.55, \ P < 0.005 \)), both of which were highest in unmined forest (canopy height: 26 ± 2.9 m; vegetation density: 78.7 ± 2.89 FV) and 30–40-year-old restoration (canopy height: 21 ± 0.0 m; vegetation density: 77.5 ± 0.36 FV). There was also a significant effect of restoration age on vegetation community composition (\( \text{Pseudo-}F_{1,15} = 9.73, \ P = 0.001 \)), with post-hoc pairwise comparisons indicating that community composition within restoration sites remained significantly different from unmined forest even after four decades of development (Figure 3.3a; Appendix 1, Table A1.3).

### 3.4.2 Insect community

Trapping yielded 15,427 insect specimens representing 169 morphospecies from 87 families (Table A1.2). The insect community was dominated by Chironomidae (non-biting midges), which accounted for 46.8% of insect specimens across all sites. Halictidae and Apidae accounted for 18.4% and 15.3% of the remaining 8,211 specimens, respectively. Indicator species analysis revealed that the youngest sites (1–5 years old) were characterised by Hymenoptera such as Apidae, Anthophoridae and Halictidae and Coleoptera such as Buprestidae and Coccinellidae (Figure 3.3b; Table A1.2), while restoration sites >5 years of age were characterised by coleopteran families such as Curculionidae, Cleridae and Chrysomelidae, the dipteran families Chloropidae and Mycetophilidae and hymenopteran families such as Braconidae (Figure 3.3b; Table A1.2).
Figure 3.3: a) Unconstrained non-metric multidimensional scaling (NMDS; Stress 0.22) showing the relative differences in vegetation composition and indicator vegetation species ($\alpha < 0.002$), and b) canonical correspondence analysis (CCA) showing the insect community composition and indicator insect families ($\alpha < 0.05$) across 41-years of post-mining restoration in the Northern Jarrah Forest. The environmental variables that significantly influenced the insect community (represented through vector overlays) were vegetation density (% of filled voxels; VDe), vegetation species richness (VSpRi) and vegetation species abundance...
(VSpAb); Points represent restoration age class centroids and standard errors; 1-5 year (■), 5-15 (▼), 15-20 (▲), 20-30 (△), 30-45 (●) and unmined remnant forest (MRF; ●).

Insect abundance was significantly higher in 1–5-year-old (296 ± 43.1 individuals) and 5–15-year-old restoration (205 ± 41.5 individuals) than in older restoration (F\textsubscript{1,100}= 20.267, \(P < 0.001\); Figure 3.2a). Similarly, insect species richness was higher in 1–5-year-old (21.6 ± 2.36 species) and 5–15-year-old restoration (20.9 ± 1.76 species) than older restoration (F\textsubscript{1,100}= 5.313, \(P = 0.023\); Figure. 3.2b). There were no significant effects of restoration age on insect Simpson’s diversity (F\textsubscript{1,100}= 0.338, \(P = 0.562\); Figure 3.2c). Patterns in insect abundance and richness over the restoration sequence were best explained by vegetation density (Abundance: \(z = -6.65\), \(P = <0.001\); Richness: \(z = -2.32\), \(P = 0.020\); Figures 3.4a and 3.4b). Neither the abundance, richness or diversity of vegetation influenced the abundance or richness of insects. However, there was a significant difference in insect community composition across restoration ages (\textit{Pseudo}-F\textsubscript{1,98} = 3.051, \(P = 0.002\)), with insect compositions converging in similarity with those of the unmined forest after approximately five years from initial restoration planting (Figure 3.3b; Table A1.4).

The CCA model displayed ~7% of the weighted variance (inertia) in insect assemblage data, and 72.5% of variance in the weighted averages and class totals for these data in its first two axes (Figure. 3.3b; CCA1: \textit{Pseudo}-F\textsubscript{1,97} = 3.91, \(P = 0.001\); CCA2: \textit{Pseudo}-F\textsubscript{1,97} = 1.54, \(P = 0.047\)). Vector analysis indicated composition varied most strongly in response to vegetation density (\(R^2 = 0.70\), \(P = 0.001\)), followed by vegetation species abundance (\(R^2 = 0.59\), \(P = 0.001\)) and vegetation species richness (\(R^2 = 0.14\), \(P = 0.008\)). Simpson’s diversity of vegetation was not a significant predictor of insect compositional variation (\(R^2 = 0.02\), \(P = 0.426\)).
Figure 3.4: Species richness (a) and abundance (b) of the insect community (comprised of Coleoptera, Diptera, Lepidoptera and Hymenoptera) across a gradient of increasing vegetation density (% of filled voxels; FV) in restored and unmined Jarrah Forest of south-western Australia. Both species richness and abundance decline as forest habitats become increasingly dense, a characteristic of aging restoration. Shaded regions around marginal effect regression lines represent 95% confidence interval.
3.5 Discussion

Previous studies have found that autonomous post-disturbance convergence of insect communities has broadly failed to occur in response to ecological restoration focussed on returning vegetation (Majer & Nichols 1998; Orabi, Moir & Majer 2010; Frick, Ritchie & Krauss 2014). However, insect communities in restored sites were similar in composition to those of adjacent unmined forest within a decade of recovery activities being undertaken, despite a lack of similar convergence in the composition of restored vegetation communities. Therefore, our study provides evidence in support of the ‘Field of Dreams’ hypothesis regarding insect communities (Watts & Didham 2006; Fernandes et al. 2019). However, our results indicate that key pollination guilds (i.e. bees) are present in much lower abundances within the closed-canopy habitats characteristic of late-stage restoration and unmined forest, potentially resulting in reduced pollination efficacy and plant reproductive success in these habitats (Menz et al. 2011). While other studies have reported close associations in ecological trajectory between vegetation and invertebrate communities (Janzen & Hallwachs 2019), especially for pollinating insects (Ghazoul 2006; Winfree, Bartomeus & Cariveau 2011b; Zirbel et al. 2017), these data suggest that the structure of restored vegetation had a stronger influence on the insect community than the vegetation community composition itself.

3.5.1 Structural drivers of reassembly

Vegetation composition has a complex role in that it both drives and is driven by the habitat structural components, as each individual plant species has unique architecture (Schaffers et al. 2008). However, it was this architecture and resulting vegetation density (as assessed in the present study using LiDAR) that had a stronger effect on the abundance, richness, and reassembly of insect communities. Our findings join a growing body of literature reporting that dense canopy vegetation constrains the abundance of bees (Hanula, Horn & O’Brien 2015; McCabe et al. 2019; Odanaka et al. 2020), while open canopies benefit them. For example, increased bee species diversity has also been reported in naturally less dense forest patches (Roberts, King & Milam 2017; Mullally et al. 2019), and in open agricultural patches in a mosaic forest landscape (Steffan-Dewenter 2002).

While insect abundance and richness peaked in the early stages of restoration (<5 years), a subtle increase in abundance and richness was also observed in the oldest restoration
sites (30–45 years restored) compared to mid- (15–20 years restored) and late-successional (20–30 years restored) sites and unmined forest. Though there were no significant differences between the 20–30 and 30–45-year-old restorations in their total density estimates, canopy vegetation was typically denser than understorey vegetation. Additionally, this age class had the lowest vegetation abundance attributed to markedly different stocking densities and forest structure in restoration conducted prior to 1988. During this time, trees were planted at a density of 625 stems/ha (Nichols and Michaelsen 1986), whereas tree stocking densities were substantially higher for post-1988 restoration, averaging over 5,000 stems/ha, comprising of approximately 80% *Eucalyptus marginata* and 20% *Corymbia calophylla* (Koch & Samsa 2007). Current practice aims to restore 600–1,400 stems/ha (Koch & Samsa 2007), and these densities may be more suitable for insects in the long-term compared to the emergent habitat from the former stocking treatments. However, if this proves not to be the case, some management of vegetation density may be required to optimise the insect biodiversity of these sites.

Forest management options, such as thinning or prescribed burning, can alter forest ecology by reducing competition, creating warmer microclimates through greater light penetration, and increasing the structural complexity of understorey vegetation (Hayes, Weikel & Huso 2003). As such, these management options have been used to improve biodiversity values in restored forest ecosystems (Arévalo & Fernández-Palacios 2005). In the Jarrah Forest, birds and reptiles have been observed to respond positively to reduced vegetation densities (Abbott & Heurck 1985; Abbott *et al.* 2003; Craig *et al.* 2010). Reduced ‘understorey clutter’ has also been associated with increased bat activity and foraging, particularly in patches cleared for forest tracks (Webala *et al.* 2011). While the process is perhaps inconsistent with most of the current best practice guidelines for ecological restoration (Miller *et al.* 2017), density-reduction treatments, may prove useful in promoting biodiversity for late-successional restoration characterised by dense understorey vegetation (Swanson *et al.* 2011; Tonietto, Ascher & Larkin 2017; Odanaka *et al.* 2020). Therefore, future research should explore role of density reductions as a management option for promoting insect communities, particularly native bees. However, any treatments considered in this process should aim to maintain old-growth trees and woody debris that provide critical habitat to a range of other taxa that were not studied here (Moir *et al.* 2005; Webala *et al.* 2011).
3.5.2 Compositional drivers of reassembly

The poor statistical association observed between vegetation community and insect community is inconsistent with some previous reports examining the factors driving insect reassembly (Moir et al. 2005; Ghazoul 2006; Winfree, Bartomeus & Cariveau 2011b; Zirbel et al. 2017). However, Schaffers et al. (2008) reported that plant community composition had a greater predictive capacity for herbivorous and predatory arthropods such as Hemiptera, Orthoptera, and Araneae compared to pollinating arthropods such as Hymenoptera and Diptera. When considered alongside habitat structural variables, the relationship between vegetation community composition and insects appears relatively weak. This may be explained by the disproportionate effect of some plants as foraging resources and the generalist nature of many plant-insect interactions, reducing the capacity for certain plants to predict the presence of specific flower-visitors (Goulson & Darvill 2004; Grundel et al. 2010). However, floral resources were not quantified here, and the inclusion of such would likely elucidate stronger trends as pollinators and flower-visitors respond positively to the presence of flowering resources (Blaauw & Isaacs 2014). Moreover, communities with higher species diversity often reflect reduced mutual dependencies and increased functional redundancy (Memmott, Waser & Price 2004; Kaiser-Bunbury et al. 2017), further weakening the predictive capacity of vegetation composition. Understanding the reciprocal effects of the functional diversity of vegetation communities on the diversity of insects, and vice versa, would showcase the dynamical properties of such networks in ways that cannot be encompassed by compositional assessments alone (Fontaine et al. 2005).

3.5.3 Compositional shifts and functional drifts

A distinct tipping point was observed in the composition of insect pollination communities in the Jarrah Forest, in that most Dipteran and some Coleopteran families were present primarily in established, late-successional restoration while many hymenopteran families, particularly within Apoidea, occurred in early-successional restoration habitats. Due to the disproportionate delivery of pollination services from bees, reduced pollination efficacy and plant reproductive success may befall late-successional habitats (Martins, Gonzalez & Lechowicz 2015). These bee communities may respond to emerging vegetation structures across developing ecological restoration, in different ways to other invertebrate groups and the mechanisms underpinning this compositional shift are complex (Brown 1991). The mechanistic underpinnings of this shift likely relate to a combination of biotic and abiotic
factors limiting bee dispersal and fitness and mediated by physiological thresholds and nutrition (Tomlinson et al. 2017b), among other candidates. Limitations to species’ energetic requirements are likely to impart considerable and cascading impacts on the ecology of restored systems (Tomlinson et al. 2014; Tomlinson et al. 2018b), yet evidence of such is sparse and the contention requires further testing. However, meticulously maintained age sequences of restoration required to confirm this are rare, and further research within those available may prove illuminating.

3.5.4 Restoration and Landscape context in community self-assembly

While insect community analysis indicated that the species composition of mid- and late-successional restoration sites converged with those of reference communities, the broader ecological context of the NJF somewhat constrains our interpretations. The reference sites were neighbouring unmined forest which was logged for timber from the late 19th century through until mid-20th century (Heberle 1997), and has since been subject to prescribed burning and forest management regimes (Sanders 1997; Bradshaw et al. 2018). These management strategies generated more dense and shaded forests than natural, undisturbed forests (Mills 1989). Additionally, Abbott, Dell and Loneragan (1989) estimate that Jarrah has a life cycle ranging between 500 and 1,000 years. It is, therefore, unlikely that unmined reference forest represents an undisturbed, mature ‘climax’ community, raising the question of the suitability of these forests as reference systems. However, virtually no pristine old-growth Jarrah Forest remains in the SWAFR (McCaw, Robinson & Williams 2011), and current government requirements for restoration and management of these forests stipulate stocking rates to meet specific silvicultural targets and harvest cycles (Gardner & Bell 2007), limiting the capacity to restore to a historical reference.

Although these data suggest that representative insect communities may reassemble in Jarrah forest landscapes without intervention following the re-establishment of native vegetation, these communities may not be reflective of “historical” or “undisturbed” communities, and this outcome may not be achieved in more degraded or highly fragmented landscapes (Knop, Herzog & Schmid 2011; Menz, Dixon & Hobbs 2013; Aavik & Helm 2018). This is because isolated habitats may experience slower rates of reassembly or be recolonised by non-target taxa, leading to longstanding compositional differences (Watts, Clarkson & Didham 2008; Cusser & Goodell 2013). However, bauxite mining in the NJF comprises a
landscape of small, irregularly-shaped mining pits scattered in an otherwise intact matrix of closed-canopy forest. Consequently, each area to be restored is relatively small, and surrounded by an adjacent source population of insects that can readily recolonise developing vegetation (Figure. 3.1), potentially lending itself to the rapid convergence of the restored insect communities with the surrounding forest. However, this outcome is distinct from that of other restoration programs that tackle clearing and fragmentation at scales exceeding thousands of hectares, such as agricultural restoration. This highlights the imperative of allocating resources towards maintaining landscapes with high degrees of connectivity and heterogeneity to facilitate biodiverse, resilient, functional, and sustainable ecological communities, whether that be through active conservation or ecological restoration. While landscape connectivity is expected to have substantial impacts on the insect community ecology and delivery of pollination services (Lundberg & Moberg 2003; Kremen et al. 2007), supporting empirical data are rare (Mitchell, Bennett & Gonzalez 2013). Therefore, concerted efforts to understand the mechanisms by which connectivity influences the structure and function of insect communities, and at what scale, are necessary to better inform restoration and landscape management.

3.5.5 Conclusions

Insect communities in the NJF responded strongly and positively to restoration efforts, despite the return of these communities not being a direct objective of ecological recovery programs. However, the landscape context at the study site may have been uniquely beneficial in facilitating this return, in that the disturbance represented a suite of small sites among a matrix of unmined forest. While the broader insect community appears to recover rapidly within this landscape, it appears that late-stage restoration and unmined forest habitats are less suitable for some pollinating taxa due to the properties of vegetation structure emerging through forest succession. As such, ecological restoration may consider trade-offs between pollinator enhancement, with attention directed towards facilitating suitable native bee habitats across all successional stages, designated land-use objectives, and other forest values. The findings presented here suggest that reduced seeding and planting densities may restore forest that is more suitable to the pollinating insect community, and future research should elucidate the efficacy and ongoing management role of thinning techniques in allowing increased light penetration to facilitate diverse understorey development and microclimate heterogeneity within presently established forest patches.
Chapter 3: Community Reassembly within the Jarrah Forest

3.6 References

Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.


Chapter 3: Community Reassembly within the Jarrah Forest


Chapter 3: Community Reassembly within the Jarrah Forest


Chapter 3: Community Reassembly within the Jarrah Forest


Shearer, B.L. & Tippett, J.T. (1989) *Jarrah dieback: the dynamics and management of Phytophthora cinnamomi in the jarrah (Eucalyptus marginata) forest of south-western Australia*. Department of Conservation and Land Management, Perth, WA.


Chapter 3: Community Reassembly within the Jarrah Forest


CHAPTER FOUR

Reproductive responses of cavity-nesting pollinators, predators and parasitoids to vegetation structure and microclimate associated with post-mining restoration

4.1 Abstract

Landscape-scale habitat alteration has a significant effect on biodiversity and biotic interactions. The delivery of essential ecosystem services may decline when important functional groups such as pollinators, predators and parasitoids are impacted by habitat loss and land-use change. The reproductive success of many organisms, particularly holometabolous insects, is strongly dependent on the abiotic (e.g., temperature) and biotic (e.g., vegetation structure) conditions of oviposition sites. In this study we analysed the effect of habitat structure and microclimate on nest-site selection and fecundity of cavity-nesting Hymenoptera by installing nesting blocks along a restoration chronosequence in Australia’s south-west. We found that forest overstorey density altered microclimate and observed increased nest-construction and fecundity within the warmer microclimates provided by early-successional forest restoration. Nest site selection and brood abundance of wasps, however, were driven by increasing vegetation density. This study strongly supports the value of early-successional and open habitats in maintaining native bee populations and suggests that thermal ecology may underpin the mechanisms driving habitat selection within heterogenous forested landscapes. This has important implications for the management of biodiverse ecosystems such as in the southwest of Western Australia. Cavity nesting bees represent an important component of the hymenopteran fauna and often fulfil specialized pollination roles that facilitate vegetation dynamics and ecosystem recovery in native ecosystems, but thermal biology is rarely considered in ecological management.
4.2 Introduction

Changes in vegetation and topography can alter microclimate and habitat structure in ways that produce cascading effects on biological communities, trophic interactions, and the delivery of ecosystem services (Denno et al. 2002; Sanders et al. 2008). Land-use changes, such as agriculture and mining, often generate highly fragmented and disturbed landscapes (Larkin, Vivian-Smith & Zedler 2006), and present unique challenges in conservation management (Kovács-Hostyánszki et al. 2017). Rehabilitation and restoration are increasingly advocated as tools to respond to losses in ecosystem services and processes (Miller et al. 2017). Restoration essentially attempts to rapidly recapitulate ecological succession to return a landscape to a representative natural ecosystem (Hobbs, Walker & Walker 2007; Prach & Walker 2011; SERA 2017), and when deployed in the face of ongoing disturbance it can result in a mosaic of differently aged ecotypes. This mosaic creates a diverse suite of abiotic and biotic conditions, with successional gradients in species abundance, richness, and diversity. While such successions are among the best-described trends in ecology (Werner 1976; Norman et al. 2006), the driving mechanisms behind these ecological patterns and habitat selection often remain poorly understood (Hale & Swearer 2017).

Habitat selection, whereby an organism chooses a new foraging or nesting site, is assumed to result from non-random distribution patterns (Hale & Swearer 2017). These patterns arise from the sensitivities of organisms to environmental cues that help differentiate between suitable habitat patches (Lindell 2008). Identifying the cues that organisms respond to can be difficult, as many environmental signals can be correlated across various spatiotemporal scales (Kemp & Ellis 2017; van Schalkwyk et al. 2019). However, such cues may drive subtlety different mechanisms that structure populations and communities (McNab 2002; Tomlinson et al. 2014).

Vegetation habitat characteristics such as structure (Araújo et al. 2006; Chapter 3) and biomass (Marques, Price & Cobb 2000; Haddad et al. 2009) exert strong influences on local insect community, potentially imposing bottom-up effects on patterns of insect abundance (Brose 2003). The resource abundance hypothesis stipulates a positive effect of vegetation cover on biodiversity and abundance, with the assumption that plants can determine the carrying capacity for insects through impacting the availability of foraging and nesting resources. (e.g. Marques, Price & Cobb 2000; Yamamoto, Yokoyama & Kawata 2007; Müller et al. 2014). In contrast, however, the abundance, richness, and diversity of Hymenoptera often
decrease in vegetation types with dense, extensive canopies (Winfree, Griswold & Kremen 2007; Rubene, Schroeder & Ranius 2015; Roberts, King & Milam 2017). The relative importance of different vegetation characteristics underlying this relationship is complex, and there have recently been calls to explore thermal parameters as a mechanistic driver of this response (Tuff, Tuff & Davies 2016; Tomlinson et al. 2018b; Tomlinson 2020).

Several studies have shown that, in addition to resource abundance and availability, microclimate plays an important role in establishing and maintaining species compositions within heterogenous landscapes (Cloudsley-Thompson 1962; Pincebourde et al. 2007; Pincebourde & Casas 2015). The effects of warming temperatures on different taxa has gained prominence in the context of global climate change (Tylianakis et al. 2008; Gilman et al. 2010; Lister & Garcia 2018), and there is evidence that even modest temperature shifts can alter the structural and functional components of an ecosystem (Peck et al. 2009; Gilman et al. 2010; Gilbert et al. 2014). However, local habitat degradation causes changes to the thermal environment at smaller scales that those caused by climate change (Tuff, Tuff & Davies 2016; Tomlinson et al. 2018b). While it is accepted that overlooking patch-scale structural variation of habitats can lead to inaccurate model estimates of thermal niches and extinction risk at the large scale (Di Marco et al. 2019; Reside et al. 2019), very little research has explored this at local scales in terms of environmental degradation or restoration (Tomlinson et al. 2018b; Garcia & Clusella-Trullas 2019; Tomlinson 2020).

Avoidance through moving to suitable microclimates is a common response of many organisms to mitigate the effects of stressful temperatures (Kearney, Shine & Porter 2009; Huey et al. 2012; Woods, Dillon & Pincebourde 2015). In their early developmental stages, however, holometabolous insects, rely entirely on the provisions provided and developmental location selected by the nesting adult (Wuellner 1999; Morato & Parentoni Martins 2006). Despite this, studies that focus on the influence of maternal oviposition-site selection on offspring have often overlooked the role of physiology and behaviour on site selection (Mousseau & Fox 1998). Evaluating maternal nest-site choice offers a unique approach to characterise habitat selection that is specific to a critical life history stage, where habitat selection by the adult has fitness implications for both offspring and maternal individuals and influences the demography and structure of the local population and ecosystem. Within restoration ecology, plant-focused studies dominate the literature; faunal perspectives, while increasing (Majer 2009), remain comparatively uncommon (Cross, Bateman & Cross 2020).
Moreover, even fewer studies have targeted the mechanistic relationship between the microclimatic conditions and habitat-selection (Nowakowski et al. 2018).

This study investigated the response of a holometabolous pollination guild of cavity-nesting Hymenoptera (CNH) to post-mining restoration within a forest ecosystem in south-western Western Australia where ecological restoration has been conspicuously well documented (Cross et al. 2019), but the insect pollination services remain largely unknown (Majer, Brennan & Moir 2007). We aimed to differentiate between the microhabitats that supported the most active and abundant nesting populations, evaluating 1) if habitat structure or microclimate best explained the associations between nesting activity and reproductive output of CNH; and 2) whether the responses among different component taxa (i.e., cavity-nesting bees and wasps) to microclimate and vegetation structure were the same. We infer likely mechanisms driving nest-site selection across a heterogenous landscape and highlight the potential for incorporating thermal biology into restoration ecology in both planning and monitoring. Knowing which factors drive habitat selection and the environmental conditions that are necessary to support crucial functional groups can aid in the development of informed and targeted restoration criteria and evidence-oriented conservation management practices.

4.3 Methods

4.3.1 Study region and study sites

This research was conducted across a chronosequence of restoration sites (Figure 4.1c) following bauxite extraction in the Northern Jarrah Forest (NJF; Thackway & Cresswell 1997). The NJF is a conservation priority at multiple jurisdictions because of its high endemism, and the large-scale habitat change that it has already undergone (Dell, Havel & Malajczuk 1989; Wardell-Johnson & Horwitz 2000; Ladiges et al. 2011). Understanding the microclimatic influences of habitat change on essential functional groups, both in terms of degradation and subsequent recovery, is an important conservation goal generally, but is specifically advocated for ecosystems in the south-west of Australia such and the Jarrah Forest (Beard, Chapman & Gioia 2000; Rix et al. 2015). Shallow mining (<6 m depth) has occurred in our study area since 1963, with approximately 550 hectares of land cleared, mined and rehabilitated annually (Koch 2007b; Koch 2007a). The resulting landscape is a matrix of unmined land and restored patches that aim to be representative of unmined forest with a diverse understory and tall, dense
eucalypt canopy (Koch 2007b), but that are also suitable for subsequent timber harvest (Abbott & Williams 2011).

Figure 4.1: Locality (a) of the Northern Jarrah Forest (shaded) in the southwest corner of Western Australia (b) and location of sites sampled across a landscape matrix of post-mining restoration (c). The polygons represent patches of restoration in a broader landscape of unmined jarrah forest last disturbed by logging approximately 80 years prior to our study. The shading gradient indicates restoration age, where the darkest shading indicates the oldest restoration. Sites in restoration plots are represented by black circles, and reference sites in unmined forest are represented by black triangles. The numbers next to each restoration site indicate the year that restoration was initiated.

Twelve survey sites were identified across the study area that encompassed a 22-year restoration chronosequence with three replicate surveys in each of four age classes (Figure 4.1c). All sites were separated by more than 1 km to ensure spatial independence beyond the flight range of most bees (Zurbuchen et al. 2010). Nine replicate sites were identified in restoration of ages 1990-2000, 2000-2010, and 2010-2020, and three unmined forest sites were chosen for reference. The sequence of bauxite extraction and rehabilitation has progressed
north over time, and so the youngest restoration is present primarily in the northern part of the study area. However, the 8.3 km extent of the area is unlikely to introduce latitudinal correlates of climatic variation (Hawkins & Felizola Diniz-Filho 2004). The selected sites were maintained for the extent of the study and each site was surveyed five times across the sampling period.

4.3.2 Study design and sampling

CNH were surveyed by establishing artificial nesting blocks, hereafter referred to as trap-nests, consisting of jarrah (*Eucalyptus marginata*) wood blocks containing 24 artificial nesting holes (11 mm diameter, ~100 mm deep; Figure 4.2). Individual cavities were fitted with 10 mm paper straws (BioPak, Bondi Junction, Australia), which allowed the contents of entire nests to be easily harvested at monthly intervals (Paini 2004; Taki *et al.* 2008; Staab *et al.* 2018). A single trap-nest post was comprised of two trap-nest blocks attached to a 1.5 m garden stake and a Lascar EL-USB-2 temperature/humidity data logger (Lascar, Whiteparish, UK) suspended within plastic jar below the two attached nesting blocks (Figure 4.2). One trap-nest post was set at each site with nesting blocks positioned approximately 1.2 m above ground level. Each trap-nest was placed at least 15 m away from the edge of the restoration patch, with one trap-nest block facing north and the other facing south. Following harvest, the nesting straws were returned to the Kings Park Science laboratory (Department of Biodiversity, Conservation and Attractions, Kings Park, Western Australia) and kept in a 25°C incubator (Thermoline Scientific, Wetherill Park, Australia) prior to brood counts and identifications. Pupal case morphotype and brood counts were determined by X-ray imaging (MultiFocus, Faxitron Bioptics, Arizona, USA). The identity of each species was confirmed by rearing brood to adulthood and emergence (Figure 4.2). For the purposes of analysis, each nesting post was considered as one sampling unit (i.e., data from individual nesting tubes were combined for total brood counts).
Remote sensing was used to assess vegetation structure at each site as a proxy of nesting availability across restoration ages. Canopy height and vegetation density were quantified with Airborne Light Detection and Ranging (LiDAR) in 2018 using an Optech ALTM 3100 (Teledyne Optech, Toronto, Canada). Vegetation point clouds were divided into a multi-layer, 50 x 50 m raster grid and categorised as understorey (<2 m), midstorey (2–10 m) and overstorey (>10 m). The total number of filled voxels (FV; volumetric pixels) was calculated for each metre height band up to maximum canopy height. Canopy height was calculated through the triangulation of the maximum height points across each site. Vegetation density, our proxy for nesting availability, was calculated as the sum of all FV for each canopy stratum (Clawges et al. 2008).

The data loggers (Lascar, Whiteparish, UK) recorded temperature and humidity at 15-minute intervals for the duration of the study. Most bees and wasps are diurnal (Szabo & Smith 1972; Lerer et al. 1982; Bloch et al. 2017), so mean temperature and humidity was calculated between 6:00 am and 6:00 pm. Temperature range was calculated as the difference between the minimum and maximum temperature for each month. All microclimatic variables were averaged across the sampling month at each site (i.e., one reading per month for each site) prior to analysis to correspond to monthly nest collection regimes.

Figure 4.2: Schematic illustration of fully constructed trap-nest with individual nesting block with 10 mm diameter removable paper straw nesting cavities with examples of capped nests; X-ray imagery of constructed nests and corresponding emergent adult cavity-nesting Hymenoptera. * Parasitoid of Megachile, † Spider predator.

4.3.3 Habit Characterisation

Remote sensing was used to assess vegetation structure at each site as a proxy of nesting availability across restoration ages. Canopy height and vegetation density were quantified with Airborne Light Detection and Ranging (LiDAR) in 2018 using an Optech ALTM 3100 (Teledyne Optech, Toronto, Canada). Vegetation point clouds were divided into a multi-layer, 50 x 50 m raster grid and categorised as understorey (<2 m), midstorey (2–10 m) and overstorey (>10 m). The total number of filled voxels (FV; volumetric pixels) was calculated for each metre height band up to maximum canopy height. Canopy height was calculated through the triangulation of the maximum height points across each site. Vegetation density, our proxy for nesting availability, was calculated as the sum of all FV for each canopy stratum (Clawges et al. 2008).

The data loggers (Lascar, Whiteparish, UK) recorded temperature and humidity at 15-minute intervals for the duration of the study. Most bees and wasps are diurnal (Szabo & Smith 1972; Lerer et al. 1982; Bloch et al. 2017), so mean temperature and humidity was calculated between 6:00 am and 6:00 pm. Temperature range was calculated as the difference between the minimum and maximum temperature for each month. All microclimatic variables were averaged across the sampling month at each site (i.e., one reading per month for each site) prior to analysis to correspond to monthly nest collection regimes.
4.3.4  Statistical analyses

The effect of restoration successional age (in years) on nesting activity and all microclimatic and structural elements of the sites was examined using linear models assuming a Gaussian error distribution (ANOVA; Kaufmann & Schering 2014). The relationship between microclimate and vegetation density was examined using linear mixed-effect models assuming Gaussian error distribution through the \textit{lmer} function in the R statistical package \textit{‘lme4’} (Bates et al. 2007) to account for the fixed effects of vegetation density at categorical height classes (overstorey, midstorey and understorey) on mean daily temperature, daily temperature range and relative humidity while holding site and sampling round as random factors to account for repeated microclimatic measurements. The effects of microclimate and vegetation stratum density on both CNH fecundity (brood abundance) and nest production (total capped nests) were tested using generalised linear mixed-effect models (GLMM) assuming a Poisson error distribution (McCulloch & Neuhaus 2005), holding site and sampling round as random factors to account for repeated monthly collections. Models were fitted using the \textit{glmer} function in the R statistical package \textit{‘lme4’} (Bates 2007). Pearson correlation coefficients (threshold <0.75) were assessed using the \textit{cor} function in the R statistical package \textit{‘PerformanceAnalytics’} (Peterson et al. 2018) to check for collinearity between environmental variables prior to model construction. Canopy height and humidity exceeded this threshold due to collinearity with overstorey density and average temperature, respectively, and were omitted from further analysis (Zuur, Ieno & Elphick 2010). Collinearity of variables below this level was not considered to significantly alter the GLMM estimates, and all other variables were retained. Following the construction of a ‘full’ model we applied a model reduction using the \textit{dredge} function within the \textit{‘MuMIn’} package (Bartoń 2014), and the models were examined by Akaike’s Information Criterion for small sample sizes (AICc; Burnham & Anderson 2002), however, model reductions did not substantially increase model parsimony (Table 4.1) and full models were maintained for final analysis.

Differences in the hymenopteran community composition between nesting sites were assessed using a two-factor non-parametric permutation multivariate analysis of variance (PERMANOVA) via the \textit{adonis} function within the ‘\textit{vegan}’ statistical package (Oksanen et al. 2007; Anderson 2014) over 999 permutations. Pair-wise post hoc comparisons were made between variables to determine which nesting sites differed from each other (Anderson 2014). Canonical Correspondence Analysis (CCA; Anderson & Willis 2003) characterised the strength and direction of microclimatic and structural effects on CNH community composition.
Total larval abundance was log$_{10}$((x+1) transformed while vegetation density was arcsine transformed prior to CCA analysis. Vectors were superimposed onto CCA biplots with significance established through 999 permutations using the `envfit` function in the `vegan` statistical package (Oksanen et al. 2007). All statistical interrogation was performed using the R statistical software in RStudio Version 1.2.5001 (R Development Core Team 2019).

Table 4.1: Parameter estimates and AICc and Log-Likelihood comparisons between full and reduced Generalized Mixed Linear Models for each response variable of: a) total larvae abundance, b), total nest production, c), taxa specific response of bee species (*Megachile aurifrons*, *M. canifrons* and *M. erythropyga*, *Hylaeus alcyoneus*) and, d) taxon specific response of wasp species (*Gasteruption breviscutum* and *Turneromyia sp.*) against explanatory variables; Average daily temperature (°C; DT$_{a}$), Temperature range (RT$_{a}$), Overstorey density (V$_{OS}$) and, Midstorey density (V$_{MS}$), Understorey Density (V$_{US}$).

<table>
<thead>
<tr>
<th>Response</th>
<th>Model</th>
<th>Parameters</th>
<th>df</th>
<th>logLik</th>
<th>AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community</td>
<td>a) Larvae</td>
<td>Full</td>
<td>DT$<em>{a}$ + RT$</em>{a}$ + V$<em>{os}$ + V$</em>{ms}$ + V$_{us}$</td>
<td>7</td>
<td>-188.946</td>
</tr>
<tr>
<td>Response</td>
<td>Reduced</td>
<td>DT$<em>{a}$ + V$</em>{us}$</td>
<td>4</td>
<td>-190.340</td>
<td>392.100</td>
</tr>
<tr>
<td>b) Nest</td>
<td>Full</td>
<td>DT$<em>{a}$ + RT$</em>{a}$ + V$<em>{os}$ + V$</em>{ms}$ + V$_{us}$</td>
<td>7</td>
<td>-137.875</td>
<td>291.751</td>
</tr>
<tr>
<td>Taxon-specific</td>
<td>Reduced</td>
<td>DT$<em>{a}$ + RT$</em>{a}$ + V$_{us}$</td>
<td>5</td>
<td>-139.074</td>
<td>292.200</td>
</tr>
<tr>
<td>c) Bees</td>
<td>Full</td>
<td>DT$<em>{a}$ + RT$</em>{a}$ + V$<em>{os}$ + V$</em>{ms}$ + V$_{us}$</td>
<td>7</td>
<td>-184.032</td>
<td>384.065</td>
</tr>
<tr>
<td>Response</td>
<td>Reduced</td>
<td>DT$<em>{a}$ + V$</em>{us}$</td>
<td>4</td>
<td>-185.403</td>
<td>382.200</td>
</tr>
<tr>
<td>d) Wasps</td>
<td>Full</td>
<td>DT$<em>{a}$ + RT$</em>{a}$ + V$<em>{os}$ + V$</em>{ms}$ + V$_{us}$</td>
<td>7</td>
<td>-92.022</td>
<td>200.043</td>
</tr>
<tr>
<td></td>
<td>Reduced</td>
<td>DT$<em>{a}$ + V$</em>{os}$ + V$_{us}$</td>
<td>5</td>
<td>-92.207</td>
<td>198.500</td>
</tr>
</tbody>
</table>

### 4.4 Results

#### 4.4.1 Sampling effort

A total of 487 nesting straws were retrieved, yielding 1514 brood. The resin bee *Megachile aurifrons* Smith, 1853 was most common, occupying 77.5% of brood cells, followed by the Gasteruptiid parasitoid *Gasteruption breviscutum* Kieffer, 1911 (9.6%) and *M. canifrons* Smith, 1853 (7.1%). The remaining brood were comprised of the Pompilid wasp *Turneromyia sp.* Banks, 1941 (3.7%), *M. erythropyga* Smith, 1853 (1.6%) and eight individual larvae of the Colletid bee *Hylaeus alcyoneus* Cockerell, 1926 (0.5%).
Chapter 4: Cavity-Nesting Hymenoptera Nest-Site Selection

Megachile aurifrons occurred in every site except the mid-successional 18-year-old restoration and was present across the entire duration of sampling (Figure 4.3a). Megachile canifrons was present at all early-successional sites, two out of three unmined sites, and one mid-successional site, but not at any late-successional sites (Figure 4.3b). We did not record M. canifrons during the February or March sampling. The parasitoid Gasteruption breviscutum occurred across all sampling months at all unmined and early-successional sites and one mid-successional 16-year-old site but was not present in any late-successional restoration (Figure 4.3c). Megachile erythropyga nested at only one unmined site during December. The pompilid Turneromyia sp. only nested at early and mid-successional sites during December and January. Hylaeus alcyoneus nested only once at a late-successional site during December.

4.4.2 The effects of restoration succession on cavity-nesting Hymenoptera

Nest construction declined with restoration age ($F_{3,46} = 10.871, P < 0.001$; Figure 4.4a) with more nests constructed per month at each site in early-successional rehabilitation (23.08 ± 3.93) than in the mid- (8.41 ± 3.22) and late-successional rehabilitation (2.08 ± 0.96). Nest construction was moderately common in unmined forest (7.00 ± 1.81). Brood abundance also declined with site age ($F_{3,46} = 6.103, P = 0.001$; Figure 4.4a) with the highest monthly brood abundance at early-successional restoration sites (66.41 ± 14.38) and the lowest abundance at late-successional restoration sites (11.50 ± 6.08). Brood abundance for both bees and wasps were higher in early-successional restoration (Bees $F_{3,46} = 4.594, P = 0.021$: Wasps $F_{3,46} = 12.666, P < 0.001$; Figure 4.4b).
Figure 4.3: Three-dimensional fitness landscape for the three most dominant species, a) *Megachile aurifrons*; b) *M. canifrons* and; c) *Gasteruption breviscutum*. Abundance was measured as the total number of offspring produced per month and shown on the z-axis. The height and colour of the landscape display the fitness gain resulting from various combinations of breeding month and forest age.
Figure 4.4: General mean ± standard error of the mean for the a) community level reproductive responses for CNH nest production (▲) and brood abundance (●); b) taxa-specific patterns of brood abundance for wasps (▲) and bees (●); c) structural densities for overstorey (▲), midstorey (●) and understorey (■) vegetation and; d) mean daily temperature (°C; ●); e) temperature range (°C; ●) and; f) relative humidity (RH%; ●), across early (ESR), mid (MSR) and late-successional (LSR) stages of restoration compared to those in unmined reference forests (MRF).
4.4.3 The effects of vegetation structure on cavity-nesting Hymenoptera

Vegetation overstorey density increased with restoration age across successional classes ($F_{3,8} = 159.41, P < 0.001$; Figure 4.4c) from dense overstorey vegetation in late-successional restoration ($7.00 \pm 0.10$) and unmined forest ($8.49 \pm 0.54$) to reduced density in mid ($2.54 \pm 0.77$) and early-successional sites ($0 \pm 0$). However, midstorey vegetation density was highest in mid-successional sites ($17.90 \pm 3.79$) compared to other restoration stages ($F_{3,8} = 14.542, P = 0.001$; Figure 4.4c). There were no significant differences in understorey density ($F_{3,8} = 2.777, P = 0.110$; Figure 4.4c) across successional classes. Canopy height increased with site age with the tallest canopies emerging in the unmined forest ($26.00 \pm 2.88$; $F_{3,8} = 24.386, P < 0.001$). While there were no effects of vegetation structure on nest production, understorey density was significantly positively related to lower total brood abundance ($z = -2.13, P = 0.033$) and reduced bee brood abundance ($z = -2.10, P = 0.036$), while increased overstory density was associated with reduced wasp brood abundances ($z = -2.16, P = 0.031$; Figure 4.5d).

4.4.4 The effects of microclimate on cavity-nesting Hymenoptera

The younger restoration sites were warmer ($34.11 \pm 0.74$) than the mid-successional ($29.34 \pm 0.67$) and late-successional restoration ($27.07 \pm 0.80$) and warmer than the mature reference forest ($28.40 \pm 0.69$; $F_{3,46} = 17.929, P < 0.001$; Figure 4.4d). Relative humidity was higher in late-successional restoration ($57.69 \pm 1.80$) and unmined forest ($55.86 \pm 1.65$) than in early-successional restoration ($48.83 \pm 1.52$; $F_{3,46} = 5.567, P < 0.001$; Figure 4.4f). Overstorey vegetation density was the only significant driver for average daily temperature ($F_{1,41} = 26.617, P < 0.001$) and humidity ($F_{1,41} = 26.962, P < 0.001$), and vegetation density had no effect on daily temperature range (Table 4.2).
**Table 4.2:** Linear relationships between the microclimatic variables of mean daily temperature (°C), daily temperature range, and mean relative humidity (%) and structural densities (% filled voxels) for overstorey (V<sub>OS</sub>), midstorey (V<sub>MS</sub>) and understorey (V<sub>US</sub>) vegetation. Significant results are bolded.

<table>
<thead>
<tr>
<th>Response</th>
<th>EV</th>
<th>Estimate</th>
<th>SE</th>
<th>F Value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily Temperature (°C)</td>
<td>V&lt;sub&gt;OS&lt;/sub&gt;</td>
<td>-0.703</td>
<td>0.136</td>
<td>26.617</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>V&lt;sub&gt;MS&lt;/sub&gt;</td>
<td>-0.041</td>
<td>0.093</td>
<td>0.093</td>
<td>0.671</td>
</tr>
<tr>
<td></td>
<td>V&lt;sub&gt;US&lt;/sub&gt;</td>
<td>-0.156</td>
<td>0.113</td>
<td>1.920</td>
<td>0.203</td>
</tr>
<tr>
<td>Temperature Range</td>
<td>V&lt;sub&gt;OS&lt;/sub&gt;</td>
<td>-0.695</td>
<td>0.337</td>
<td>4.252</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>V&lt;sub&gt;MS&lt;/sub&gt;</td>
<td>-0.069</td>
<td>0.229</td>
<td>0.090</td>
<td>0.771</td>
</tr>
<tr>
<td></td>
<td>V&lt;sub&gt;US&lt;/sub&gt;</td>
<td>-0.147</td>
<td>0.280</td>
<td>0.275</td>
<td>0.614</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>V&lt;sub&gt;OS&lt;/sub&gt;</td>
<td>0.909</td>
<td>0.175</td>
<td>26.962</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>V&lt;sub&gt;MS&lt;/sub&gt;</td>
<td>-0.003</td>
<td>0.119</td>
<td>0.001</td>
<td>0.981</td>
</tr>
<tr>
<td></td>
<td>V&lt;sub&gt;US&lt;/sub&gt;</td>
<td>0.244</td>
<td>0.145</td>
<td>2.821</td>
<td>0.132</td>
</tr>
</tbody>
</table>

Warmer temperatures (DT<sub>a</sub>) and greater temperature ranges (RT<sub>a</sub>) were associated with increased nest construction (DT<sub>a</sub>: z = 3.49, P < 0.001; RT<sub>a</sub>: z = -2.63, P = 0.009; Figure 4.5a), and warmer temperatures were also associated with higher brood abundance (z = 2.83, P = 0.005; Figure 4.5b, Figure A2.1). There were different effects for different taxonomic groups, however, in that bee brood abundance was higher at warmer sites (z = 2.68, P = 0.007; Figure 4.5c), but wasp brood abundance was not influenced by site temperature (z = 1.910, P = 0.056; Table 4.3).
Table 4.3: Parameter estimates (PE) and standard errors (SE) for each response variable included in the selected Generalised Mixed Linear Models; a) total larvae abundance, b) total nest production, c) taxa specific response of bee species (*Megachile aurifons*, *M. canifrons*, *M. erythropyga* and *Hylaeus alcyoneus*) and, d) taxa specific response of wasp species (*Gasteruption breviscutum* and *Turneromyia sp.*) against explanatory variables; Average daily temperature (°C; DT<sub>a</sub>), Temperature range (T<sub>aR</sub>), Overstorey density (V<sub>OS</sub>) and, Midstorey density (V<sub>MS</sub>), Understorey Density (V<sub>US</sub>) and; the z-statistic. Asterisks indicate significance level as follows: ‘*’ P < 0.05; ‘**’ P < 0.01; ‘***’ P < 0.001.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Community Response</th>
<th>Taxa-Specific Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>a) All Larvae</strong></td>
<td><strong>b) All Nests</strong></td>
</tr>
<tr>
<td></td>
<td>PE ± SE</td>
<td>z</td>
</tr>
<tr>
<td>DT&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.263 ± 0.09**</td>
<td>2.88</td>
</tr>
<tr>
<td>T&lt;sub&gt;aR&lt;/sub&gt;</td>
<td>-0.059 ± 0.061</td>
<td>-0.98</td>
</tr>
<tr>
<td>V&lt;sub&gt;US&lt;/sub&gt;</td>
<td>-0.343 ± 0.161*</td>
<td>-2.13</td>
</tr>
<tr>
<td>V&lt;sub&gt;MS&lt;/sub&gt;</td>
<td>0.084 ± 0.084</td>
<td>1.00</td>
</tr>
<tr>
<td>Marginal R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.619</td>
<td>0.537</td>
</tr>
<tr>
<td>Conditional R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.810</td>
<td>0.820</td>
</tr>
</tbody>
</table>
Figure 4.5: Relationship between the most significant predictive variables (x-axes) for each reproductive response as determined by generalised linear mixed effect models: a) total nest construction; b) total brood abundance; c) bee brood abundance and d) wasp brood abundance.
4.4.5 Compositional variation

The nesting community composition changed with restoration age ($Pseudo-F_{1,46} = 9.46, P = 0.001$), primarily because the early successional restoration sites, particularly the 2014 restoration, presented increased Megachilid abundances. Both vegetation patterns and microclimate were important explanatory factors of the community assemblage of CNH (Figure 4.6), together accounting for 19.46% of the observed variation (CCA model with two axes - CCA1: $Pseudo-F_{1,29} = 5.822, P = 0.048$; CCA2: $Pseudo-F_{1,29} = 0.950, P = 0.900$). Mean daily temperature was the only microclimatic element to significantly influence CNH community structure ($R^2 = 0.36, P = 0.001$), and $V_{OS}$ was the only vegetation structural element to significantly influence CNH community composition ($R^2 = 0.30, P = 0.004$).

Figure 4.6: Canonical correspondence analysis biplot visualising the effect microclimatic and structural variables on the cavity-nesting hymenopteran community sampled within early-successional (■), mid-successional (▲), late-successional (♦) and unmined forest (○). Response variables included within CCA model: mean daily temperature (D.T_a), Daily temperature range (R.T_a), understorey vegetation density (% filled voxels; V_Us), overstorey vegetation density (% filled voxels; V_Os). The explanatory effect was only significant for daily temperature and overstorey density (permutation test, $P < 0.01$)
4.5 Discussion

By characterising site-level variation in vegetation density and thermal conditions, we were able to quantify the contributions of both these drivers on both CNH fecundity and nest construction, and the composition of the nesting community. We found that denser forest structures were associated with cooler microclimates, which, in turn, were associated with the patterns of nest abundance and nest construction within a matrix of cleared, mined, and restored land. Although elements of both vegetation structure and microclimate influenced the nesting community, at our sites, thermal conditions arose as the dominant driver of cavity-nesting Hymenoptera nest construction and fecundity. Female cavity-nesting bees in the NJF selected nesting sites with warmer microclimates, which occurred in early successional restoration that has previously been found to host the most diverse bee communities in the region (Chapter 3). Cavity-nesting wasps also selected for the warmer, early-successional restoration, however, wasps were found to be responsive to the emergent properties of overstory vegetation than microclimate.

4.5.1 Structural and microclimatic effects on reproductive ecology

The prevailing paradigm in hymenopteran nesting ecology is that the population dynamics of cavity nesters are primarily limited by the accessibility of nest sites and available cavities (Harmon-Threatt 2020), in accordance with the resource abundance hypothesis (Yamamoto, Yokoyama & Kawata 2007). In contrast to this, dense Jarrah Forest, which we inferred to provide greater natural availability of nesting resources, did not appear to promote nesting Hymenoptera. In fact, as vegetation density increased, there was a decrease in reproductive activity and nest construction at our experimental trap nests. Though understory vegetation density arose as a significant predictor of larval abundance, temperature played a greater role in explaining the observed trends.

Microclimatic differences were a strong correlate of vegetation density at our Jarrah Forest sites and temperature arose as the strongest predictor of both reproductive output and nest construction. There was an approximately 8.5°C increase in mean daily temperature between early and late stage restoration in the NJF, which is broadly consistent with reports from other forest communities (Lebrija-Trejos et al. 2011). The cooler microclimates within older stands likely result from increased shade and humidity and decreased solar radiation.
(Lebrija-Trejos et al. 2011), light transmittance (Angelini et al. 2015), and wind speed (Crall et al. 2020). The associations between vegetation structure and microclimate support recent arguments regarding the role of thermal biology in structuring the response of animals to changing landscapes and environmental conditions (Tuff, Tuff & Davies 2016). Such associations can be used to project habitat suitability estimates (Tomlinson 2020), and energy expenditure (Tomlinson et al. 2018b), across landscapes through topo-climatic and mechanistic modelling with the adaptability to encompass changing environmental conditions and diverse taxonomic foci.

4.5.2 Differential effects of habitat structure and microclimate on bees and wasps

Thermal conditions have a well-established role in organism fitness, reproductive output and species distribution limits in ectotherms (Huey & Berrigan 2001; Frazier, Huey & Berrigan 2006), so we expected that temperature might play a substantial role in the reproductive biology and nesting activity of cavity-nesting Hymenoptera in the Jarrah Forest. Our overarching results supported this expectation, and were also consistent with earlier reports of increased nesting activity in warmer microclimates by other Hymenoptera (Forrest & Chisholm 2017), Lepidoptera (Kührt, Samietz & Dorn 2005; Kührt, Samietz & Dorn 2006), Hemiptera (Schilman & Lazzari 2004) Orthoptera (Stahlschmidt & Adamo 2013), and Diptera (Fogleman 1979). However, this trend in our study was primarily driven by the response of bees, who dominated the nesting community, rather than wasps.

There are powerful potential physiological mechanisms that underpin the nest site selection of solitary bees as maternal nest-site selection should be under strong selection to optimise the development and fitness of progeny (Jaenike & Holt 1991). Within tolerance limits, warmer developmental conditions increase the rate of ontogenetic development (Ratte 1984; Sibly & Atkinson 1994; Radmacher & Strohm 2011), which means faster generation times, and potentially increased voltinism, in warmer microhabitats (Forrest, Cross & CaraDonna 2019). However, in some cases modifications to development rate and species voltinism may have multi-scale consequences through inducing phenological asynchrony with interacting species, within and among trophic levels (Kudo & Ida 2013; Damien & Tougeron 2019). However, it is equally plausible that site-selection results from constraints on the physiological performance of cavity-nesting adults. Ectotherm performance is limited by body temperature (Angilletta, Huey & Frazier 2010), and microclimatic conditions ultimately
constrain the realised body temperatures of insects (Sinclair et al. 2016) and their capacity to pursue life processes such as movement and foraging (Huey & Stevenson 2015). The thresholds of thermal tolerance can be varied, even between Hymenoptera from the same ecosystem, and can contribute to different patterns in seasonal emergence and reproductive activity (e.g. Tomlinson et al. 2015). Although we did not identify their precise thermal optima or tolerance thresholds, average daily temperatures above 33°C appear to be preferred by the dominant summer-flying species (e.g., Megachile aurifrons) nesting in the Jarrah Forest. As such, we propose that physiological mechanisms may underpin the processes that structure the populations and communities of these cavity-nesting insects in the NJF (McNab 2002; Bradshaw 2003), but the precise limits and pathways of these mechanisms, for example how they influence larval development or adult performance, remain to be described for these taxa.

In contrast to solitary bees, we found that the nesting ecology of wasps was not strongly related to microclimate, and was under greater influence of vegetation density, as has been reported for wasps in other systems (Schüepp et al. 2011; Coudrain, Herzog & Entling 2013; Rubene, Schroeder & Ranius 2015). Increased structural complexity and vegetation density may influence the populations structures of wasps through limiting visual and chemical cues necessary to detect and locate host or prey (Stoltz, Vinson & MacKinnon 1976). Unfavourable habitat structures have also been suggested to negatively impact wasp populations due to restricted dispersal rates and higher degrees of specialisation (Steffan-Dewenter 2002; Santoro et al. 2011), or reduced prey abundance (Chow 2000). However, support for these effects is equivocal (Coudrain, Herzog & Entling 2013), and is likely to be a result of complex and interacting effects that are species-specific and system-dependent (Forrest & Chisholm 2017). Moreover, the differences observed between the responses of bees and wasps to habitat structure and microclimate highlight the challenge in generalising such effects of environmental and land use change on different taxonomic groups (Ewers & Didham 2006).

4.5.3 Limitations of interpretation

We found that the CNH communities of the Jarrah Forest were less speciose and diverse than other communities where trap nests have been deployed (e.g. Murphy 2016), likely resulting from the specificity of cavity size and summer phenology. While it is also possible that our artificial nesting blocks were less attractive to the wider cavity-nesting community in more established forest because nest sites were not naturally limiting, the trends observed here
are consistent with earlier surveys that report on reduced hymenopteran abundance and richness in more established restoration and mature Jarrah Forest (Chapter 3). As such, we conclude that dense Jarrah Forest is less suitable to certain hymenopteran communities than more open habitat, despite the likelihood of increased nesting resource availability. However, the unique mosaic structure of the restored landscape has previously been suggested to strongly influence the dispersal of insect pollinators among the restored landscape (Chapter 3). Therefore, we advise caution in extrapolating to other systems which may feature greater habitat homogeneity or reduced habitat connectivity.

4.5.4 Conclusions and management implications

We have demonstrated that early-successional restoration provides an important habitat for nesting and reproduction of solitary bees, apparently due to the warmer microclimates that result from its open canopy structure. We anticipate nest site selection is largely driven by physiological traits of both adults and offspring, with warmer conditions promoting increased physiological performance, but advocate further research to quantify this potential mechanism. Given the association between open canopy structure and warmer nesting microclimates, the increased structural density that accumulates as restoration matures can cool the microclimate and present reproductive and physiological limitations to summer-flying CNH and other thermophilus insects. The ecological importance of open, early successional habitat patches is frequently overlooked and poorly represented within typical forest management regimes (Franklin et al. 2002), and within restoration planning, which often targets a representative reference community (Miller et al. 2017). Furthermore, if these dense habitats are sustained and homogenous at large scales they may substantially reduce the regional biodiversity of some insect pollinators, and creating canopy gaps or open areas within dense restoration may increase CNH abundance, population stability, distribution throughout the landscape (Mullally et al. 2019). It has previously been suggested that vegetation thinning may be a beneficial management strategy in these forests (Chapter 3), and we provide further support here that such a suggestion may direct forest management policy and practice towards sustaining a heterogenous landscape featuring a mix of successional stages and their biological legacies alongside old-growth forests.
4.6 References

Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.


Chapter 4: Cavity-Nesting Hymenoptera Nest-Site Selection


Murphy, M.V. (2016) Interactive effects of land-use change and rainfall decline on insect species networks. *Doctor of Philosophy, The University of Western Australia*.


CHAPTER FIVE

Interspecific variation in the thermal tolerance and performance of solitary bees and their parasitoid associate.

5.1 Abstract

Some of the most intimately linked biological interactions involve host-parasite systems. However, shifting temperatures, stemming from habitat or climate change, could disrupt these interactions due to dissimilar thermal tolerance and performance influencing the distribution and population biology of component species. To understand these dynamics, we modelled thermal tolerance and performance of metabolic rates of the congeneric host species *Megachile aurifrons* Smith, 1853 and *Megachile canifrons* Smith, 1853 (Hymenoptera: Megachilidae) and a parasitoid associate, *Gasteruption breviscutum* Kieffer, 1911 (Hymenoptera: Gasteruptiidae) between 20-45°C using fluorescence respirometry. The parasitoid wasp had a significantly higher maximum metabolic rate than the host bees which may correspond to faster developmental rates and population growth at temperatures optimal to the parasitoid. However, the host species both had a greater thermal performance breadth with upper thermal limits (CT_{max}) at least 4.3°C higher than the parasitoid. This raises the potential for host species to exploit ‘enemy-free space’ in microclimates that impart thermal stress to the parasitoid, providing a potential mechanistic explanation for nest-site selection by the bees. Collectively, the responses of both the parasitoid and host to different warming scenarios including clearing, habitat modification or climate change, may lead to range shifts, reduced abundance, shifted phenologies and decoupled multitrophic interactions if suitable thermal landscapes are not maintained.
5.2 Introduction

Highly fragmented landscapes threaten biodiversity and the effective management of ecosystems (Allen et al. 2018). Conservation and ecological restoration are essential to mitigate further losses, and ambitious recovery goals have been proposed for the next decade (Aronson et al. 2020; Waltham et al. 2020). Ecosystems, however, represent complex networks between species, and their environment (Cadenasso, Pickett & Grove 2006; Kuuluvainen 2009). Insight into the patterns and processes underpinning population and community dynamics provide a critical foundation for improving ecosystem-scale restoration initiatives (Palmer, Ambrose & Poff 1997; Miller et al. 2017; Chapter 1). However, quantifying the structural or compositional dynamics of populations and communities to environmental change is a key challenge because species can display markedly different responses to biotic (e.g., habitat structure and resource availability; Chapter 3 and 4) and abiotic (e.g., microclimate; Chapter 4) conditions.

Some of the most intimately linked biological interactions involve host-parasite systems, which can modulate community compositions and ecosystem functioning in both natural and modified landscapes (Godfray & Godfray 1994). Parasitoids can exert top-down control on trophic structure by regulating the abundance of their host species, which in turn, influence parasitoid population abundance and distribution (Walker & Jones 2001; Chidawanyika, Mudavanhu & Nyamukondiwa 2019). However, the mechanisms underlying the response of host-parasite interactions to changing environmental conditions are inherently complex (Brooks & Hoberg 2007), and governed by a suite of abiotic conditions such as temperature (Jeffs & Lewis 2013; Furlong & Zalucki 2017; Iltis et al. 2020), wind (Juillet 1964; Casas 1989) and barometric pressure (Crespo & Castelo 2012; Austin, Guglielmo & Moehring 2014). Among these, temperature is one of the most pervasive abiotic influences on organism physiology (Huey 1991; Angilletta Jr, Niewiarowski & Navas 2002), and is unequivocally recognised as a major factor driving host-parasitoid interactions (Hance et al. 2007; Le Lann et al. 2011; Jeffs & Lewis 2013). Characterising such responses and understanding species’ thermal requirements or tolerance thresholds can be invaluable to explaining community and population dynamics within and across thermal landscapes.

Thermal biology has an established lineage within ecological and comparative physiology (Angilletta Jr, Niewiarowski & Navas 2002; Somero 2011), and the thermal limits of organisms have recently gained renewed attention due to the mechanistic link between physiological traits and species responses to habitat modification and climate change.
Hosts and their parasitoids have intricately coupled life cycles, however, each species may have different thermal tolerances and respond differently to temperature (Gehman, Hall & Byers 2018). For example, parasitoids are often less thermally tolerant than their host species (Agosta, Joshi & Kester 2018; Machekano, Mvumi & Nyamukondiwa 2018; Mutamiswa, Chidawanyika & Nyamukondiwa 2018), and generally possess lower thermal optima (Topt; the temperature that coincides with peak performance; Tomlinson 2019) and critical thermal maximums (CTmax; where performance equals zero; Tomlinson 2019) when compared to their host species (reviewed in Furlong & Zalucki 2017). Additionally, increased specialisation tends to be more common at higher trophic levels (Araújo, Bolnick & Layman 2011). Therefore, parasitoids may also reflect ‘specialist’ (stenothermic) thermal performance curves, characterised by higher peak performances ($R_{\text{max}}$) at the cost of reduced performance breadth (Huey & Hertz 1984). If hosts and their associated parasitoids have substantially different thermal performance traits (e.g., CT$_{\text{max}}$, Topt and R$_{\text{max}}$), and different adaptive capacities, then divergence of their respective niches in response to environmental change may disrupt population dynamics, sustainability, and ultimately ecological functions (Brooks & Hoberg 2007; Franke et al. 2019). As higher trophic levels frequently exhibit higher degrees of specialisation, increasing their susceptibility to shifting population dynamics and environmental change (Sznajder & Harvey 2003; Araújo, Bolnick & Layman 2011), quantifying the thermal requirements of both the host and parasitoid species can help elucidate the impacts of changing temperatures at local and regional scales.

The aim of this study was to determine differences in habitat use by sympatric cavity-nesting Hymenoptera in a mosaic landscape of ecological restoration and remnant forest (Chapter 4). As such, we evaluated the physiological performance and thermal tolerance of two dominant Megachile Latreille, 1802 and a parasitoid associate, Gasteruption Latreille, 1776 from the Northern Jarrah Forest (NJF) in Western Australia’s south-western biodiversity hotspot (Gioia & Hopper 2017). More specifically, we sought to quantify interspecific differences in thermal performance between the larvae of these species, with the expectation that the parasitoid would have higher maximum performance than the hosts within conditions ideal to the parasitoid. However, we expected Gasteruption to have a lower thermal optimum and narrower thermal tolerance range, congruent with previous studies within host-parasitoid networks (Furlong & Zalucki 2017).
5.3 Methods

5.3.1 Study Species

*Megachile* is a large cosmopolitan genus of the Megachilidae, comprised of predominantly cavity-nesting bees that typically nest in pre-existing or naturally formed holes in wood (Paini 2004). We studied two species that have previously been reported to be common species in restoration habitats in the NJF (Chapter 4). *Megachile aurifrons* Smith, 1853, has an Australian wide distribution, and *M. canifrons* Smith, 1853 has a comparatively smaller geographic range limited to southern regions of Australia. Both species have distinct habitat preferences in the NJF, preferentially nesting in open, early successional habitat that are warmer than the surrounding forest (Chapter 4).

The Gasteruptiidae is a distinctive parasitoid wasp family comprised of two extant subfamilies, Gasteruptiinae and Hyptiogastrinae (Parslow, Schwarz & Stevens 2020a). The Gasteruptiinae feature four genera, of which *Gasteruption* is the most speciose (506 spp.) with a global distribution (Parslow, Schwarz & Stevens 2020b). Adults are commonly nectarivorous, however, it is likely at least some species also feed on pollen (Jennings & Austin 1994). The larvae are predator-inquilines that feed on both the larvae and provisions supplied by the host species (Parslow, Schwarz & Stevens 2020b). While the group parasitise a diverse array of species, cavity-nesting bees such as *Megachile* account for the majority of the host species records (Parslow, Schwarz & Stevens 2020b). As with the *Megachile* spp. that we studied, *Gasteruption* were often found parasitising bee nests in open, early-successional forest habitats, but their preferences are more complicated than those of the bees they parasitise and are not intrinsically related to microclimate (Chapter 4).

5.3.2 Species collection and rearing

We deployed twelve artificial nesting blocks (trap-nests), consisting of two independent Jarrah wood blocks drilled with 24 x 11 mm diameter holes to a depth of approximately 100 mm and filled with 10mm diameter paper straws (BioPak, Bondi Junction, Australia) attached to a 1.5 m wooden stake. Nesting posts were deployed in January and harvested after one month of nesting activity. The nesting straws were transported to the Kings Park Science laboratory (West Perth), where they were maintained in a 25°C incubator for six weeks prior to respirometry measurements (Thermoline Scientific, Wetherill Park, Australia). Prior to
respiration trials, pupal case morphotype and species identity were determined by X-ray imaging (MultiFocus, Faxitron Bioptics, Arizona, USA). Six species of cavity-nesting Hymenoptera were found within the nesting tubes, and identity of each species was confirmed by rearing several brood to adulthood and emergence (Chapter 4; Figure 4.2). All larvae included in respiration measurements were sampled from the same nesting period and measured during their final larval instar (prepupa with cocoon) to ensure minimal variation in larvae age.

5.3.3 Respiration measurement

We used a novel fluorescence respirometer (Q2 Technology; ASTEC Global; The Netherlands), following Tomlinson et al. (2018a) to measure oxygen (O2) consumption of the study species in a closed respirometry chamber (Figure 5.1). This technology works by exciting a fluorescent metal organic dye on the underside of the chamber lid, which becomes increasingly fluorescent in response to reduced O2-levels in the sealed chamber (ASTEC Global; Scafaro et al. 2017). The machine was programmed to conduct repeated measurements with a built in fluorometer, combining the theory behind a closed system respirometer with the capacity of a flow-through system to repeatedly sample the chamber atmosphere throughout a metabolic trial (Vleck 1987; Withers 2001; Tomlinson et al. 2018a). Larvae (identified by X-ray imagery) were extracted from nesting tubes and immediately placed in unsealed 500 μl vials (respirometry chambers) and exposed to a single temperature treatment, where partial pressures of O2 were measured every 10 minutes. The fluorometric O2-analyser was calibrated prior to each set of measurements (i.e., every 10 minutes) using two reference vials; one representing atmospheric O2-standards and the other an O2-depleted sodium dithionite solution (Scafaro et al. 2017). Forty-five M. aurifrons, 45 M. canifrons and 30 G. breviscutum were each subject to a single temperature treatment between 20°C and 45°C. Measurements were ceased upon a 20% decrease in O2 (i.e., 80% remaining), to avoid any substantial decreases in metabolic performance due to atmospheric hypoxia (Hoback & Stanley 2001; Harrison, Greenlee & Verberk 2018). Therefore, the average trial lasted just over two hours (2.29 ± 0.23 hours).
5.3.4 Statistical analysis

All statistical analyses were undertaken using the R statistical software in RStudio Version 1.2.5001 (R Development Core Team 2019) and data are presented as means ± standard errors unless otherwise stated. We tested for differences in the mass of *M. aurifrons*, *M. canifrons* and *G. breviscutum* larvae for all experimental temperatures using a general linear model followed by a Tukey post-hoc test (Abdi & Williams 2010). Data were visually inspected for normality and the assumptions of general linear models were verified. Where we

Figure 5.1: Diagram of the Q2 fluorescence respirometer and experimental layout afforded by the machine’s architecture. A single 500 μL vial served as a closed respirometry chamber for an individual cavity-nesting hymenopteran larva (a) that was placed within a 48-well measurement plate (b). Each row (1-3) was assigned a species while each column (A-F) represents a potential replicate measurement for each respective species. Following Tomlinson et al. (2018a), the fourth row was filled with ‘blank vials’ (B) to capture the effects of changing atmospheric pressures between repeated measures. Two vials left of plate indicate 0% oxygen (x) and atmospheric oxygen (o) that were used as calibration readings prior to measurement. The dashed line represents the recording sequence followed by the fluorometer. Plates were assigned one of four available temperatures (c). These were set at nominal intervals of ~2.5 °C between 20–48°C.
found significant differences in body mass, all subsequent metabolic responses were scaled by an exponent of 0.75 (Mb$^{0.75}$; mlO$_2$.g$^{-0.75}$.h$^{-1}$; Chown et al. 2007).

The thermal performance of *M. aurifrons*, *M. canifrons* and *G. breviscutum*, were characterised through a non-linear curve-fitting approach (Ritz & Streibig 2008). We compared three candidate models (see Table 5.1) previously reported to describe ectothermic thermal performance in biologically meaningful terms (e.g., thermal optima and critical thermal maxima; Tomlinson 2019). The three-parameter beta-distribution function (Yan & Hunt 1999) was determined as the model of best fit for both adult and larval responses by Akaike’s Information Criterion for small sample sizes (AICc; Burnham & Anderson 2002). The selected model was built into the *drc* package (Ritz & Streibig 2012) and fit to untransformed data to obtain the parameters describing the peak metabolic rate (R$_{max}$), thermal optimum (T$_{opt}$) and critical maximum temperature (CT$_{max}$) for all species. From the model estimates, T$_{pref}$ was estimated following equation 12.2 proposed in Tomlinson (2019), and performance breadth was calculated as the difference between T$_{pref}$ and T$_{opt}$. Pairwise comparisons were conducted between species regarding the parameter estimates (R$_{max}$, T$_{opt}$ and CT$_{max}$) via an approximate t-test of ratios using the ‘compParm’ function in the *drc* package (Ritz & Streibig 2012).

### 5.4 Results

#### 5.4.1 Parameter estimates

The T$_{opt}$ estimates for *M. aurifrons* and *M. canifrons* resolved at 36.6 ± 1.45°C and 33.5 ± 1.67°C, respectively, while the T$_{opt}$ estimates for the parasitoid wasp, *G. breviscutum*, was 33.6 ± 0.85°C. (Table 5.1, Figure 5.2). The upper critical thermal tolerance limits (CT$_{max}$) for *M. aurifrons* and *M. canifrons* were estimated at 49.0 ± 3.17°C and 46.4 ± 3.10°C, respectively, while the CT$_{max}$ for the parasitoid wasp, *G. breviscutum*, was estimated at 42.1 ± 1.77°C (Table 5.1, Figure 5.2). The R$_{max}$ for *M. aurifrons* and *M. canifrons* were estimated at 0.05 ± 0.004 mlO$_2$.g$^{-0.75}$.h$^{-1}$ and 0.04 ± 0.004 mlO$_2$.g$^{-0.75}$.h$^{-1}$, respectively, while the R$_{max}$ for the parasitoid wasp, *G. breviscutum*, was estimated at 0.06 ± 0.005 mlO$_2$.g$^{-0.75}$.h$^{-1}$ (Table 5.2, Figure 5.2).

#### 5.4.2 Parameter comparisons

*Megachile aurifrons* and *M. canifrons* shared statistically indistinguishable R$_{max}$ estimates ($t = -0.889$, $P = 0.376$; Table 5.3), however, R$_{max}$ was significantly higher for the
parasitoid *G. breviscutum*, compared to *M. aurifrons* ($t = -2.753, P = 0.007$; Table 5.3) and *M. canifrons* ($t = -3.842, p < 0.001$; Table 5.3). There were no statistically significant differences between $T_{opt}$ estimates for *M. aurifrons* and *M. canifrons* ($t = -1.442, P = 0.152$), nor between *G. breviscutum* and *M. canifrons* ($t = -0.064, P = 0.949$; Table 5.3), or *G. breviscutum* and *M. aurifrons* ($t = 1.718, P = 0.089$; Table 5.3). There were no statistically significant differences between *M. aurifrons* and *M. canifrons* larval $CT_{\text{max}}$ estimates ($t = -0.519, P = 0.605$; Table 5.3), nor between *G. breviscutum* and *M. canifrons* ($t = 0.966; P = 0.336$) or *G. breviscutum* and *M. aurifrons* ($t = 1.164, P = 0.071$; Table 5.3)
Table 5.1: Candidate model equations for the characterisation the thermal performance of cavity-nesting Hymenoptera. The beta-distribution function (Yan and Hunt, 1999) resolved the best fit for both global models and all other candidate models deviated more than six AIC units from this model.

<table>
<thead>
<tr>
<th>Eq</th>
<th>Non-linear Model</th>
<th>Reference</th>
<th>Equation</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bi-exponential model</td>
<td>(Logan et al. 1976;</td>
<td>[ VCO_2 = y_0 (e^{kt_a} - e^{-t_a - T_{opt}}) ]</td>
<td>-596.87</td>
</tr>
<tr>
<td></td>
<td>Tomlinson et al. 2015)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Exponentially modified logistic model</td>
<td>(Kovac et al. 2007;</td>
<td>[ VCO_2 = R_{min} + \left( \frac{b}{1 + e^{d(t_{opt}- T_a)}} \right) - e^{T_a - CT_{max}} ]</td>
<td>-602.68</td>
</tr>
<tr>
<td></td>
<td>Tomlinson 2019)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Beta-distribution model</td>
<td>(Yan &amp; Hunt 1999)</td>
<td>[ VCO_2 = R_{max} \left( \frac{ct_{max} - T_a}{CT_{max} - T_{opt}} \right) \left( \frac{t_a}{T_{opt}} \right)^{-ct_{opt}} ]</td>
<td>-608.76</td>
</tr>
</tbody>
</table>

- \( VCO_2 \) = Metabolic rate (rate of carbon dioxide production)
- \( y_0 \) = Intercept of the curve where \( T_a \) equates to 0
- \( k \) = Metabolic scaling exponent
- \( T_a \) = Experimental temperature
- \( T_{opt} \) = Thermal optimum where \( VCO_2 \) reaches its numerical peak (i.e., \( R_{max} \))
- \( R_{min} \) = Minimum metabolic rate
- \( R_{max} \) = Maximum metabolic rate, coincides with \( T_{opt} \).
- \( b \) = Difference between \( R_{max} \) and \( R_{min} \)
- \( CT_{max} \) = Upper thermal tolerance limit where performance intersects horizontal axis (i.e., 0)
Table 5.2: Mass and parameter estimates for the thermal performance and tolerance of cavity-nesting Hymenoptera in a host-parasitoid system. \( R_{\text{max}} \) (upper performance asymptote), \( T_{\text{opt}} \) (thermal optimum) and \( CT_{\text{max}} \) (critical thermal maximum), were quantified by fitting a beta-distributional model (Yan and Hunt, 1999), while \( T_{\text{pref}} \) (thermal preference) and performance breadth were calculated following Tomlinson (2019), and performance breadth was calculated as the difference between \( T_{\text{pref}} \) and \( T_{\text{opt}} \).

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Mass (mg)</th>
<th>( R_{\text{max}} )</th>
<th>( T_{\text{opt}} )</th>
<th>( CT_{\text{max}} )</th>
<th>( T_{\text{pref}} )</th>
<th>Breadth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Megachile aurifrons</em> (Host)</td>
<td>45</td>
<td>162.7 ± 0.01</td>
<td>0.05 ± 0.004</td>
<td>36.6 ± 1.45</td>
<td>48.9 ± 3.17</td>
<td>30.4</td>
<td>6.2</td>
</tr>
<tr>
<td><em>Megachile canifrons</em> (Host)</td>
<td>45</td>
<td>118.3 ± 0.01</td>
<td>0.04 ± 0.004</td>
<td>33.5 ± 1.67</td>
<td>46.4 ± 3.10</td>
<td>27.1</td>
<td>6.4</td>
</tr>
<tr>
<td><em>Gasteruption breviscutum</em> (Parasitoid)</td>
<td>30</td>
<td>166.2 ± 0.01</td>
<td>0.06 ± 0.005</td>
<td>33.6 ± 0.85</td>
<td>42.1 ± 1.77</td>
<td>29.4</td>
<td>4.2</td>
</tr>
</tbody>
</table>
Figure 5.2: Thermal performance curves modeled from the beta-distribution functions of allometrically corrected resting metabolic rates for three interacting species of a host-parasitoid system: *Megachile aurifrons* (solid line; ○), *M. canifrons* (dashed line; ▲) and *Gasteruption breviscutum* (dotted line; ■)
Chapter 5: Interspecific Physiological Variation

**Table 5.3:** Pairwise comparisons of parameters estimated from the three-parameter beta-distribution model (Eq 3; Table 5.1) for metabolic rate of cavity-nesting Hymenoptera hosts (*Megachile aurifrons* and *Megachile canifrons*) and parasitoid (*Gasteruption breviscutum*) at various constant temperatures. Asterisks indicate significance level as follows: ‘*’ P < 0.05; ‘**’ P < 0.01; ‘***’ P < 0.001.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Interspecific Comparison</th>
<th>Ratio</th>
<th>Std. Error</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{max}$</td>
<td>$M. canifrons / M. aurifrons$</td>
<td>0.892</td>
<td>0.121</td>
<td>-0.889</td>
<td>0.376</td>
</tr>
<tr>
<td></td>
<td>$M. canifrons / G. breviscutum$</td>
<td>0.661</td>
<td>0.088</td>
<td>-3.842</td>
<td>0.000 ***</td>
</tr>
<tr>
<td></td>
<td>$M. aurifrons / G. breviscutum$</td>
<td>0.741</td>
<td>0.094</td>
<td>-2.753</td>
<td>0.007 **</td>
</tr>
<tr>
<td>$T_{opt}$</td>
<td>$M. canifrons / M. aurifrons$</td>
<td>0.916</td>
<td>0.058</td>
<td>-1.442</td>
<td>0.152</td>
</tr>
<tr>
<td></td>
<td>$M. canifrons / G. breviscutum$</td>
<td>0.996</td>
<td>0.056</td>
<td>-0.064</td>
<td>0.949</td>
</tr>
<tr>
<td></td>
<td>$M. aurifrons / G. breviscutum$</td>
<td>1.088</td>
<td>0.051</td>
<td>1.718</td>
<td>0.089</td>
</tr>
<tr>
<td>$CT_{max}$</td>
<td>$M. canifrons / M. aurifrons$</td>
<td>0.947</td>
<td>0.102</td>
<td>-0.519</td>
<td>0.605</td>
</tr>
<tr>
<td></td>
<td>$M. canifrons / G. breviscutum$</td>
<td>1.102</td>
<td>0.106</td>
<td>0.966</td>
<td>0.336</td>
</tr>
<tr>
<td></td>
<td>$M. aurifrons / G. breviscutum$</td>
<td>1.164</td>
<td>0.090</td>
<td>1.822</td>
<td>0.071</td>
</tr>
</tbody>
</table>

### 5.5 Discussion

This study quantified the interspecific differences in thermal performance between the larvae of two dominant *Megachile* species and an associated parasitoid, *Gasteruption breviscutum* to provide a mechanistic basis for habitat selection across a mosaic of mixed-successional restoration (Chapter 2). Metabolic rates were characterised by asymmetrical, unimodal humped shaped curves, consistent with previously reported thermal performance responses of ectotherms (Angilletta Jr 2006). Despite expectations that there would be different thermal optima and maxima, $R_{max}$ was the only parameter to differ significantly between the larvae of three interacting species of a host-parasite network. However, the comparison between the breadth and shape of the performance curves supports the contention that “a jack of all temperatures is a master of none” (Huey & Hertz 1984), whereby cavity-nesting host bees possess wider, generalist performance, while the parasitoid possess narrower, specialist performance. Understanding the extent of offset between the thermal performance of hosts and their parasitoids can aid in the prediction of how such sympatric interacting species may respond to habitat, land-use or climate change.
5.5.1 Thermal performance and the pace of life

As far as we are aware, this is the first study to test for differences in metabolic rate between host insects and their parasitoid associates. As such, there are no direct comparisons for these data. However, increased metabolic scope is generally assumed to correlate with increased development rate and ‘pace of life’ (Pettersen, White & Marshall 2016). The relationship between organism growth and metabolic rate is often assumed to be both positive and causative (but see Martinez, Menze & Agosta 2017), and suggested to be driven by various mechanisms such as mitochondrial efficiency and enzyme function (Kingsolver & Woods 1997; Martinez, Menze & Agosta 2017). Therefore, it is likely that warmer microclimates (e.g., early successional forest) may facilitate accelerated development for the cavity-nesting Hymenoptera investigated here, especially in conditions where ambient temperatures remain close to $T_{opt}$, where $R_{max}$ is achieved for much of the day (Angilletta, Huey & Frazier 2010; Tomlinson 2019).

Considering the ~50% increase in $R_{max}$ from host to parasitoid, it is plausible that *G. breviscutum* may outperform *M. aurifrons* and *M. canifrons*, particularly in habitats that maintain temperatures approximating the thermal optima of the parasitoid. In this scenario, increased development, population growth and parasitism rates are likely for the parasitoid, assuming that the energetic requirements to complete such accelerated development are met (Altermatt 2010). While the maintenance of a higher metabolic rate may accelerate development, the energetic demand is also increased and the optimisation of nutrition is constrained by maternal behaviour and ability to locate and parasitise appropriate host nests (Monticelli et al. 2019). Consequently, trade-offs between thermal requirements and larval nutrition may occur if optimal thermal habitats do not support abundant host populations to parasitise. At an interspecific scale, however, the specific enzymatic and energetic pathways contributing to holometabolous maturation in insects are poorly understood (Folguera et al. 2010b). Therefore, further investigations into the biochemical and biophysical ecology of these taxa may deliver greater insights into the mechanisms underpinning the physiological variation observed here that can mediate nest-site selection and niche space.
5.5.2 Thermal tolerance

While increased metabolic activity may favour developmental rate in *Gasteruption*, their upper thermal limits are substantially lower than those of *Megachile* which may confer a potential competitive advantage to the heat-tolerant host species in warmer microclimates. Although the *Megachile* larvae develop more slowly, they are able to develop at a similar pace across a wider range of temperatures, increasing their resilience to landscape level changes in microclimate (Seebacher *et al.* 2015), assuming that metabolic rate is analogous to development rate (Gibert & De Jong 2001; Folguera *et al.* 2010a; Hyun *et al.* 2017). The parasitoid wasp could be more vulnerable to thermal shifts resulting from localised habitat change, or more broadly from global climate change, due to their reduced thermal tolerance compared to the host species. While the T\(_{\text{opt}}\) of *M. canifrons* aligns with that of *G. breviscutum* such that both species T\(_{\text{opt}}\) resolve at approximately 33.5°C, *M. aurifrons* is estimated to have a T\(_{\text{opt}}\) approximately 3°C higher than both other species while the CT\(_{\text{max}}\) of both *Megachile* resolved between 4.3-6.9°C higher than the parasitoid (Figure 5.2). Although these differences were not found to be statistically significant here, the magnitude of these differences in thermal tolerance are substantial, and almost certainly have a biological relevance that was not detected statistically. A low number of wasp larvae were available, and there was high variability in their individual metabolic responses, especially at high temperatures (approx. 500% at 37°C; Figure 5.2). It is likely the lack of statistical significance between T\(_{\text{opt}}\) and CT\(_{\text{max}}\) estimates were driven by the small sample sizes and the conservative nature of the pairwise comparisons test employed here (Ritz & Streibig 2008), and we are unconvinced that the thresholds reported are genuinely indistinguishable.

Generally, however, host species tend to reflect higher T\(_{\text{opt}}\) and CT\(_{\text{max}}\) estimates than their parasitoids (reviewed in Furlong & Zalucki 2017). For example, Lepidopteran hosts such as *Heliothis virescens* Fabricius, 1777, *Putella xylostella* Linnaeus, 1758 and *Spodoptera exigua* Hübner, 1808 have T\(_{\text{opt}}\) estimates between 4–10°C warmer than their parasitoids *Trichogramma acacioi* Brun, Gomez de Moraes & Soares 1984, *Diadegema semiclausum* Hellén, 1949 and *Microplitis manila* Bouché, 1834, respectively (Gulzar *et al.* 2012; Qiu *et al.* 2012; Ngowi *et al.* 2017), while their CT\(_{\text{max}}\) estimates ranged between 2–8°C higher. The data presented here are broadly consistent with previous findings, and we suspect that the thermal performance niches of host and parasitoid are indeed different, and the sample size failed to provide the statistical power to conclusively identify the difference in thermal tolerance. As such, it is likely that even non-statistically significant differences in thermal performance could
have substantial biological relevance, particularly in the face rapid environmental change or long-term climate change, and general deviations in thermal profiles should not be ignored.

5.5.3 Generalist-specialist trade-off

There is often a trade-off between maximum metabolic performance and performance breadth (but see Huey & Hertz 1984). Specialists perform better over a narrower suite of conditions at the cost of tolerance, while generalists operate well across a broad suite of conditions at the cost of maximal performance. Our interspecific comparisons suggest a higher degree of thermal specialisation within the parasitoid wasp when compared to the host species. Generally, performance declines more precipitously at temperatures beyond the $T_{opt}$ in a thermal specialist than in generalists (Levins 1968; Huey & Hertz 1984). As such, a broader window of thermal tolerance by *Megachile spp.* may allow for compensatory behaviour to reduce nest parasitism through the selection of warmer nesting sites (i.e., enemy-free space hypothesis; Hirayama & Kasuya 2010; Sadek, Hansson & Anderson 2010).

Early-successional restoration sites, where over 50% of all *M. aurifrons* nests were constructed (Chapter 4), accumulated an average of 17.9 ± 1.3 days above this 38.5°C threshold, according to microclimatic data recorded there in between December 2019 and April 2020 (Chapter 4; Figure A2.1). Additionally, these open canopy sites accumulated an average of 17.9 ± 2.8 days within *M. aurifrons* performance breadth (30.4−36.6°C; Figure A3.1a) while only exceeding the tolerance threshold of 49°C for a cumulative average of 4.6 ± 3.3 days (Appendix 2; Figure A3.1b). Comparatively, the early-successional sites were less suitable to the parasitoids and only presented a cumulative average of 11.3 ± 1.2 days within the performance breadth temperatures (29.4−33.6°C; Figure A3.1a) while exceeding the tolerance thresholds for 11.5 ± 2.7 days (Figure A3.1b). Moreover, the declining performance function of *G. breviscutum* intersects that of *M. aurifrons* at 38.5°C where *G. breviscutum* has already experienced a 27.5% decrease in metabolic performance (Figure 5.2), and early successional restoration supports thermal conditions that favour *M. aurifrons* up to four times as often as other site ages (Figure A3.1c). Megachilid hosts may have a substantial competitive advantage over the parasitoid within such conditions given that the parasitoid is more likely to experience thermal stress that may terminally compromise larval development (Sibly & Atkinson 1994; Moiroux, Brodeur & Boivin 2014; Martinez, Menze & Agosta 2017; Chidawanyika, Mudavanhu & Nyamukondiwa 2019). Therefore, these calculations suggest that one likely
driver of *M. aurifrons* preference to nest in warm, exposed microclimates in the NJF is the pursuit of enemy-free space where host population growth and fitness is likely to greatly exceed that of the parasitoid.

### 5.5.4 Implications for restoration and landscape management

The findings from this study have strong implications for restoration efforts in the NJF, where there are several restoration sites of varying successional ages. Higher abundances of cavity-nesting Hymenoptera were found in early successional restoration sites that are hotter, and a decrease in their abundance as sites establish and become cooler due to increased canopy cover (Chapter 4). While nesting in warmer microclimates may be more advantageous to the host species, the habitat patches currently utilised may also become less suitable as vegetation establishes over time without management intervention. Consequently, establishing vegetation may cause the dislocation of host and parasitoid distributions, shifts in generational development times and emergence, and ultimately, trophic cascades (Agosta, Joshi & Kester 2018). As the study species play important ecological roles as pollinators and parasitoids, possible management techniques such as canopy thinning may serve to increase microclimatic heterogeneity within dense, closed canopy restoration and increase habitat suitability for these species (Taki *et al.* 2010; Odanaka *et al.* 2020). Additionally, ensuring high degrees of habitat connectivity can allow for movement and dispersal between sites of different restoration ages and between restored and unmined forest. (Knop, Herzog & Schmid 2011; Tomlinson *et al.* 2018b). Niche envelope modelling at high spatio-temporal resolution can help practitioners predict likely responses of such species to environmental change and could provide mechanistic insight into the consequences of proposed management actions (Tomlinson *et al.* 2018b; Tomlinson 2020). However, the metabolic and thermal performance data alone cannot offer enough insight into specific spatial distribution patterns. Therefore, empirical data on habitat selection and use, fitness, and physiology can be coupled with various environmental or climatic scenarios to gain insight into habitat suitability under changing conditions.

### 5.5.5 Limitations to interpretation

Beyond the potential statistical limitations that we have already identified, the greatest limit to our interpretation here is a lack of comparative context as studies comparing sympatric
species are underrepresented in the literature, arguably due to the preconception of a lower likelihood of discerning major physiological differences (Garland Jr & Adolph 1994). As noted, this is potentially the first study that compares the maximum metabolic performance of host and parasitoid larvae and therefore, the prevalence of dissimilar maximal metabolic performance traits between hosts and parasitoids cannot be generalised. While adaptation may be at least partly responsible for the physiological variation observed between the examined species, phylogenetic independence of these responses cannot be assumed (Stone, Nee & Felsenstein 2011), particularly between *M. aurifrons* and *M. canifrons* owing to phylogenetic resemblance. Therefore, it is not possible to attribute the differences described to either phylogeny or adaptation to environmental conditions. Comparisons between populations may provide greater inference into macroevolutionary processes (Garland Jr & Adolph 1991; Garland Jr, Harvey & Ives 1992; Angilletta Jr, Niewiarowski & Navas 2002). However, the precise mechanisms underpinning the variation between host and parasitoid larvae are unknown and the specific genetic, enzymatic, and energetic pathways contributing differences in thermal performance cannot be established here and further studies are necessary to provide appropriate physiological context for this work.

**5.5.6 Conclusions**

*Megachile* host larvae appear to possess a more ‘generalist’ thermal performance with a higher thermal tolerance and wider breadth compared to the *Gasteruption* parasitoid larvae that possess a more ‘specialist’ thermal performance. This study highlights the potential competitive advantages associated with being either a thermal generalist (i.e., increased tolerance thresholds) or a specialist (i.e., increased metabolic performance). In an ecological context, we feel that these differences in the thermal tolerance of the larvae may substantially explain the nesting behavior of the *Megachile*, because seeking warmer microhabitats should allow their larvae to develop at near-peak efficiency for most of the summer while also substantially impairing their parasitoids. Combined, the responses of both the parasitoid and host to different warming scenarios including clearing, habitat modification or climate change, may lead to range shifts, reduced abundance, shifted phenologies and decoupled multitrophic interactions. However, in the context of Jarrah Forest restoration, management techniques such as canopy thinning may serve to maximise microclimatic heterogeneity and promote the stability and sustenance of these intricate trophic interactions.
5.6 References

Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.


Chapter 5: Interspecific Physiological Variation


Chapter 5: Interspecific Physiological Variation


Chapter 5: Interspecific Physiological Variation


CHAPTER SIX

Ontogenetic variation of the physiological performance and thermal tolerance within *Megachile aurifrons* (Hymenoptera: Megachilidae).

6.1 Abstract

Temperature plays a major role in many aspects of insect biology and ecology such as metabolism, growth, movement, and reproduction. The impact of temperature varies strongly with age, especially in holometabolous insects with discrete developmental stages. Understanding this variation is particularly important for informing restoration initiatives directed towards minimising demographic bottlenecks and localised species extirpation. In this context, this study aimed to gain a mechanistic insight into the role of temperature in driving nest-site selection across a mixed-successional forest restoration landscape. Here, we examined the ontogenetic variation of thermal tolerance and performance of adults and larvae of our model species, *Megachile aurifrons* Smith, 1853 (Hymenoptera: Megachilidae), by measuring standard metabolic rate using fluorescence respirometry. Adult *M. aurifrons* possessed a thermal optimum (T_{opt}) ~8°C higher than larvae and showed 52-fold increase in maximum metabolic rate (R_{max}) but displayed no statistically significant differences in upper thermal tolerance limits (CT_{max}) than their larvae. The preference for, and selection of, early-successional restoration sites for oviposition maximises the performance of both offspring and maternal adults, however, the cooler microclimates of closed-canopy forest substantially limit adult performance, providing one explanation for the reduced abundance and fecundity of these pollinators in late-successional and unmined Northern Jarrah Forest (NJF). If a tight mosaic of mixed successional forest facilitating an heterogenous thermal landscape is not maintained, the nesting and foraging behavior by *M. aurifrons* may be reduced, thus limiting its potential as a pollination vector.
6.2 Introduction

The ecological niche is a multivariate space that represents the biotic and abiotic conditions for persistence of any given species and is key for understanding habitat selection and distributions (Grinnell 1917; Hutchinson & MacArthur 1959; Tracy & Christian 1986). Thermal conditions form a critical dimension of a species’ ecological niche, playing a central role in the regulation of physiological processes such as respiration, metabolism and growth (Huey & Berrigan 2001; Angilletta Jr & Angilletta 2009). Consequently, thermal effects can influence functional and behavioural traits such as locomotion and reproduction, and translate broadly into large-scale ecological processes such as dispersal, selection and adaptation (Huey & Berrigan 2001; Peck et al. 2009). The effects of temperature across all biological levels make it one of the most pervasive abiotic influences on terrestrial ecosystems and species persistence will depend on the ability of individuals to respond to changing thermal conditions (Williams et al. 2008; Berg et al. 2010; Somero 2010). Therefore, understanding species tolerance and vulnerability to temperature is one of the most pressing issues in contemporary ecology in the face of rapid habitat and climate change.

The field of biophysical ecology applies thermodynamic principles to inform mechanistic models of physiological processes (Porter & Gates 1969; Gates 1980; Tracy 1982), essentially seeking to quantify the relationship and interaction between an organism and its environment. Interspecific comparisons are common in biophysical ecology and related disciplines such as functional morphology and comparative physiology (Withers 1992; Carey 2005), often comparing the different correlates between species-specific physiological traits (e.g., thermal and drought tolerance) and abiotic conditions (e.g., temperature and moisture availability). While there is undeniable value in interspecific comparisons of metabolic rate, there are also substantial insights to be gained by comparing individuals of the same species at different stages of life history, especially in understanding the changing niches of holometabolous species (Chown, Chown & Nicolson 2004).

Intraspecific variation in response to thermal stress can differentially impact the survivorship of specific developmental stages (Arias, Poupin & Lardies 2011; Alonso, Salgado & Palacín 2016), each of which clearly influences the adaptiveness of the species as a whole. The differences in thermal tolerance associated with ontogenies, ageing and senescence are well recognised, however, the intraspecific variation driven by these factors are often overlooked within comparative insect physiology (Chown, Gaston & Robinson 2004; Bowler
Ontogenetic physiological variation can be critical for holometabolous insects, particularly those with sessile juvenile stages that cannot behaviourally thermoregulate or seek refuge like mobile adults. Therefore, their survival relies entirely on the reproductive decisions of the maternal insects (Folguera et al. 2010a). Despite the critical role of temperature and thermal tolerance in constraining a species’ fundamental and realised niches, ontogenetic variation is rarely integrated into biophysical frameworks of climate or habitat change impacts (Kingsolver et al. 2011).

Comparisons of physiological traits can be explored to understand distributions and potential responses to changing environments (Gaston et al. 2009; Wilczek et al. 2010). Habitat modification and land-use change cause localised changes in microclimate and are two of the major drivers of biodiversity loss (Foley et al. 2005). Physiological traits also have ecological implications in this context as distinct life history stages may be differentially equipped to deal with the thermo-energetic consequences of modified environments (De Chazal & Rounsevell 2009; Tran et al. 2017). There have been calls to incorporate thermal biology into studies of fragmentation and ecological restoration (Tuff, Tuff & Davies 2016), especially of critical functional groups like insect pollinators (Heinrich 1975; Abrol 2005; McCallum, McDougall & Seymour 2013), though very few studies have done so to date (Chapter 2). This study aims to compare the ontogenetic physiological variation of a dominant cavity-nesting species within the Northern Jarrah Forest (NJF), *Megachile aurifrons* Smith, 1853 (Hymenoptera: Megachilidae). This species is a key insect pollinator, and provides a broad range of pollination services, as well as potentially being important in some specialist pollination symbioses (Scaccabarozzi et al. 2020). *Megachile aurifrons* has a holometabolous life cycle where larval and pupal development occurs within pre-existing or naturally formed holes in wood (Paini 2004). The adults have an exclusive summer-flying phenology while megachilid brood often enter an overwintering diapause as prepupae (Tepedino & Parker 1988; Paini 2004; Yocum et al. 2006), potentially imparting a broader range of thermal conditions.

Using *M. aurifrons* as a model species, this study explores how the developmental shifts in the thermal biology of key insect pollinators may contribute to their nest-site selection. To quantify this, thermal performance functions were constructed for female adults and larvae and compared physiological performance and thermal tolerance traits with the expectation that larvae would have a wider performance breadth, due to their developmental phenology and immobile nature, while emergent adults would have a narrower performance breadth than larvae due to their capacity to behaviourally thermoregulate (Ma et al. 2018). It was also
expected that adults would possess significantly higher metabolic performance ($R_{\text{max}}$), thermal optimum ($T_{\text{opt}}$) and critical thermal tolerance ($CT_{\text{max}}$) than larvae, stemming primarily from the energetic and thermal requirements associated with the maintenance and activation of flight muscle (Beenakkers, Van der Horst & Van Marrewijk 1984). By quantifying thermal performance of metabolic rate, rather than other performance traits like locomotory efficiency (Kellermann et al. 2019), we can also infer some of the consequences of temperature on the energetics of $M. \text{aurifrons}$ development and gain insight into the suitability of nesting sites based on their thermal profiles while providing mechanistic interpretations of nest-site selection previously reported for the species (Chapter 4)

### 6.3 Methods

#### 6.3.1 Species collection and rearing

We collected the experimental population from the NJF in the southwest of Western Australia by installing twelve artificial nesting blocks (trap-nests). These consisted of two independent Jarrah wood blocks drilled with 24 x 11 mm diameter holes to a depth of approximately 100 mm and filled with 10 mm diameter paper straws (BioPak, Bondi Junction, Australia) attached to a 1.5 m wooden stake. Nesting straws were collected monthly between November 2019 and April 2020 to encompass the nesting phenology of $M. \text{aurifrons}$, and the nesting straws were transported to the Kings Park Science laboratory (West Perth), where they were stored in a 25°C incubator (Thermoline Scientific, Wetherill Park, Australia) for six weeks prior to respirometry measurements.

#### 6.3.2 Respirometry measurements

Fluorescence respirometry was used to repeatedly measure oxygen ($O_2$) consumption of individual cavity-nesting Hymenoptera larvae or adults in a closed chamber using $Q_2$ Technology (ASTEC Global, The Netherlands), following Tomlinson et al. (2018a). This technology works by exciting a fluorescent metal organic dye on the underside of the chamber lid, which becomes increasingly fluorescent in response to reduced $O_2$-levels in the sealed chamber (ASTEC Global; Scafaro et al. 2017). The machine was programmed to conduct repeated measurements with a built in fluorometer, combining the theory behind a closed
system respirometer with the capacity of a flow-through system to repeatedly sample the chamber atmosphere throughout a metabolic trial (Vleck 1987; Withers 2001; Tomlinson et al. 2018a). This means that real-time measurements of pO₂ can be quantified over long trial periods in an otherwise stable atmosphere without disturbing either the test subjects, or the experimental atmosphere.

Forty-five *M. aurifrons* larvae were extracted from nesting tubes, weighed, and immediately introduced into 500 µl vials (metabolic chambers) and exposed to a single temperature treatment between 20°C and 45°C (Figure 5.1). Within a week of emergence, respirometry trials were conducted on individual adults that were supplied a diet of honey diluted with water at a 1:2 ratio. Forty-five females were subject to a single temperature treatment between 20°C and 46°C. Experimental temperatures were maintained within ±1°C for the entire duration of the respirometry trial. We did not test experimental temperatures below 20°C because the Q₂ Technology is equipped with a heater, but not a cooling system, and cannot produce experimental atmospheres cooler than ambient conditions.

Repeated measurements were obtained through the automated data collection algorithm that measures the partial pressure of O₂ in a standard-predetermined sequence at user-defined intervals (ASTEC Global; Scafaro et al. 2017); in our case every two minutes for adults, and every 10 minutes for larvae. To avoid potential influences of hypoxia or hypercapnia on the physiological state of the subjects (Hoback & Stanley 2001; Harrison, Greenlee & Verberk 2018), measurements were ceased upon a 20% decrease in O₂ (i.e. 80% remaining). The average exposure period to each experimental temperature was approximately two hours for larvae (1.88 ± 0.28 h), 30 minutes for females (0.51 ± 0.07 h) and 42 minutes for males (0.69 ± 0.16 h).

6.3.3 Statistical analysis

We tested for differences in the mass of *M. aurifrons* adult females and larvae for all experimental temperatures using a general linear model followed by a Tukey post-hoc test (Abdi & Williams 2010). Data were visually inspected for normality through graphical analysis and the assumptions of general linear models were verified. All subsequent metabolic responses were allometrically corrected by a mass-specific scaling exponent of $M_b^{0.75}$ (ml.CO₂.g⁻¹.h⁻¹; Chown et al. 2007). Measured metabolic rates were standardised to a
common temperature of 25°C using a $Q_{10}$ factor of 2.5. Adults and larvae were compared to the allometric regression of insect metabolic rates reported by Chown et al. (2007) and Maino and Kearney (2014), respectively, and statistically compared to allometric expectations using paired students $t$-tests (Zar 1999).

The thermal performance of metabolic rates of *M. aurifrons* larvae and adult females, were characterised through a non-linear curve-fitting approach. We compared three candidate models (Table 6.1) that previously have been suggested as descriptions of ectothermic thermal performance for their biologically meaningful parameter estimates (e.g., thermal optima and critical thermal maxima; Tomlinson 2019). The three-parameter beta-distribution function (Yan & Hunt 1999) was determined as the model of best fit for both adult and larvae responses by Akaike’s Information Criterion for small sample sizes, using the *thermperf* package (Bruneaux 2017). The selected model was built into the *drc* package (Ritz & Streibig 2012) and the data subject to a boxcox transformation ($\lambda = -0.01$; CI: -0.022, 0.041). From the quantified $R_{\max}$, $T_{\text{opt}}$ and $CT_{\max}$ model estimates, $T_{\text{pref}}$ was estimated following equation 12.2 proposed in Tomlinson (2019), and performance breadth was calculated as the difference between $T_{\text{pref}}$ and $T_{\text{opt}}$. Pairwise comparisons of the parameter estimates ($R_{\max}$, $T_{\text{opt}}$ and $CT_{\max}$) were conducted between developmental stages via an approximate $t$-test using the `compParm` function in the *drc* package (Ritz & Streibig 2012). All statistical analyses were undertaken using R statistical software in RStudio Version 1.2.5001 (R Development Core Team 2019) and presented as means ± standard errors unless otherwise stated.
Table 6.1: Candidate model equations examined for use in the characterisation the thermal performance of adult (A) and larvae (L) *Megachile aurifrons*. The beta-distribution function (Yan and Hunt, 1999) emerged as the best fit for both global models and was selected for further analysis.

<table>
<thead>
<tr>
<th>Eq</th>
<th>Model</th>
<th>Reference</th>
<th>Equation</th>
<th>AIC (A, L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bi-exponential function</td>
<td>(Logan et al. 1976; Tomlinson et al. 2015)</td>
<td>$VCO_2 = y_0 (e^{k(T_a - T_{opt})}$</td>
<td>175.7, -228.2</td>
</tr>
<tr>
<td>2</td>
<td>Exponentially modified logistic function</td>
<td>(Kovac et al. 2007; Tomlinson 2019)</td>
<td>$VCO_2 = R_{min} + \left( \frac{b}{1 + e^a(T_{so} - T_a)} \right) - e^{T_a - CT_{max}}$</td>
<td>178.8, -226.6</td>
</tr>
<tr>
<td>3</td>
<td>Beta-Distribution function</td>
<td>(Yan &amp; Hunt 1999)</td>
<td>$VCO_2 = R_{max} \left( \frac{CT_{max} - T_{opt}}{CT_{max} - T_{opt}} \right) \frac{T_a}{T_{opt}} - CT_{max} - T_{opt}$</td>
<td>175.0, -232.2</td>
</tr>
</tbody>
</table>

$VCO_2$ = Metabolic rate (rate of carbon dioxide production)

$y_0$ = Intercept of the curve where $T_a$ equates to 0

$k$ = Metabolic scaling exponent

$T_a$ = Experimental temperature

$T_{opt}$ = Thermal optimum where $VCO_2$ reaches its numerical peak (i.e., $R_{max}$)

$R_{min}$ = Minimum metabolic rate

$R_{max}$ = Maximum metabolic rate, coincides with $T_{opt}$.

$b$ = Difference between $R_{max}$ and $R_{min}$

$CT_{max}$ = Upper thermal tolerance limit where performance intersects horizontal axis (i.e., 0)
6.4 Results

6.4.1 Body mass and allometric comparisons

Larval *M. aurifrons* (162.7 ± 0.01 mg) were heavier than adults (117.1 ± 0.01 mg; $F_{1,88} = 28.23$, $P < 0.001$). Adult *M. aurifrons* conformed to allometric expectations reported by Chown *et al.* (2007) ($F_{1,389} = 1811.12$, $R^2 = 0.82$; $P < 0.001$, Figure 6.1a). There was no significant difference in the mass-specific metabolic rates that were predicted by Chown *et al.* (2007) and observed mass-specific metabolic rate of females ($t_{44} = -1.217$, $P = 0.229817$), that represented 109.7% of the allometric prediction. However, the metabolic rate of larvae was significantly lower than the allometric expectations of Chown *et al.* (2007) for adult insects ($t_{44} = 26.302$, $P < 0.001$), representing only 4.7% of the metabolic rate of a similarly sized adult. When compared to allometric predictions of the metabolic rates of larval *M. aurifrons* (Maino & Kearney 2014), however, there were no significant differences between the observed and expected mass-specific metabolic rates of larval ($t_{44} = 1.526$, $P = 0.1342$; Figure 6.1b).

6.4.2 Thermal performance and tolerance comparisons

Adults had significantly higher $R_{\text{max}}$ estimates ($2.11 ± 0.21 \text{ mLO}_2.\text{g}^{-0.75}.\text{h}^{-1}$; Table 6.2; Figure 6.2) compared to larvae ($0.04 ± 0.01 \text{ mLO}_2.\text{g}^{-0.75}.\text{h}^{-1}$; $t_{84} = 7.179$, $P < 0.001$; Table 6.3). Thermal optimum ($T_{\text{opt}}$) estimates were also significantly higher for adults (43.81 ± 3.12°C; Table 2; Figure 6.2) than for larvae (34.84 ± 0.92°C; $t_{84} = 2.445$, $P = 0.016$; Table 6.3). $CT_{\text{max}}$ estimates for larvae (48.15 ± 1.57°C; Table 6.2, Figure 6.2) and adults (53.84 ± 5.22°C; Table 6.2, Figure 6.2) were statistically indistinguishable ($t_{84} = 1.043$, $P = 0.300$; Table 6.3).
Figure 6.1: Relationship between log$_{10}$ scaled mass (g) and metabolic rate (μW) $Q_{10}$ corrected to 25°C for a) adult *Megachile aurifrons* females (●) plotted against 391 other insect taxa (○) compiled from Chown *et al.* (2007) and; b) *M. aurifrons* larvae (♦) plotted against metabolic data across 25 other species during larval development (○) assimilated from Maino and Kearney (2014). Both developmental stages scaled as expected against their respective interspecific allometries though larvae had significantly lower mass-specific metabolic rates compared to adults. Solid lines represent the interspecific allometries for insect adults (a) and larvae (b), and dashed lines represent 95% confidence intervals.
Table 6.2: Mass and thermal performance estimates of *Megachile aurifrons* adults and larvae. Parameter estimates $R_{\text{max}}$ (upper performance asymptote), $T_{\text{opt}}$ (thermal optimum) and $C_{\text{Tmax}}$ (critical thermal maximum), were estimated by fitting a beta-distributional model (Yan and Hunt, 1999), while $T_{\text{pref}}$ (thermal preference) was calculated following Tomlinson (2019) equation 12.2, and performance breadth was calculated as the difference between $T_{\text{pref}}$ and $T_{\text{opt}}$.

<table>
<thead>
<tr>
<th>Life-Stage</th>
<th>n</th>
<th>Mass (mg)</th>
<th>$R_{\text{max}}$</th>
<th>$T_{\text{opt}}$ (°C)</th>
<th>$C_{\text{Tmax}}$ (°C)</th>
<th>$T_{\text{pref}}$ (°C)</th>
<th>Breadth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult (♀)</td>
<td>45</td>
<td>117.1 ± 0.01</td>
<td>2.11 ± 0.214</td>
<td>43.8 ± 3.24</td>
<td>53.8 ± 5.22</td>
<td>38.8</td>
<td>5.02</td>
</tr>
<tr>
<td>Larvae</td>
<td>45</td>
<td>162.7 ± 0.01</td>
<td>0.04 ± 0.004</td>
<td>35.2 ± 1.08</td>
<td>48.1 ± 1.57</td>
<td>28.7</td>
<td>6.45</td>
</tr>
</tbody>
</table>

Table 6.3: Pairwise comparisons of parameters estimated from the three-parameter beta-distribution model (Eq 3 in Table 6.1) for metabolic rate of *Megachile aurifrons* at various constant temperatures. Asterisks indicate significance level as follows: ‘∗’ $P < 0.05$; ‘∗∗’ $P < 0.01$; ‘∗∗∗’ $P < 0.001$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comparison</th>
<th>Ratio</th>
<th>Std. Error</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\text{max}}$</td>
<td>Adult Female / Larvae</td>
<td>52.270</td>
<td>7.142</td>
<td>7.179</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>$T_{\text{opt}}$</td>
<td>Adult Female / Larvae</td>
<td>1.244</td>
<td>0.100</td>
<td>2.455</td>
<td>0.016 *</td>
</tr>
<tr>
<td>$C_{\text{Tmax}}$</td>
<td>Adult Female / Larvae</td>
<td>1.119</td>
<td>0.114</td>
<td>1.043</td>
<td>0.300</td>
</tr>
</tbody>
</table>
Figure 6.2: Thermal performance curves modeled from the beta-distribution function (Eq 3 in Table 6.1) of allometrically corrected resting metabolic rates for female adult (dotted line; ▲) and larval (solid line; ○) *Megachile aurifrons*. Note the two different vertical scales pertaining to adults and larvae respectively.
6.5 Discussion

Although there is a broad and expanding literature on the interspecific variations in insect thermal performance (Chown, Addo-Bediako & Gaston 2002; Chown et al. 2007; Hoffmann, Chown & Clusella-Trullas 2013), far less is known about the intraspecific, intersexual or ontogenetic variation of thermal performance, or the underlying environmental correlates (but see Folguera et al. 2010a; Maino & Kearney 2014). This study represents the first comparative assessment of ontogenetic physiological variation in the Australian endemic bee, *Megachile aurifrons*. All of the measured metabolic rates followed asymmetrical, unimodal hump shaped curves, as expected for the thermal performance of ectothermic species (Angilletta Jr 2006). Larvae had significantly lower metabolic rates than adult bees, even after accounting for their mass differences while the critical thresholds of metabolic performance appear to shift to higher temperatures with development as adults had a $T_{\text{opt}}$ and $CT_{\text{max}}$ estimates 8.6°C and 5.7°C higher than larvae, respectively.

6.5.1 Thermal performance comparisons

It is well established that body mass plays an integral role in modulating metabolic responses across a broad suite of taxa (Brown et al. 2004; Price et al. 2012). Adult *M. aurifrons* adhere to the allometric relationship for adult insects reported by (Chown et al. 2007). While larval metabolic rates were substantially lower than the allometric expectations for adults, they conform to the interspecific allometric relationship for insect larvae presented by Maino and Kearney (2014). These allometric comparisons suggest two things: first, that the measurements using the $Q_2$ technology, which has not been used to measure insect metabolic rates prior to our study, are consistent with the extant literature; second, they highlight an ontogenetic increase in metabolic rate, where adults generally have substantially higher energetic expenditure for their mass at a standard temperature of 25°C.

There was a 52-fold increase in $R_{\text{max}}$ between prepupal larvae of *M. aurifrons* and female adults. This conforms to expectation that larvae will have significantly lower metabolic rates compared to adults as insects follow a U-shaped trajectory in metabolic rates as they develop, where pre-pupal stages generally reflect the lowest point on the curve (Melampy & Willis 1939; Schmolz, Kősece & Lamprecht 2005; Maino & Kearney 2014). The increase in metabolic rate is likely to result from changes in metabolically active tissue, energy demand and oxidative capacity between developmental stages arising from morphological and
physiological reorganisation (Hoekstra et al. 2018). The rapid decline in metabolic rate during larval development is likely to be the product of histolysis and autophagy of larval tissues (Merkey et al. 2011), and substantial remodelling of the tracheal system. However, the increase in metabolic rate associated with later stages of development has been attributed to morphogenesis and the construction of adult appendages during metamorphosis (Odell, 1998), and terminal differentiation of metabolically active flight muscle prior to eclosion (Merkey et al. 2011). However, the maintenance and activation of mitochondria-rich flight muscle is likely to account for the majority of the energetic budget for adult bees (Suarez 2000). Additionally, species with complex life-histories and developmental stages often occur across markedly different conditions as the larvae and adults emerge in different seasons, and a growing body of literature points towards stage-specific physiological adaptations pertaining to specific seasonal climates (i.e., the beneficial acclimation hypothesis; Leroi, Bennett & Lenski 1994; Angilletta Jr & Angilletta 2009). However, empirical data to support functional and mechanistic explanations for ontogenetic variation in thermal performance are remarkably limited and further research is necessary to quantify these dynamics in M. aurifrons and across ectotherms more broadly.

6.5.2 Thermal tolerance comparisons

There is increasing evidence for ontogenetic variation in thermal performance and tolerance thresholds of insects (Folguera et al. 2010b; Kingsolver & Buckley 2020). Adult $T_{\text{opt}}$ estimates increased by at least 8°C from those of the larvae and such an increase supports the hypothesis that ontogenetic shifts will be reflected by a shift of tolerance thresholds to warmer temperatures. However, there was no statistically significant difference reflected in the $CT_{\text{max}}$ estimates, suggesting that M. aurifrons develop a narrower performance breadth across ontogenies. The most likely drivers of these trends include differences driven by variation in mobility and occupied microhabitat (Kingsolver et al. 2011; Pincebourde & Casas 2015; Woods, Dillon & Pincebourde 2015). Adult Megachile are likely to be more exposed to daily temperature fluctuations, however, they also have the greatest potential for behavioural thermoregulation due to increased mobility and flight (Ma et al. 2018). This is in stark contrast to larvae, which have no opportunity to behaviourally mitigate stressful temperatures while potentially being exposed to a wider suite of seasonal conditions (Marais & Chown 2008; Barton, Clusella-Trullas & Terblanche 2019; English & Barreaux 2020). However, when
considering the 5.7°C increase in CT\textsubscript{max} estimated from larvae to adults, it seems more plausible that \textit{M. aurifrons} display a complete shift to higher tolerance thresholds than a narrowing of performance breadth and the high degrees of variability at these critical thresholds obscure the statistical interpretations. Nevertheless, optimal temperatures for organism metabolic performance are mediated by complex physiological systems and are likely to be ecologically and evolutionarily constrained (Angilletta Jr, Niewiarowski & Navas 2002; Chown \textit{et al.} 2010; Buckley, Ehrenberger & Angilletta Jr 2015). Whether the stage-dependent responses are products of selection strategies, acclimation, or merely reflections of shifting physiological morphology is unclear and the mechanisms involved are poorly understood (Stillwell \textit{et al.} 2010), warranting further study.

The intraspecific differences in tolerance thresholds are described, but the energetic consequences have substantial importance for the nesting ecology of these bees. The breadth of the larval response means that they perform close to their optimum across a wide range of temperatures, and that they suffer minimal thermal stress when T\textsubscript{a} exceeds their optimum. If larval development rate relates to metabolic rates, this means that the larvae should be developing nearly as rapidly as they can if the female nests in sites that are perpetually close to the thermal optimum of her larvae (Ruel & Ayres 1999; Martin & Huey 2008), without suffering excessive cost when the nests get too hot. It also means, however, that both males and females are going to be highly active in warm parts of the landscape, which may increase their energetic and foraging requirements and their activity as pollination vectors in these communities similar to other endemic bee species in the region (Tomlinson \textit{et al.} 2015), over and above other insect groups with lower energetic and thermal tolerances, such as beetles (Tomlinson 2020).

6.5.3 Implications for landscape management

One of the major aims of this study was to develop a mechanistic understanding of patterns of nest site selection and fecundity previously described for \textit{M. aurifrons} in a mosaic landscape of post-mining Jarrah Forest restoration (Chapter 4). Female \textit{M. aurifrons} prefer to oviposit in the warmest nesting sites across the restored matrix, at sites that average between 30-36°C (Chapter 4). These thermal environments align more closely to the thermal optima of larvae than they do for adults, suggesting that \textit{M. aurifrons} oviposition site selection conforms with the preference-performance hypothesis (Jaenike 1978; Valladares & Lawton 1991). This
hypothesis stipulates that maternal insects will preferentially oviposit in sites that maximise the optimal conditions for performance and development of their offspring (Gripenberg et al. 2010). By selecting microclimates to maximise larval metabolism and presumably development rate (Gibert & De Jong 2001; Folguera et al. 2010a; Arnqvist et al. 2017), maternal adults may increase the number of generations per year and maximise population numbers assuming that variation in developmental rate is at least partially driven by metabolic physiology (Hansen, Bentz & Turner 2001). Therefore, supplementing early-successional restoration sites that are limited in natural nesting cavities with artificial nests can encourage large populations of bees that are likely to have rapid generation times and high foraging activity. However, as the larvae examined here were removed from field conditions and reared in controlled laboratory conditions, further research is required to ensure the warm, fluctuating temperatures typical of early-successional sites translate to successful emergence as metabolic physiology and thermal acclimation can have persistent fitness impacts that was not investigated here.

The early-successional restoration and unmined forest both averaged a cumulative 17.4 days that satisfied the thermal conditions optimal to the larvae (defined as temperatures between $T_{\text{pref}}$ and $T_{\text{opt}}$) compared to mid- (16.2 days) and late-successional restoration (16.1 days; Figure A4.1). This provides further support for the hypothesis that ‘mother knows best’, however, the lower nesting rate observed in the unmined forest (Chapter 4), irrespective of the thermal suitability of these habitats to larvae, can be explained by the reduced suitability of these sites to the maternal bees (5.28 days; Figure A4.1). Furthermore, adult $M. \text{aurifrons}$ can be predicted to experience a 62% decrease from peak metabolic performance in cooler habitats, such as the unmined forest habitat, where the mean daily temperature was 28.4℃ in summer months (Chapter 4). While these conditions remain within the tolerance thresholds for the species, the capacity to forage (Balderrama, de Almeida & Núñez 1992), provision and ultimately, deliver pollination services (Arroyo, Armesto & Primack 1985), may be limited within these conditions as a result of reduced metabolic performance.

6.5.4 Future research directions

Early-successional restoration appears to optimise the thermal performance of $M. \text{aurifrons}$ across both life-stages. However, habitat suitability is as much defined by resource availability and other abiotic pressures (e.g. moisture availability) as it is by environmental
temperatures (Hirzel & Le Lay 2008). Early-successional habitats may not facilitate sustainable populations if the metabolic demands cannot be met by the locally available nutritional resources (Tomlinson et al. 2018b), or if the hygric conditions are suboptimal. In the context of resource availability, the NJF features a highly connected, heterogenous ecological matrix that is likely to support a diverse array of nectar and pollen resources (Havel, Dell & Malajczuk 1989). However, this conjecture is beyond the scope of this research to validate and requires descriptive and predictive statistical models for pollen and nectar resources across time and space, or insight into the hygric performance of the target taxa. Energetic barriers may not arise within highly connected heterogenous landscapes. However, in other contexts such as recently cleared habitat, highly fragmented landscapes or large-scale monocultural restoration, energetic deficits are likely to emerge (Tomlinson et al. 2018b). In this scenario, small warm patches may increase the energetic requirements of *M. aurifrons* and increase the foraging and subsequent pollination activity, increasing the value of *M. aurifrons* as a pollination vector. Regardless, fostering microclimatic heterogeneity and a diverse mix of nectariferous plants can promote both pollinator diversity, performance and the delivery of pollination services (Woodard & Jha 2017).

6.5.5 Conclusions

This study emphasises the ontogenetic variability of thermal performance and sensitivity, and how habitat modification can differentially impact the performance of developmental stages and the behaviour of adult *M. aurifrons*. The performance parameters estimated here are consistent with biological expectations and understanding how different developmental stages and performance traits respond to temperature can alter our predictions for the fitness consequences of environmental change. Adult *M. aurifrons* had a higher $R_{\text{max}}$ than larvae, but a narrower thermal performance breadth while larvae had a lower $T_{\text{opt}}$ than adults that aligned closely with the preferred nesting sites. When combined with estimates of the ‘thermal quality’ of nesting habitats, it appears that both adult and offspring performance drives nest-site selection, broadly consistent with the preference-performance hypothesis. While the unmined forest provides a similar duration of optimal conditions for development, the metabolic costs imposed on the maternal adults by the cooler environments are far greater and the energetic implications towards these key pollinators may impact pollination activity at a landscape scale. If a tight mosaic of mixed successional forest facilitating an heterogenous
thermal landscape is not maintained, the nesting and foraging behavior by *M. aurifrons* may be reduced, thus limiting its potential as a pollination vector. These results indicate one, but not necessarily the only, causal explanation for why these pollinators preferentially select for the open-canopy, early successional restoration sites within the heterogeneous forest landscape. However, without understanding the precise enzymatic or metabolic mechanisms underlying ontogenetic variation, the explanation for such variation remains elusive. Furthermore, without understanding the interacting effects of other physiological responses, such as desiccation resistance, inference into consequences of environmental change remain limited. Therefore, the integration of ecophysiology and biophysical ecology to draw synergistic links between patterns, processes, and the mechanistic drivers of such can rapidly elucidate impacts of environmental change which can be translated into proactive management action.
6.6 References

Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.


CHAPTER SEVEN:

General discussion and concluding remarks

7.1 Overview

This thesis represents the first comprehensive study of which I am aware assessing the environmental dynamics influencing the response of insect pollinators to post-mining restoration within the Northern Jarrah Forest (NJF) region of Western Australia. Generally, this research aimed to characterise the biotic and abiotic conditions driving insect pollinator community dynamics by addressing how the impacts of vegetation composition, structure and microclimate influenced insect pollinator reassembly and habitat use. More specifically, I provide an updated perspective on the prevalence of fauna and ecophysiology within the restoration ecology literature in the form of a literature review, which is followed by four experimental chapters that demonstrate how traditional biodiversity surveys can be complimented with laboratory based physiological experiments to provide a mechanistic insight into the patterns and processes underpinning restoration. In this discussion, I summarise the key findings of this research (Figure 7.1) and provide future directions for restoration science, practice, and policy. Last, I synthesise the outcomes from experimental chapters to highlight opportunities for the integration of novel ecophysiological approaches by adapting an existing adaptive management framework.
Figure 7.1: A summary of the findings from the experimental chapters of this thesis highlighting an interaction between the thermal gradient (a; blue reflects cooler conditions while red represents warmer conditions), where late successional restoration is generally cooler than early stages, and vegetation density, represented by the green cells, is generally increased within late-successional restoration (b). Species abundance (c) and species richness (d) both increase within early successional restoration in response to reduced vegetation density (x-axes reflect vegetation structural gradient). The reproductive output (brood abundance) of cavity-nesting Hymenoptera increased with increasing temperatures (e; x-axes reflect thermal gradient) and was highest within early-successional restoration, and peak metabolic performances of cavity-nesting Hymenoptera aligned closely with the thermal profiles of early-successional restoration (f).
7.2 Summary of findings

7.2.1 Chapter Two

Chapter Two examined the extent to which fauna, habitat selection, ecophysiology and thermal biology are integrated into the broader restoration ecology literature. Although restoration science is advancing, we generally lack insight into the complex physiological mechanisms (particularly thermal mechanisms) that can govern the responses of fauna to restoration initiatives and management. The review highlighted a significant gap between the expanding body of scientific literature and integration of ecophysiology in restoration, and formed the approach used in the experimental chapters of this thesis.

Generally, ecophysiology can be integrated into any restoration scenario where the physiological knowledge improves the ability to describe, explain or predict ecological patterns and the processes underpinning restoration outcomes. More broadly, restoration ecophysiology is a discipline applying physiological theory and practice to deliver a mechanistic insight into the responses to, and consequences of, environmental degradation and subsequent restoration. Restoration ecophysiology can help understand the complex relationships between an organism’s physiological performance and its external environment to infer the implications for population, community, and ecosystem dynamics across a restoration landscape. However, restoration ecophysiology should also draw upon the well-established knowledge, perspectives, and approaches of other disciplines (e.g., landscape ecology, functional biology and community ecology) as physiology alone is insufficient to address the broad suite of applied challenges and priorities within restoration. While insect pollinators were the specific focus of this thesis, the approach taken here is generally applicable and transferable across taxa and restoration contexts.

7.2.2 Chapter Three

Investigations into the community level responses of insect pollinators to habitat structure and vegetation composition across a post-mining restoration chronosequence resulted in four main conclusions:
1. Even after approximately three decades of succession, the vegetation community composition was not fully restored and remained far from resembling the composition of the unmined reference community.

2. The insect pollinators in the NJF seem to have responded in accordance with the ‘Field of Dreams’ hypothesis despite the lack of convergence within the vegetation community. However, mature forests (e.g., late successional restoration and unmined forest) did not support an insect pollinator community as abundant or rich as early successional restoration.

3. The insect pollinator community was more responsive to the emergent properties of habitat structure (i.e., increasing vegetation density) than the abundance, richness, diversity, or composition of the vegetation community itself.

4. Increased vegetation density was associated with reduced pollinator abundance and species richness (Figure 7.1c and 7.1d)

The recovery of degraded habitats through various means of ecological restoration and rehabilitation is a slow process, often taking many decades to ‘successfully’ recover, if full recovery occurs at all (Suding & Gross 2006). Generally, insect pollinator communities responded well to ecological post-mining restoration, in accordance to the ‘Field of Dreams Hypothesis’ (Palmer, Ambrose & Poff 1997), despite their return and diversity not being a specific target of the restoration agenda and the lack of compositional convergence among the vegetation community. Though vegetation communities are well-known mediators of invertebrate communities (Janzen & Hallwachs 2019), the role of vegetation community in structuring pollinator community was relatively weak. Rather, I found the insect pollinator community to be related strongly to vegetation density, particularly in the canopy. I also noted that some dominant Dipteran families (e.g., Tipulidae, Mycetophilidae and Chloropidae) were more dominant within the older ecological recovery sites, conforming to the suggestion that such taxa prefer closed-canopy habitats (Økland, Götmark & Nordén 2008). This contrasts with Coleoptera (e.g., Nitidulidae and Dermestidae) and Hymenoptera (Apidae, Halictidae, Colletidae), which indicated strong preferences towards the early-successional restoration. This suggested that these populations favour the early-successional habitat characterised by open, low-growing woody vegetation as opposed to the tall, closed-canopy structure typical of late-successional ecological recovery and unmined forest. From these results I speculated that vegetation structure is a significant driver of community composition as it can directly and indirectly impact several abiotic (i.e. temperature, Chapter Three, Four and Five) and biotic
(e.g. vegetation structure, Chapter Three and Four; interspecific interactions, Chapter Five; maternal effects, Chapter Six) factors that can either facilitate or impede on the ecological requirements of certain species (Brown 1991).

7.2.3 Chapter Four

The effects of temperature on different taxa have come to the forefront of research and conservation priorities in light of climate change projections over the last few decades (Tylianakis et al. 2008; Gilman et al. 2010; Lister & Garcia 2018). Several studies have shown that, in addition to resource abundance and availability, microclimatic conditions play an important role in establishing and maintaining species aggregations (Cloudsley-Thompson 1962; Pincebourde et al. 2007; Pincebourde & Casas 2015). In a closer inspection of the role of structure and microclimate in shaping biodiversity responses to restoration, Chapter Four demonstrated that:

1. Early successional restoration provides an important habitat for nesting and reproduction of solitary bees and wasps (Figure 7.1e).
2. Although elements of both vegetation structure and microclimate influenced the nesting community, at our sites, thermal conditions arose as the dominant driver of cavity-nesting bee nest construction and fecundity.
3. The nesting biology of cavity nesting wasps, on the other hand, while still indicating preferences for early-successional restoration, was more responsive to the emergent properties of overstorey vegetation than microclimate.

Changes in microclimate and habitat structure as a result of vegetative and topographical alteration can have cascading effects on biological communities, trophic interactions and the delivery of ecosystem services (Stangler, Hanson & Steffan-Dewenter 2015). The re-establishment of disturbed ecosystems often generates heterogeneous landscapes, with mosaic-like patterns of vegetation representing various stages of successional maturation (Larkin, Vivian-Smith & Zedler 2006) and different thermal environments. In summary, early successional restoration facilitated the most abundant populations of cavity-nesting hymenoptera and highlighted taxon-specific nest-site selection cues, with cavity-nesting wasps responding to vegetation structure, as opposed to temperature, which primarily motivated nesting patterns by cavity-nesting bees. However, this experimental chapter was
unable to disentangle the potential role of interspecific antagonistic relationships or intraspecific requirements in driving nest-site selection.

7.2.4 Chapter Five

While drawing associations between environmental conditions and habitat selections through measures of abundance and richness can elucidate patterns behind faunal responses to habitat change, this approach is limited in its capacity to draw inference into the processes, causes or mechanisms underpinning responses (Chown, Chown & Nicolson 2004). The results from Chapter Four highlight that cavity-nesting hymenopteran nest construction and fecundity are correlated with the warmer temperatures of early-successional habitats. However, these findings do not establish any mechanistic insight into the processes that underpin the patterns of nest-site selection. Therefore, in Chapter 5, I compared the physiological limitations and thermal biology of cavity-nesting Hymenoptera within a host-parasitoid system. This approach aimed to understand how variations of thermal tolerance might provide an underlying mechanism to explain differences in habitat use observed in Chapter Four. In doing so, Chapter Five demonstrated:

1. Cavity-nesting host bees exhibit wider, ‘generalist’ thermal performance curves compared to the narrower ‘specialist’ performance curve of the parasitoid wasp.
2. The parasitoid wasp larvae had a higher maximum metabolic rate ($R_{max}$) compared to the Megachilid host bees.
3. The tolerance thresholds resolved between 4.3 and 6.9°C higher in the Megachilid host larvae compared to the parasitoid wasp.
4. The estimated thermal optima broadly align with daily temperatures averages of early-successional restoration (~34°C; Figure 7.1f)

I characterised interspecific and intergeneric differences in thermal performance between larvae of two dominant Megachile species and an associated parasitoid, Gasteruption breviscutum. The physiological data, combined with estimates of thermal habitat suitability, allude to the potential for M. aurifrons to have a competitive advantage through increased tolerance thresholds and the capacity to move to enemy-free space. However, patterns of artificial nest-site selection do not indicate the significant reduction in parasitism levels in warmer microclimates that may be expected if thermal biology alone dictated the host-
parasitoid dynamic (Chapter Four). Therefore, empirical studies to examine the potentially deleterious effects of warmer microclimates on parasitoids may be necessary, as population level fitness responses were beyond the scope of both these studies.

7.2.5 Chapter Six

Nest-site selection for appropriate oviposition conditions is assumed to be under strong selective pressures such that site selection is correlated with suitability and success of progeny development (Jaenike & Holt 1991). However, it is equally plausible that site-selection results from constraints on the physiological performance of maternal adults. To understand the differential effects of warming across ontogenies, I measured the metabolic performance of both larvae and adult female M. aurifrons. This chapter demonstrated that:

1. Larvae have significantly lower energetic expenditure for their mass at a standard temperature and there is a 52-fold increase in maximum resting metabolic rates across developmental stages.
2. Larvae have significantly lower thermal optima when compared with adults.
3. Adult M. aurifrons exhibit a narrower performance breadth and increased thermal specificity than larvae, which is likely compensated for through behavioural thermoregulation.
4. The preference-performance hypothesis appears to, at least partially, explain M. aurifrons nest-site selection such that the temperature conditions of preferred nesting sites align closely with the $T_{opt}$ of larvae to maximise development and performance.

The preferred nest-sites present thermal environments that align more closely to the thermal optima of larvae (~34°C) than for adults (~43–48°C), suggesting that M. aurifrons oviposition site selection conforms with the preference-performance hypothesis (Jaenike 1978; Valladares & Lawton 1991). This hypothesis predicts that maternal insects will preferentially oviposit in sites that maximise the optimal conditions for performance and development of their offspring (Gripenberg et al. 2010). However, empirical data to support functional and mechanistic explanations for ontogenetic variation in thermal performance is remarkably limited and further research is necessary to quantify these dynamics in M. aurifrons and across ectotherms more broadly.
Chapter 7: General Discussion

7.3 Priorities for future research and action

7.3.1 Taxonomic perspectives

While ecophysiology provides a very insightful approach to understanding and explaining biodiversity responses to environmental change, to understand the ecology and distribution of a species, it is essential to first identify it accurately. Though numerous studies have contributed to such inventories and databases on invertebrate biodiversity, the taxonomic knowledge of many invertebrates is still limited, and even less is known about the biological, functional and physiological traits for all but a few species (Cardoso et al. 2011). Adequate baseline data on what species are present, and the abundances at which they occur, lay the foundation for any restoration context, and are particularly important in highly diverse ecosystems where many of the key agents remain unidentified, or even unknown to science (Braby & Williams 2016). Simply contributing to the knowledge base of invertebrate taxonomy, abundance and distribution at local and regional scales is no trivial goal. This knowledge should be pursued as a research target with urgency considering the growing number of studies reporting global declines in insect populations and associated ecological cascades (Potts et al. 2010; Hallmann et al. 2017; Sánchez-Bayo & Wyckhuys 2019).

7.3.2 Physiological perspectives

An organism’s physiology plays a central role in defining their fundamental niche, and a secondary role in determining the realised niche through behavioural responses to certain environmental cues (Huey 1991). Thermal physiology is critical to understanding species behaviour, fitness and dispersal limits (Barton, Clusella-Trullas & Terblanche 2019). However, the consequences of other ecophysiological mediators such as salinity and moisture, will also play non-mutually exclusive roles in driving species responses to environmental change (Boyle, Shogren & Brawn 2020). Without insight into other regulating factors, the understanding of a species’ niche becomes limited (Porter & Tracy 1983). In contrast to temperature, we have a poor mechanistic understanding of the hygric physiology of terrestrial ectotherms despite the importance of precipitation regimes in driving much of the biology for terrestrial taxa (Chown, Sørensen & Terblanche 2011; Boyle, Shogren & Brawn 2020). Without understanding the relationship between aridity or precipitation we cannot fully predict how ectotherms will respond to microclimatic shifts in humidity and related factors under
global climate change or local environmental change. Many of the approaches applied to
determine a species’ thermal niche can be extended to that of their hygric niche. For example,
correlative studies to determine the general associations between a species abundance,
reproductive phenology, and habitat selection provide a solid foundation to understand the
range of hygric conditions that a species can endure. Where possible, however, efforts should
be directed towards drawing mechanistic links between moisture-mediated performance,
fitness, population dynamics and community responses, as they are currently understood for
thermal performance. Modelling approaches taken from other disciplines, such as that of a
hydrothermal niche may be useful in characterising the interplay between an organism’s
thermal and hygric niches. This can increase the dimensionality of species distribution models
and attempts to tackle this complex interplay may increase the predictive and explanatory
capacity of ecophysiology in driving inter- and intraspecific responses to microclimatic
variation in any applied context. Understanding moisture limitations and thermal and moisture
interactions may also provide a means to predict species responses to drought and increases in
temperature under climate change.

Specific to the target taxa and restoration context explored within my thesis, the next
logical step would be to conduct mechanistic modelling generated from the parameter estimates
of thermal performance and tolerance for *Megachile aurifrons* that may describe how
management actions might alter the thermo-energetics of this native pollinator. Combing
remote sensing of forest structural attributes with temperature correlates (as established within
Chapter Two) can generate landscape-scale insights into habitat suitability for this species, at
least from a thermal perspective. The physiological and spatial data obtained here could also
be used to derive estimates of habitat suitability within various management scenarios, such as
thinning, or in the context of predicted climate change. However, the reliability of these models
could be substantially increased through incorporating dynamic energy budgets (Kooijman &
Kooijman 2010), and can be used to recognise the consequences of thinning to resource
availability that may result in sublethal effects on *M. aurifrons* through local resources not
meeting the energetic requirements.

7.3.3 Methodological perspectives

It is also important to note the challenges of translating results obtained within
controlled laboratory conditions into reliable predictions of complex *in situ* field conditions
Unexpected responses can emerge between modelled projections of organism responses and reality. Therefore, further research is needed to explore the ecological energetics of target taxa that reflect the energetic costs of existing within any given environment. A broad suite of isometric and telemetric approaches exist to test field metabolic rates (FMR), each with varying degrees of applicability depending on the environment and organism (reviewed in Tomlinson et al. 2014). One approach includes the measurement of radioactive rubidium ($^{86}$Rb). This approach has been suggested as a relatively simple, inexpensive, avenue for measuring FMR (Peters 1996; Bradshaw & Bradshaw 2007; Tomlinson, Mathialagan & Maloney 2014), and one that may be particularly useful for insects (Tomlinson et al. 2013; Tomlinson, Mathialagan & Maloney 2014). Quantifying FMR provides the opportunity to test hypotheses about the physiological constraints imposed on organisms from environmental change in ways that controlled laboratory assessments cannot encompass (Tomlinson et al. 2014). However, FMR approaches are rare within applied ecology, particularly when considering small ectothermic organisms, and warrant greater incorporation into future research efforts.

7.3.4 Management perspectives

Collaborations can enable unparalleled opportunities for targeted research and can increase the validity of proposed frameworks through evidence-based investigation (David, Dixon & Menz 2016). One such way to foster greater collaboration between ecophysiology and restoration ecology is to engage with adaptive management. Adaptive management is often defined as a structured, cyclical process of decision-making that accounts for change and uncertainty in a “learning by doing” fashion from which informed adaptive action is implemented to improve upon previous efforts (Williams 2011; McDonald et al. 2016). Adaptive management is considered as what should be the standard approach to ecological restoration (McDonald et al. 2016; SERA 2017), and should be designed with the input of both researchers and practitioners (Taylor, Kremsater & Ellis 1997; Morghan, Sheley & Svejcar 2006). The most direct approach to adaptive management is to conduct regular site monitoring to establish whether the current management and restoration actions are working to achieve restoration goals, targets, and objectives. However, the implementation of an adaptive management framework can range from a trial-and-error approach, to deliberate monitoring.
schemes or the integration of experimental design and explicit hypothesis testing (Bormann, Haynes & Martin 2007; Williams 2011).

Species-specific physiology and site-specific conditions (and manipulation of such) have high applicability in the design, implementation, monitoring and adaptive management of almost any restoration initiative (Figure 7.2). While traditional biodiversity monitoring (e.g., species richness, abundance and composition) can describe patterns of spatial and temporal variation, they are limited in their capacity to provide mechanistic or causal interpretation (Lawton 1999; Verberk, Van Noordwijk & Hildrew 2013). However, by simply integrating experimental physiology into restoration management, hypothesis testing can delineate cause-and-effect relationships between limiting environmental factors and predict species responses to environmental change (e.g., successional pathways, climate change, drought) or management practices (e.g., canopy thinning, prescribed burning). Ecophysiology, for example, can characterise physiological variation and the implications of such to restoration through identifying vulnerable species (Chapter Five) or developmental stages (Chapter Six). This information can help practitioners facilitate management strategies to minimise stress, maximise habitat suitability and increase fitness of conservation significant species or other target taxa. Alternatively, this insight can be applied to invasive or pest species and the reverse can apply, such that management actions increase barriers to fitness to minimise invasion or inform biological control practices (Schmitz & Barton 2014; Tougeron et al. 2016). Ecophysiology can also be used to establish trigger points and clear measurable indicators, through drawing upon physiological data of tolerance thresholds or performance parameters to infer habitat suitability (Chapter Five; Chapter Six). Collectively, monitoring physiological responses to changing environmental conditions in conjunction with broader patterns of biodiversity and fitness responses can provide an encompassing perspective of whether current restoration practices are working as expected, or whether practice needs to be adjusted (Cooke & O’Connor 2010). Furthermore, adaptive management is inherently flexible, and can integrate new physiological traits and measures as understanding develops and can be equally applied to other ecosystems and taxa.

There are several speculations as to why there has been negligible integration of animal physiology into restoration ecology. There are concerns about scientists communicating their findings solely to other scientists through peer-reviewed literature (Bertuol-Garcia et al. 2018), of restoration ecology becoming an “echo-chamber” (Menz, Dixon & Hobbs 2013), and the value of the research being poorly communicated to practitioners (Seavy & Howell 2010).
Conversely, the practical outcomes of restoration projects are often not published in peer-review journals, restricting feedback from practitioners to the scientific community (Sunderland et al. 2009), and also restricting the communication of lessons learned to only local groups, regardless of the global challenge that ecological restoration represents. Collectively, this highlights a potential divide between the science and practice of ecological restoration generally. More specifically, however, physiology has been criticised for dealing with inadequate biological, spatial and temporal scales (Cooke & O’Connor 2010), and of having objectives that do not align with the knowledge needs of practitioners and decision-makers (Cooke & Suski 2008; Cooke & O’Connor 2010). Increased communication between both physiologists and ecologists, and between science and practice within all stages of restoration programs, can facilitate interdisciplinary objectives and beneficial outcomes to all parties. Therefore, practitioners and ecologists should collaborate further and facilitate bi-directional flows of knowledge to achieve the shared goal of maximising the efficiency and success of the restoration process (Baker, Eckerberg & Zachrisson 2014; Young et al. 2014; David, Dixon & Menz 2016).
**Figure 7.2:** Schematic overview representing how organism physiology can be used across an adaptive management cycle to describe, predict, and explain organism responses to ecological restoration, contribute to the evaluation of restoration trajectories and inform management actions to feed back into future restoration planning and implementation in a flexible, iterative process of decision making and knowledge acquisition.
7.3.5 **Opportunities for adaptive management in Jarrah Forest restoration**

Chapter Three and Chapter Four of this thesis established that pollinator abundance, richness and reproductive output were all significantly reduced in late-succession restoration (Figure 7.1). While these sites were structurally similar to the surrounding unmined forest, the dense, closed canopy vegetation generated much cooler microclimates and was not conducive for an abundant pollinator community. Building upon this, Chapters Five and Six collectively highlighted that some of the native pollinators may select for warmer microclimates due to their thermal requirements around 33-37°C to facilitate maximum metabolic performance. Therefore, to meet the goal of sustaining an abundant and biodiverse pollinator community across all stages of restoration development, land-managers may look towards adaptive management actions. For example, this may consist of a reactive approach whereby management actions such as canopy thinning within restoration stands that are already established or a proactive approach such as reduced seeding densities in future restoration sites, or indeed a combination of both.

7.4 **Concluding remarks**

The evaluation of restored habitats can be enhanced through drawing synergistic links between patterns, their motivating processes, especially when tied to demographic responses or ecological service provision. The chapters within this thesis combine to highlight a feasible way of incorporating animal physiology into restoration ecology by drawing strong connections between community ecology, functional biology, and ecophysiology. However, this thesis serves as just one example of how restoration ecophysiology can be readily incorporated into restoration monitoring, and how it can bridge the gap between descriptive (correlative), explanatory and predictive science when combined with traditional field surveys.

The experimental adaptive management framework illustrates how physiological data can make considerable contributions to understanding organism responses to restoration. This is achieved through using ecophysiology to describe physiological variation, explain emerging patterns of biodiversity, predict future responses, and inform management actions to achieve the goal, targets, and objectives of ecological restoration to maximise the likelihood of restoration success (Figure 7.2). This is not a new framework; it instead identifies how to incorporate novel measurements into an established planning and monitoring structure to gain new insights into ecological restoration. This approach may foster the interdisciplinary
collaborations necessary to promote the co-development of future research questions that can provide the practical science needed to inform case-by-case restoration initiatives (Cabin et al. 2010; Perring et al. 2015; Suding et al. 2015). This integrative, interdisciplinary approach may have been an overlooked element in allowing ecophysiology to play a substantial and informative role in ecological restoration, and I am hopeful that applying this framework to other ecophysiological traits will offer exciting new pathways to increase restoration success and respond to the challenges that we have set for ourselves in the Anthropocene.
7.5 References

Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.


percent decline over 27 years in total flying insect biomass in protected areas. *PloS one*, **12**, e0185809.


Chapter 7: General Discussion


Appendix

Appendix 1: Build it and they will come: Insect pollinator community reassembly in spatiotemporally heterogenous forest restoration.

<table>
<thead>
<tr>
<th>Genus Species</th>
<th>Abundance</th>
<th>1-5</th>
<th>5-15</th>
<th>15-20</th>
<th>30-45</th>
<th>MRF</th>
<th>Stat</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia acuminata</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.354</td>
<td>0.116</td>
</tr>
<tr>
<td>Acacia alata</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.500</td>
<td>0.022</td>
</tr>
<tr>
<td>Acacia browniana</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.189</td>
<td>1.000</td>
</tr>
<tr>
<td>Acacia drummondii</td>
<td>181</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.796</td>
<td>0.001</td>
</tr>
<tr>
<td>Acacia extensa</td>
<td>55</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.502</td>
<td>0.044</td>
</tr>
<tr>
<td>Acacia horridula</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
<td>1.000</td>
</tr>
<tr>
<td>Acacia lateriticola</td>
<td>74</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.833</td>
<td>0.001</td>
</tr>
<tr>
<td>Acacia longifolia</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.289</td>
<td>1.000</td>
</tr>
<tr>
<td>Acacia nervosa</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
<td>1.000</td>
</tr>
<tr>
<td>Acacia pulchella</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.551</td>
<td>0.012</td>
</tr>
<tr>
<td>Acacia urophylla</td>
<td>24</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.354</td>
<td>0.336</td>
</tr>
<tr>
<td>Adenanthos barbiger</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.577</td>
<td>0.005</td>
</tr>
<tr>
<td>Aira caryophyllea</td>
<td>135</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
<td>1.000</td>
</tr>
<tr>
<td>Allocasuarina fraseriana</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.246</td>
<td>0.882</td>
</tr>
<tr>
<td>Allocasuarina humilis</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
<td>1.000</td>
</tr>
<tr>
<td>Amphypogon amphipogonoides</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.469</td>
<td>0.051</td>
</tr>
<tr>
<td>Genus Species</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>Banksia grandis</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.408</td>
</tr>
<tr>
<td>Billardiera sp. 1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.354</td>
</tr>
<tr>
<td>Billardiera variifolia</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.420</td>
</tr>
<tr>
<td>Boronia fastigiata</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.559</td>
</tr>
<tr>
<td>Bossiaea aquifolium</td>
<td>1060</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.736</td>
</tr>
<tr>
<td>Bossiaea eriocarpa</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Bossiaea ornata</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.383</td>
</tr>
<tr>
<td>Bossiaea pulchella</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Burchardia congesta</td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.764</td>
</tr>
<tr>
<td>Caladenia ferruginea</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.354</td>
</tr>
<tr>
<td>Caladenia sp. 1</td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.367</td>
</tr>
<tr>
<td>Calothamnus lateralis</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Calothamnus quadrifidus</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.354</td>
</tr>
<tr>
<td>Chamaescilla corymbosa</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.198</td>
</tr>
<tr>
<td>Chenopodium carinatum</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.236</td>
</tr>
<tr>
<td>Chenopodium sp. 1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.204</td>
</tr>
<tr>
<td>Chorizema dicksonii</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.236</td>
</tr>
<tr>
<td>Chorizema ilicifolium</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.463</td>
</tr>
<tr>
<td>Clematis pubescens</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.289</td>
</tr>
<tr>
<td>Conostylis pusilla</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.289</td>
</tr>
<tr>
<td>Conostylis setigera</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.417</td>
</tr>
<tr>
<td>Corchorus macropetalus</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Genus Species</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>Corymbia calophyla</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.500</td>
</tr>
<tr>
<td>Dampiera linearis</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.408</td>
</tr>
<tr>
<td>Daviesia cordata</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Daviesia preissii</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Daviesia rhombifolia</td>
<td>41</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.588</td>
</tr>
<tr>
<td>Dioscorea hastifolia</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.524</td>
</tr>
<tr>
<td>Disa bracteata</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.289</td>
</tr>
<tr>
<td>Diuris longifolia</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.289</td>
</tr>
<tr>
<td>Drakaea sp. 1</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.408</td>
</tr>
<tr>
<td>Drosera erythrohiza</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.289</td>
</tr>
<tr>
<td>Drosera pallida</td>
<td>199</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.580</td>
</tr>
<tr>
<td>Eucalyptus marginata</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.500</td>
</tr>
<tr>
<td>Eucalyptus megacarpa</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.500</td>
</tr>
<tr>
<td>Eucalyptus patens</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Eucalyptus resinifera</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.707</td>
</tr>
<tr>
<td>Gamochaeta coarctata</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.433</td>
</tr>
<tr>
<td>Gompholobium marginatum</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Gompholobium preissii</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Haemodorum sp. 1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.289</td>
</tr>
<tr>
<td>Hakea amplexicaulis</td>
<td>13</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.320</td>
</tr>
<tr>
<td>Hakea ilicifolia</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Hakea lissocarpha</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.204</td>
</tr>
<tr>
<td>Genus Species</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Hakea undulata</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.408</td>
</tr>
<tr>
<td>Hardenbergia comptoniana</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.354</td>
</tr>
<tr>
<td>Hemiandra sp. l</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Hibbertia acerosa</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.354</td>
</tr>
<tr>
<td>Hibbertia amplexicaulis</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.343</td>
</tr>
<tr>
<td>Hibbertia commutata</td>
<td>198</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.534</td>
</tr>
<tr>
<td>Hibbertia huegeli</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Hibbertia perfoliata</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.364</td>
</tr>
<tr>
<td>Hibbertia racemosa</td>
<td>18</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.500</td>
</tr>
<tr>
<td>Hovea chorisemifolia</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.714</td>
</tr>
<tr>
<td>Hypocalymma angustifolia</td>
<td>157</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.661</td>
</tr>
<tr>
<td>Hypocalymma cordifolium</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Hypochaeris glabra</td>
<td>426</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.500</td>
</tr>
<tr>
<td>Kennedia coccinea</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.471</td>
</tr>
<tr>
<td>Labuchea punctata</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.354</td>
</tr>
<tr>
<td>Lagenophora huegeli</td>
<td>132</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.572</td>
</tr>
<tr>
<td>Lasiopetalum floribundum</td>
<td>112</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.624</td>
</tr>
<tr>
<td>Lechenaultia biloba</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.204</td>
</tr>
<tr>
<td>Lepidosperma squamatum</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.204</td>
</tr>
<tr>
<td>Leucopogon capitellatus</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.309</td>
</tr>
<tr>
<td>Leucopogon nutans</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.149</td>
</tr>
<tr>
<td>Leucopogon propinquus</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.289</td>
</tr>
<tr>
<td><strong>Genus Species</strong></td>
<td><strong>Abundance</strong></td>
<td><strong>1-5</strong></td>
<td><strong>5-15</strong></td>
<td><strong>15-20</strong></td>
<td><strong>20-30</strong></td>
<td><strong>30-45</strong></td>
<td><strong>MRF</strong></td>
<td><strong>Stat</strong></td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------</td>
<td>---------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td><em>Leucopogon verticillatus</em></td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.612</td>
</tr>
<tr>
<td><em>Levenh hookia stipitata</em></td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.310</td>
</tr>
<tr>
<td><em>Lomandra caespitosa</em></td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.405</td>
</tr>
<tr>
<td><em>Lomandra hermaphrodita</em></td>
<td>31</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.552</td>
</tr>
<tr>
<td><em>Lomandra integra</em></td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.378</td>
</tr>
<tr>
<td><em>Lomandra micrantha</em></td>
<td>94</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.725</td>
</tr>
<tr>
<td><em>Lomandra preissii</em></td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.565</td>
</tr>
<tr>
<td><em>Lomandra sonderi</em></td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.682</td>
</tr>
<tr>
<td><em>Lomandra sp. 1</em></td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.204</td>
</tr>
<tr>
<td><em>Lomandra spartea</em></td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.491</td>
</tr>
<tr>
<td><em>Lotus subbiflorus</em></td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td><em>Loxocarya cinerea</em></td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.500</td>
</tr>
<tr>
<td><em>Macrozamia riedlei</em></td>
<td>42</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.813</td>
</tr>
<tr>
<td><em>Microtis media</em></td>
<td>16</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.451</td>
</tr>
<tr>
<td><em>Mirbelia dilatata</em></td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.505</td>
</tr>
<tr>
<td><em>Neurachne alopecuroidea</em></td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.351</td>
</tr>
<tr>
<td><em>Opercularia apiciflora</em></td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.412</td>
</tr>
<tr>
<td><em>Opercularia echinocephala</em></td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.441</td>
</tr>
<tr>
<td><em>Orchidaceae sp. 1</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td><em>Patersonia babianoides</em></td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.289</td>
</tr>
<tr>
<td><em>Pentapeltis pettigera</em></td>
<td>51</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.707</td>
</tr>
<tr>
<td><em>Persoonia longifolia</em></td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.577</td>
</tr>
<tr>
<td>Genus Species</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>Phyllanthus calycinus</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.236</td>
</tr>
<tr>
<td>Pimelea suaveolens</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.236</td>
</tr>
<tr>
<td>Platysace filiformis</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.408</td>
</tr>
<tr>
<td>Platysace tenuissima</td>
<td>252</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.667</td>
</tr>
<tr>
<td>Pseudognaphalium luteo-album</td>
<td>30</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.387</td>
</tr>
<tr>
<td>Pteridium esculentum</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.707</td>
</tr>
<tr>
<td>Pterostylis nana</td>
<td>198</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.528</td>
</tr>
<tr>
<td>Pterostylis recurva</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.354</td>
</tr>
<tr>
<td>Pterostylis sp. 1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.258</td>
</tr>
<tr>
<td>Pterostylis vittata</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.373</td>
</tr>
<tr>
<td>Pyrorchis nigricans</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.289</td>
</tr>
<tr>
<td>Ranunculus colonorum</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.333</td>
</tr>
<tr>
<td>Rytidosperma caespitosum</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.265</td>
</tr>
<tr>
<td>Scaevola calliptera</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.231</td>
</tr>
<tr>
<td>Senecio diaschidea</td>
<td>17</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.243</td>
</tr>
<tr>
<td>Senecio quadridentatus</td>
<td>65</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.496</td>
</tr>
<tr>
<td>Sphaerolobium medium</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.408</td>
</tr>
<tr>
<td>Stylidium amoenum</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
<tr>
<td>Stylidium piliferum</td>
<td>57</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.405</td>
</tr>
<tr>
<td>Tetraria capillaris</td>
<td>115</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.714</td>
</tr>
<tr>
<td>Tetrarrhena laevis</td>
<td>64</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.813</td>
</tr>
<tr>
<td>Tetratheca hirsuta</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.612</td>
</tr>
<tr>
<td>Genus Species</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td><em>Thelymitra crinita</em></td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.426</td>
</tr>
<tr>
<td><em>Thelymitra sp. 1</em></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.500</td>
</tr>
<tr>
<td><em>Thomasia sp. 1</em></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.408</td>
</tr>
<tr>
<td><em>Thysanotus dichotomus</em></td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.567</td>
</tr>
<tr>
<td><em>Thysanotus manglesianus</em></td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.500</td>
</tr>
<tr>
<td><em>Thysanotus multiflorus</em></td>
<td>43</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.669</td>
</tr>
<tr>
<td><em>Thysanotus sp1.</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.354</td>
</tr>
<tr>
<td><em>Thysanotus sp2.</em></td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.229</td>
</tr>
<tr>
<td><em>Thysanotus thyrsoides</em></td>
<td>47</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.417</td>
</tr>
<tr>
<td><em>Trachymene pilosa</em></td>
<td>56</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.500</td>
</tr>
<tr>
<td><em>Trichocline spatulata</em></td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.408</td>
</tr>
<tr>
<td><em>Tricoryne elatior</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.289</td>
</tr>
<tr>
<td><em>Trymalium ledifolium</em></td>
<td>264</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.794</td>
</tr>
<tr>
<td><em>Velleia trinervis</em></td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.408</td>
</tr>
<tr>
<td><em>Vulpia myuros</em></td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.408</td>
</tr>
<tr>
<td><em>Xanthorrhoea gracilis</em></td>
<td>88</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.802</td>
</tr>
<tr>
<td><em>Xanthorrhoea preissii</em></td>
<td>105</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.900</td>
</tr>
<tr>
<td><em>Xanthosia atkinsoniana</em></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.185</td>
</tr>
<tr>
<td><em>Xanthosia candida</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.289</td>
</tr>
</tbody>
</table>
Table A1.2: Results of an indicator species (morphospecies reported at family level resolution) analysis reported at family level for in the insect pollinator community across sampled restoration sites (age range in years) and unmined forest (MRF) sampled in the Northern Jarrah Forest. The age class columns (1-5, 5-15… MRF) indicate (with ones) which restoration site ages were preferred by the species. The final two columns represent the associate statistic (Stat) and p-value (P) of permutational test conducted via multilevel pattern analysis using the `multipatt` function in the `indispecies` statistical package for R (Cáceres & Legendre 2009). NAs in the P value column correspond to those species which occur in all restoration age classes.

<table>
<thead>
<tr>
<th>Morphospecies</th>
<th>Abundance</th>
<th>1-5</th>
<th>5-15</th>
<th>15-20</th>
<th>20-30</th>
<th>30-45</th>
<th>MRF</th>
<th>Stat</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acroceridae sp. 1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Aphelinidae sp. 1</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.350314</td>
<td>0.171</td>
</tr>
<tr>
<td>Aphelinidae sp. 2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Aphelinidae sp. 3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Apidae sp. 1</td>
<td>714</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.633835</td>
<td>0.018</td>
</tr>
<tr>
<td>Apidae sp. 2</td>
<td>467</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.828406</td>
<td>0.001</td>
</tr>
<tr>
<td>Apidae sp. 3</td>
<td>66</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.541364</td>
<td>0.124</td>
</tr>
<tr>
<td>Apidae sp. 4</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.367899</td>
<td>0.075</td>
</tr>
<tr>
<td>Asilidae sp. 1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Asilidae sp. 2</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Aulacidae sp. 1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Bethylidae sp. 1</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.25</td>
<td>0.654</td>
</tr>
<tr>
<td>Bethylidae sp. 2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.333333</td>
<td>0.135</td>
</tr>
<tr>
<td>Bibionidae sp. 1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.258199</td>
<td>0.355</td>
</tr>
<tr>
<td>Braconidae sp. 1</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.368956</td>
<td>0.122</td>
</tr>
<tr>
<td>Morphospecies</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
<td>$P$</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>------</td>
<td>-------</td>
<td>-----</td>
</tr>
<tr>
<td>Braconidae sp. 2</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.472448</td>
<td>0.013</td>
</tr>
<tr>
<td>Braconidae sp. 3</td>
<td>25</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.472882</td>
<td>0.229</td>
</tr>
<tr>
<td>Braconidae sp. 4</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.316228</td>
<td>0.103</td>
</tr>
<tr>
<td>Braconidae sp. 5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.288675</td>
<td>0.105</td>
</tr>
<tr>
<td>Braconidae sp. 6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>0.1</td>
</tr>
<tr>
<td>Buprestidae sp. 1</td>
<td>41</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.520964</td>
<td>0.002</td>
</tr>
<tr>
<td>Buprestidae sp. 2</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Buprestidae sp. 3</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.288675</td>
<td>0.43</td>
</tr>
<tr>
<td>Buprestidae sp. 4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.272166</td>
<td>0.717</td>
</tr>
<tr>
<td>Buprestidae sp. 5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.258199</td>
<td>0.393</td>
</tr>
<tr>
<td>Calliphoridae sp. 1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.25</td>
<td>0.658</td>
</tr>
<tr>
<td>Calliphoridae sp. 2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Calliphoridae sp. 3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Carabidae sp. 1</td>
<td>92</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.610368</td>
<td>NA</td>
</tr>
<tr>
<td>Carabidae sp. 2</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.408248</td>
<td>0.045</td>
</tr>
<tr>
<td>Carabidae sp. 3</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.320759</td>
<td>0.227</td>
</tr>
<tr>
<td>Carabidae sp. 4</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Cecidomyiidae sp. 1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Cerambycidae sp. 1</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.30429</td>
<td>0.451</td>
</tr>
<tr>
<td>Cerambycidae sp. 2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Chalcididae sp. 1</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Chalcididae sp. 2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.288675</td>
<td>0.107</td>
</tr>
<tr>
<td>Morphospecies</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
<td>P</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Chalcididae sp. 3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.333333</td>
<td>0.168</td>
</tr>
<tr>
<td>Chironomidae sp. 1</td>
<td>7216</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.995086</td>
<td>NA</td>
</tr>
<tr>
<td>Chloropidae sp. 1</td>
<td>491</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.859504</td>
<td>0.009</td>
</tr>
<tr>
<td>Chrysomelidae sp. 1</td>
<td>82</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.514496</td>
<td>NA</td>
</tr>
<tr>
<td>Chrysomelidae sp. 2</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.416692</td>
<td>0.021</td>
</tr>
<tr>
<td>Chrysomelidae sp. 3</td>
<td>35</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.475595</td>
<td>0.149</td>
</tr>
<tr>
<td>Chrysomelidae sp. 4</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.399957</td>
<td>0.122</td>
</tr>
<tr>
<td>Chrysomelidae sp. 5</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Chrysomelidae sp. 6</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Ciidae sp. 1</td>
<td>275</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.641689</td>
<td>NA</td>
</tr>
<tr>
<td>Ciidae sp. 2</td>
<td>28</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.362287</td>
<td>0.901</td>
</tr>
<tr>
<td>Cleridae sp. 1</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.288675</td>
<td>0.332</td>
</tr>
<tr>
<td>Cleridae sp. 2</td>
<td>30</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.425777</td>
<td>0.342</td>
</tr>
<tr>
<td>Cleridae sp. 3</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.360458</td>
<td>0.216</td>
</tr>
<tr>
<td>Cleridae sp. 4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.408248</td>
<td>0.013</td>
</tr>
<tr>
<td>Cleridae sp. 5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Coccinellidae sp. 1</td>
<td>100</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.597472</td>
<td>0.001</td>
</tr>
<tr>
<td>Coccinellidae sp. 2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Coccinellidae sp. 3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Colletidae sp. 1</td>
<td>39</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.362517</td>
<td>0.657</td>
</tr>
<tr>
<td>Colletidae sp. 2</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.318063</td>
<td>0.819</td>
</tr>
<tr>
<td>Colletidae sp. 3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
</tbody>
</table>
### Appendix

<table>
<thead>
<tr>
<th>Morphospecies</th>
<th>Abundance</th>
<th>1-5</th>
<th>5-15</th>
<th>15-20</th>
<th>20-30</th>
<th>30-45</th>
<th>MRF</th>
<th>Stat</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colletidae sp. 4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Colletidae sp. 5</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Colletidae sp. 6</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.383893</td>
<td>0.081</td>
</tr>
<tr>
<td>Colletidae sp. 7</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.288675</td>
<td>0.423</td>
</tr>
<tr>
<td>Colletidae sp. 8</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Conopidae sp. 1</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Corylophidae sp. 1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Corylophidae sp. 2</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Crabronidae sp. 1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Cryptophagidae sp. 1</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.316228</td>
<td>0.113</td>
</tr>
<tr>
<td>Culicidae sp. 1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.258199</td>
<td>0.376</td>
</tr>
<tr>
<td>Curculionidae sp. 1</td>
<td>255</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.754053</td>
<td>0.002</td>
</tr>
<tr>
<td>Curculionidae sp. 2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Curculionidae sp. 3</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.328395</td>
<td>NA</td>
</tr>
<tr>
<td>Curculionidae sp. 4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Curculionidae sp. 5</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Dermentidae sp. 1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.288675</td>
<td>0.119</td>
</tr>
<tr>
<td>Dolichopodidae sp. 1</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.272166</td>
<td>0.72</td>
</tr>
<tr>
<td>Drosophilidae sp. 1</td>
<td>49</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.319373</td>
<td>0.916</td>
</tr>
<tr>
<td>Elateridae sp. 1</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.333333</td>
<td>0.726</td>
</tr>
<tr>
<td>Elateridae sp. 2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Elateridae sp. 3</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.353145</td>
<td>0.11</td>
</tr>
<tr>
<td>Morphospecies</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
<td>P</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>----------------</td>
<td>------------</td>
<td>---------</td>
</tr>
<tr>
<td>Elateridae sp. 4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Empididae sp. 1</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.246183</td>
<td>0.886</td>
</tr>
<tr>
<td>Empididae sp. 2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Empididae sp. 3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Empididae sp. 4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Eucharitidae sp. 1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.333333</td>
<td>0.156</td>
</tr>
<tr>
<td>Eucnemidae sp. 1</td>
<td>159</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.540616</td>
<td>0.151</td>
</tr>
<tr>
<td>Eucnemidae sp. 2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Eucnemidae sp. 3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Eulophidae sp. 1</td>
<td>74</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.604614</td>
<td>0.085</td>
</tr>
<tr>
<td>Eulophidae sp. 2</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.358952</td>
<td>0.158</td>
</tr>
<tr>
<td>Eulophidae sp. 3</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.34029</td>
<td>0.751</td>
</tr>
<tr>
<td>Eulophidae sp. 4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Eupelmidae sp. 1</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.275241</td>
<td>0.664</td>
</tr>
<tr>
<td>Eurytomidae sp. 1</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Fergusoninidae sp. 1</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Figitidae sp. 1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Formicidae sp. 1</td>
<td>242</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.789949</td>
<td>0.011</td>
</tr>
<tr>
<td>Formicidae sp. 2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.288675</td>
<td>0.111</td>
</tr>
<tr>
<td>Formicidae sp. 3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Formicidae sp. 4</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.333333</td>
<td>0.152</td>
</tr>
<tr>
<td>Formicidae sp. 5</td>
<td>25</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.372485</td>
<td>0.162</td>
</tr>
<tr>
<td>Morphospecies</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
<td>P</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-----------</td>
<td>------------</td>
<td>--------</td>
</tr>
<tr>
<td>Formicidae sp. 6</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Formicidae sp. 7</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.389249</td>
<td>0.17</td>
</tr>
<tr>
<td>Halictidae sp. 1</td>
<td>334</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.753085</td>
<td>0.001</td>
</tr>
<tr>
<td>Halictidae sp. 2</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.408248</td>
<td>0.022</td>
</tr>
<tr>
<td>Halictidae sp. 3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Halictidae sp. 4</td>
<td>97</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.613953</td>
<td>0.001</td>
</tr>
<tr>
<td>Halictidae sp. 5</td>
<td>170</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.707257</td>
<td>0.001</td>
</tr>
<tr>
<td>Halictidae sp. 6</td>
<td>534</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.707629</td>
<td>0.001</td>
</tr>
<tr>
<td>Halictidae sp. 7</td>
<td>370</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.522119</td>
<td>0.004</td>
</tr>
<tr>
<td>Halictidae sp. 8</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Hepialidae sp. 1</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.360993</td>
<td>0.091</td>
</tr>
<tr>
<td>Hesperiidae sp. 1</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.343216</td>
<td>0.193</td>
</tr>
<tr>
<td>Hesperiidae sp. 2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.333333</td>
<td>0.133</td>
</tr>
<tr>
<td>Ichneumonidae sp. 1</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.316228</td>
<td>0.117</td>
</tr>
<tr>
<td>Ichneumonidae sp. 2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Latridiidae sp. 1</td>
<td>193</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.833621</td>
<td>0.001</td>
</tr>
<tr>
<td>Leiodidae sp. 1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.288675</td>
<td>0.432</td>
</tr>
<tr>
<td>Leiodidae sp. 2</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.25</td>
<td>0.639</td>
</tr>
<tr>
<td>Limoniidae sp. 1</td>
<td>423</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.560112</td>
<td>NA</td>
</tr>
<tr>
<td>Lucanidae sp. 1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Lycaenidae sp. 1</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.288675</td>
<td>0.752</td>
</tr>
<tr>
<td>Megachilidae sp. 1</td>
<td>38</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.383482</td>
<td>NA</td>
</tr>
<tr>
<td>Morphospecies</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
<td>P</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-----------</td>
<td>----------</td>
<td>-------</td>
</tr>
<tr>
<td>Megachilidae sp. 2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Megachilidae sp. 3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Megachilidae sp. 4</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.353553</td>
<td>0.322</td>
</tr>
<tr>
<td>Megaspilidae sp. 5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.258199</td>
<td>0.369</td>
</tr>
<tr>
<td>Megaspilidae sp. 6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.288675</td>
<td>0.107</td>
</tr>
<tr>
<td>Meloidae sp. 1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Mordellidae sp. 1</td>
<td>231</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.583564</td>
<td>0.101</td>
</tr>
<tr>
<td>Muscidae sp. 1</td>
<td>45</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.542326</td>
<td>0.003</td>
</tr>
<tr>
<td>Muscidae sp. 2</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.263523</td>
<td>0.89</td>
</tr>
<tr>
<td>Mycetophilidae sp. 1</td>
<td>269</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.489588</td>
<td>0.071</td>
</tr>
<tr>
<td>Mycetophilidae sp. 2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.288675</td>
<td>0.121</td>
</tr>
<tr>
<td>Nepticulidae sp. 1</td>
<td>35</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.375124</td>
<td>0.871</td>
</tr>
<tr>
<td>Nitidulidae sp. 1</td>
<td>234</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.377913</td>
<td>0.534</td>
</tr>
<tr>
<td>Nitidulidae sp. 2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Nitidulidae sp. 3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Noctuidae sp. 1</td>
<td>18</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.333333</td>
<td>0.186</td>
</tr>
<tr>
<td>Nymphalidae sp. 1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.333333</td>
<td>0.186</td>
</tr>
<tr>
<td>Oecophoridae sp. 1</td>
<td>205</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.664211</td>
<td>NA</td>
</tr>
<tr>
<td>Oecophoridae sp. 2</td>
<td>60</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.474858</td>
<td>NA</td>
</tr>
<tr>
<td>Oecophoridae sp. 3</td>
<td>40</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.547274</td>
<td>0.004</td>
</tr>
<tr>
<td>Phoridae sp. 1</td>
<td>549</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.720838</td>
<td>NA</td>
</tr>
<tr>
<td>Pieridae sp. 1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Morphospecies</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
<td>P</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>--------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Platygastridae sp. 1</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.314819</td>
<td>0.939</td>
</tr>
<tr>
<td>Platygastridae sp. 2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Platygastridae sp. 3</td>
<td>33</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.486875</td>
<td>0.069</td>
</tr>
<tr>
<td>Platygastridae sp. 4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.258199</td>
<td>0.378</td>
</tr>
<tr>
<td>Platygastridae sp. 5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.288675</td>
<td>0.125</td>
</tr>
<tr>
<td>Platygastridae sp. 6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Pompilidae sp. 1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Pompilidae sp. 1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Pompilidae sp. 1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Pteromalidae sp. 1</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.275241</td>
<td>0.706</td>
</tr>
<tr>
<td>Pteromalidae sp. 2</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.354526</td>
<td>0.348</td>
</tr>
<tr>
<td>Pteromalidae sp. 3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Ptinidae sp. 1</td>
<td>143</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.570007</td>
<td>0.013</td>
</tr>
<tr>
<td>Ptinidae sp. 2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.258199</td>
<td>0.349</td>
</tr>
<tr>
<td>Ptinidae sp. 3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Ptinidae sp. 4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.288675</td>
<td>0.442</td>
</tr>
<tr>
<td>Scarabaeidae sp. 1</td>
<td>66</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.513426</td>
<td>0.228</td>
</tr>
<tr>
<td>Scarabaeidae sp. 2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Scarabaeidae sp. 3</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Scarabaeidae sp. 4</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.288675</td>
<td>0.423</td>
</tr>
<tr>
<td>Scarabaeidae sp. 5</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
</tr>
<tr>
<td>Morphospecies</td>
<td>Abundance</td>
<td>1-5</td>
<td>5-15</td>
<td>15-20</td>
<td>20-30</td>
<td>30-45</td>
<td>MRF</td>
<td>Stat</td>
<td>P</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>--------</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>Scarabaeidae sp. 6</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Scirtidae sp. 1</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Scoliidae sp. 1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sepsidae sp. 1</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sphecidae sp. 1</td>
<td>25</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.471405</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Sphecidae sp. 2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.288675</td>
<td>0.392</td>
<td></td>
</tr>
<tr>
<td>Staphylinidae sp. 1</td>
<td>60</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.602283</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Staphylinidae sp. 2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.333333</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Staphylinidae sp. 3</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.33121</td>
<td>0.321</td>
<td></td>
</tr>
<tr>
<td>Staphylinidae sp. 4</td>
<td>23</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.406736</td>
<td>0.394</td>
<td></td>
</tr>
<tr>
<td>Staphylinidae sp. 5</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Syrphidae sp. 1</td>
<td>49</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.46898</td>
<td>0.211</td>
<td></td>
</tr>
<tr>
<td>Syrphidae sp. 2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tabanidae sp. 1</td>
<td>15</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.34277</td>
<td>0.662</td>
<td></td>
</tr>
<tr>
<td>Tachinidae sp. 1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.258199</td>
<td>0.346</td>
<td></td>
</tr>
<tr>
<td>Tachinidae sp. 1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.258199</td>
<td>0.367</td>
<td></td>
</tr>
<tr>
<td>Tenebrionidae sp. 1</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.364</td>
<td>0.142</td>
<td></td>
</tr>
<tr>
<td>Tephritidae sp. 1</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.309735</td>
<td>0.425</td>
<td></td>
</tr>
<tr>
<td>Tephritidae sp. 2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tephritidae sp. 3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.235702</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Throscidae sp. 1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.333333</td>
<td>0.183</td>
<td></td>
</tr>
<tr>
<td>Tiphidae sp. 1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.258199</td>
<td>0.368</td>
<td></td>
</tr>
</tbody>
</table>
| Taxonomic Family       | Distribution
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tipulidae sp. 1</td>
<td>3 0 0 1 1 1 0 0.25 0.637</td>
</tr>
<tr>
<td>Torymidae sp. 1</td>
<td>1 0 0 0 0 1 0 0.288675 0.105</td>
</tr>
<tr>
<td>Vespidae sp. 1</td>
<td>4 1 0 0 0 0 0 0.235702 1</td>
</tr>
<tr>
<td>Vespidae sp. 2</td>
<td>2 1 0 1 0 0 0 0.235702 1</td>
</tr>
<tr>
<td>Zopheridae sp. 1</td>
<td>5 0 0 1 1 0 0 0.235702 1</td>
</tr>
</tbody>
</table>
Table A1.3: Post-hoc pairwise tests for one-way permutational multivariate analysis of variance on ranked Bray-Curtis dissimilarity matrix for \((\log_{10} + 1)\) transformed vegetation community data across corresponding survey site restoration years. Non-significant comparisons (bolded) indicate that compositions from each site are statistically indistinguishable.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>0.028*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2015</td>
<td>0.025*</td>
<td>0.114*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2011</td>
<td>0.030*</td>
<td>0.031*</td>
<td>0.032*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2010</td>
<td>0.028*</td>
<td>0.022*</td>
<td>0.032*</td>
<td>0.024*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2007</td>
<td>0.027*</td>
<td>0.038*</td>
<td>0.028*</td>
<td>0.033*</td>
<td>0.034*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2003</td>
<td>0.028*</td>
<td>0.024*</td>
<td>0.024*</td>
<td>0.029*</td>
<td><strong>0.089</strong></td>
<td><strong>0.159</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2002</td>
<td>0.020*</td>
<td>0.027*</td>
<td>0.036*</td>
<td>0.025*</td>
<td>0.034*</td>
<td>0.031*</td>
<td><strong>0.249</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2001</td>
<td>0.035*</td>
<td>0.028*</td>
<td>0.035*</td>
<td>0.040*</td>
<td>0.027*</td>
<td>0.036*</td>
<td>0.026*</td>
<td>0.030*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1996</td>
<td>0.023*</td>
<td>0.029*</td>
<td>0.034*</td>
<td>0.041*</td>
<td>0.030*</td>
<td>0.020*</td>
<td>0.030*</td>
<td>0.038*</td>
<td>0.024*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1994</td>
<td>0.033*</td>
<td>0.034*</td>
<td>0.038*</td>
<td>0.026*</td>
<td>0.023*</td>
<td>0.028*</td>
<td><strong>0.134</strong></td>
<td>0.027*</td>
<td>0.027*</td>
<td>0.031*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1991</td>
<td>0.031*</td>
<td>0.032*</td>
<td>0.032*</td>
<td>0.027*</td>
<td>0.032*</td>
<td>0.024*</td>
<td>0.023*</td>
<td>0.024*</td>
<td>0.023*</td>
<td><strong>0.110</strong></td>
<td><strong>0.204</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1985</td>
<td>0.031*</td>
<td>0.031*</td>
<td>0.028*</td>
<td>0.022*</td>
<td>0.034*</td>
<td>0.017*</td>
<td>0.030*</td>
<td>0.026*</td>
<td>0.037*</td>
<td>0.040*</td>
<td>0.023*</td>
<td>0.041*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1979</td>
<td>0.030*</td>
<td>0.033*</td>
<td>0.031*</td>
<td>0.029*</td>
<td>0.027*</td>
<td>0.031*</td>
<td>0.035*</td>
<td>0.033*</td>
<td>0.022*</td>
<td>0.029*</td>
<td>0.032*</td>
<td>0.036*</td>
<td>0.030*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MRF1</td>
<td>0.029*</td>
<td>0.038*</td>
<td>0.038*</td>
<td>0.029*</td>
<td>0.029*</td>
<td>0.033*</td>
<td>0.032*</td>
<td>0.022*</td>
<td>0.021*</td>
<td>0.028*</td>
<td>0.026*</td>
<td>0.024*</td>
<td>0.041*</td>
<td>0.032*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MRF2</td>
<td>0.024*</td>
<td>0.034*</td>
<td>0.032*</td>
<td>0.031*</td>
<td>0.028*</td>
<td>0.026*</td>
<td>0.032*</td>
<td>0.034*</td>
<td>0.023*</td>
<td>0.030*</td>
<td>0.042*</td>
<td>0.033*</td>
<td>0.039*</td>
<td>0.029*</td>
<td>0.032*</td>
<td>-</td>
</tr>
<tr>
<td>MRF3</td>
<td>0.037*</td>
<td>0.023*</td>
<td>0.035*</td>
<td>0.025*</td>
<td>0.039*</td>
<td>0.037*</td>
<td>0.029*</td>
<td>0.025*</td>
<td>0.035*</td>
<td>0.031*</td>
<td>0.023*</td>
<td>0.027*</td>
<td>0.028*</td>
<td>0.035*</td>
<td>0.027*</td>
<td>0.030*</td>
</tr>
</tbody>
</table>
Table A1.4: Post-hoc pairwise tests for one-way permutational multivariate analysis of variance on ranked Bray-Curtis dissimilarity matrix for (log10 + 1) transformed insect pollinator community data across sampled sites defined by their year of restoration. Significant comparisons (bolded) indicate that compositions from each site are statistically different.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2018</td>
<td>0.690</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2017</td>
<td>0.268</td>
<td>0.275</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>0.126</td>
<td>0.139</td>
<td>0.715</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>0.145</td>
<td>0.066</td>
<td>0.368</td>
<td>0.919</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>0.057</td>
<td>0.024*</td>
<td>0.083</td>
<td>0.258</td>
<td>0.529</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>0.045*</td>
<td>0.009**</td>
<td>0.041*</td>
<td>0.073</td>
<td>0.322</td>
<td>0.392</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>0.032*</td>
<td>0.003**</td>
<td>0.022*</td>
<td>0.035*</td>
<td>0.173</td>
<td>0.485</td>
<td>0.880</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>0.038*</td>
<td>0.008**</td>
<td>0.019*</td>
<td>0.045*</td>
<td>0.219</td>
<td>0.507</td>
<td>0.401</td>
<td>0.817</td>
<td>0.452</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>0.017*</td>
<td>0.004**</td>
<td>0.009**</td>
<td>0.025*</td>
<td>0.141</td>
<td>0.479</td>
<td>0.724</td>
<td>0.814</td>
<td>0.883</td>
<td>0.775</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>0.066</td>
<td>0.029*</td>
<td>0.052</td>
<td>0.310</td>
<td>0.600</td>
<td>0.427</td>
<td>0.186</td>
<td>0.184</td>
<td>0.187</td>
<td>0.555</td>
<td>0.316</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1994</td>
<td>0.052</td>
<td>0.008**</td>
<td>0.030*</td>
<td>0.069</td>
<td>0.455</td>
<td>0.452</td>
<td>0.399</td>
<td>0.545</td>
<td>0.475</td>
<td>0.818</td>
<td>0.502</td>
<td>0.524</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1991</td>
<td>0.030*</td>
<td>0.006**</td>
<td>0.052</td>
<td>0.143</td>
<td>0.31</td>
<td>0.425</td>
<td>0.181</td>
<td>0.319</td>
<td>0.335</td>
<td>0.528</td>
<td>0.473</td>
<td>0.957</td>
<td>0.809</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1985</td>
<td>0.033*</td>
<td>0.016*</td>
<td>0.028*</td>
<td>0.278</td>
<td>0.535</td>
<td>0.651</td>
<td>0.479</td>
<td>0.611</td>
<td>0.276</td>
<td>0.572</td>
<td>0.75</td>
<td>0.543</td>
<td>0.407</td>
<td>0.263</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1979</td>
<td>0.040*</td>
<td>0.011*</td>
<td>0.022*</td>
<td>0.154</td>
<td>0.279</td>
<td>0.584</td>
<td>0.325</td>
<td>0.264</td>
<td>0.08</td>
<td>0.300</td>
<td>0.164</td>
<td>0.204</td>
<td>0.443</td>
<td>0.185</td>
<td>0.685</td>
<td></td>
</tr>
<tr>
<td>MRF1</td>
<td>0.044*</td>
<td>0.011*</td>
<td>0.021*</td>
<td>0.214</td>
<td>0.405</td>
<td>0.589</td>
<td>0.13</td>
<td>0.168</td>
<td>0.087</td>
<td>0.349</td>
<td>0.304</td>
<td>0.609</td>
<td>0.326</td>
<td>0.536</td>
<td>0.802</td>
<td>0.684</td>
</tr>
</tbody>
</table>

**Significant differences at p < 0.01.**

*Significant differences at p < 0.05.
Appendix 2: Reproductive responses of cavity-nesting pollinators, predators and parasitoids to vegetation structure and microclimate associated with post-mining restoration

Figure A2.1 The patterns of reproductive activity in terms of brood abundance (yellow) and nest construction (blue) for cavity-nesting Hymenoptera plotted against the average daily temperature per month for each site per monitoring round (month: 1) December; 2) January; 3) February; and 4) March). The daily temperature was calculated for times between 6:00am and 6:00pm.
Appendix 3: Interspecific variation in the thermal tolerance and performance of solitary bees and their parasitoid associate.

Figure A3.1: Cumulative mean time across early (ESF), mid (MSF) and late-successional (LSF) stages of forest restoration and unmined reference forests (MRF) sites: a) within the optimal preferred conditions (defined as temperatures between thermal preference ($T_{pref}$) and thermal optima ($T_{opt}$) of *Megachile aurifrons* (■) and *Gasteruption breviscutum* (●); b) exceeding the critical thermal maximum for *M. aurifrons* (■) and *G. breviscutum* (●); and c) above 38.5°C (■), representing thermal conditions that allow *M. aurifrons* (host) to outperform *G. breviscutum* (parasitoid).
Appendix 4: Interspecific variation in the thermal tolerance and performance of solitary bees and their parasitoid associate.

Figure A4.1: Cumulative mean time a) within the optimal preferred conditions (defined as temperatures between thermal preference ($T_{\text{pref}}$) and thermal optima ($T_{\text{opt}}$) of *Megachile aurifrons* larvae (■) and adults (●) across early (ESF), mid (MSF) and late-successional (LSF) stages of forest restoration and unmined reference forests (MRF) sites.