Insects as Food in Aotearoa New Zealand

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Abstract

Edible insects have been proposed as a sustainable future protein source for a growing global population. Overseas, interest has seen investment in start-up companies specialising in producing insects for human consumption. Yet little is known of the edible insect species in Aotearoa New Zealand or whether New Zealanders are willing to consume insects. To address this, I investigated the current landscape of edible insects in Aotearoa. I created a catalogue of species with traditional and contemporary uses, the companies that are producing insect-based products and the social and legislative landscape they exist in. I then explored the social licence for insects as food, using a questionnaire to examine the perceptions and experiences of 620 participants, finding an openness to insects as food, influenced by prior experience, knowledge, and a low food neophobia score. However, this was tempered by sentiment analysis showing insect ingredients were viewed more negatively than other food ingredients suggesting an unconscious bias. The huhu beetle, *Prionoplus reticularis*, was widely listed by participants as an edible insect in Aotearoa, suggesting a potential candidate for future farming. However, surprisingly little is known about the biology of this well-known beetle. To address this, I used GCMS to explore the metabolomic profile of this species along a latitudinal gradient. I found that a random forest model trained on this data was able to predict the geographic location of a larvae with reasonable accuracy, providing a possible tool to detect the provenance of edible insects in the future. I investigated the beetles' scramble competition mating system, and, using static allometry and flight mills, I found evidence of the interplay of sexual selection and stabilising selection on traits associated with male fitness in this species. I also found evidence of sexual dimorphism in the species, indicating the importance of female chemical cues to male mate searching. Using scanning electron microscopy, I investigated the antennal ultrastructure of male and female *P. reticularis*, describing unique, sexually dimorphic sensilla in this species. This thesis serves as a starting point for the development of edible insects in Aotearoa.

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Chapter 1 A General Introduction to Insects as Food

"If we could get over our phobia of eating insects you could solve world starvation. It's high protein, no fat, extreme nutrients, and they're just abundant, they're everywhere. But, no, we're not going to do that!"

There likely comes a point in every doctoral candidate's journey, where critically acclaimed, Academy Award winning actor, Nicolas Cage, summarises four years of their work in an offhand comment to *Entertainment Weekly* (Collis, 2023). Whilst insects do contain fat, Mr. Cage correctly points out the potential of edible insects to address concerns of protein supply as humanity's global population increases, but also acknowledges the perceived disgust and reticence of many cultures, but particularly in the west, to consume insects. However, it is not just Hollywood stars suggesting insects could be a solution to our food security concerns.

With the global population expected to reach 9 billion by 2050, the Food and Agriculture Organisation of the United Nations (FAO) estimates that the global agricultural demand will increase by 50 percent of the demand in 2013 (Food and Agriculture Organization of the United Nations, 2017). In light of this challenge, there has been increased interest in the place of insects within the human diet. Entomophagy is already a common practice in many cultures, with over 1,500 recorded species of insects consumed globally (Van Itterbeeck & Pelozuelo, 2022) by several hundred-million people (van Huis et al., 2022). Globally, few insect species are reared commercially at an industrial scale for food, with the majority being wild harvested. The primary species reared commercially for human consumption are crickets (e.g. *Acheta domestica*, *Gryllus bimaculatus*, *Teleogryllus commodus*) and mealworms (e.g. *Alphitobius diaperinus*, *Tenebrio molitor*,

Zophobas morio) (van Huis, 2016). However, interest in eating insects has only increased in western cultures over the past decade.

The production of insects can be achieved through wild-harvest, semi-domestication, or farming. Wild harvesting has been practiced by humans for millennia and is particularly effective for gregarious species of insects allowing for efficient collection. The wild harvesting of insects can be practiced as a form of biocontrol for pest species in agriculture whilst supplementing protein in the diet. A successful example can be seen with *Patanga succincta* locusts which were a major agricultural pest in Thailand prior to a successful campaign to use them as food (Hanboonsong, 2010). Semi-domestication involves manipulating the environment to facilitate the rearing of insects for part of their lifecycle. For example, palm trees in some South-East Asian countries will be cut down to provide palm weevil breeding sites (Yen, 2015). Farming incorporates the entire lifecycle of the insect in an environmentally controlled environment with varying degrees of automation. Possibly the oldest example of farming insects is silk farming, which has been practiced for over 5000 years. Silk production was the primary objective here with the de-cocooned pupae a by-product that was used as food (Cherry, 1987).

The lower environmental impacts of rearing insects for food are often cited to increase the sustainability of our food production systems, compared to conventional sources of animal protein production (Halloran et al., 2017; Oonincx et al., 2010; Oonincx & de Boer, 2012). Life cycle assessments, an analytical tool to assess the environmental impact of a product, suggest that land-use and greenhouse gas emissions of insect-rearing are lower than other forms of livestock (Bosch et al., 2019; Halloran et al., 2017; Nikkhah et al., 2021; Oonincx & de Boer, 2012), however, this is dependent on the rearing methods used (Bosch et al., 2019; Oonincx & de Boer, 2012). In comparison to traditional livestock, insects, which are poikilotherms, have a favourable food conversion ratio (FCR) with some species able to utilise low quality or non-traditional feedstocks that may otherwise be seen as a waste stream (e.g., some cerambycid larvae can

consume forestry waste, mealworms can utilise vegetable scraps and brewer's yeast). The FCR (kg feed/kg of fresh weight) for mealworms is 2.2 which compares to 2.3 in poultry, 4.0 in swine, and 2.7 – 8.8 in beef (depending on the feed used) (Oonincx & de Boer, 2012).

Like other livestock, the rearing of insects also produces greenhouse gasses (GHGs) and waste comprised of exuviae (moulted insect skin) and frass (faeces and undigested substrate) which also produces GHGs. Insects, and their frass, produce much lower carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) than cattle making them a more environmentally sustainable solution than conventional livestock (Oonincx et al., 2010). The production of ammonia (NH_3), which causes nitrification of waterways and indirectly N_2O production, is also lower and, combined with the ability to utilise waste streams from other areas of primary production, the frass can be used in the agricultural system as a fertiliser enabling a circular economy (Dicke, 2018; Oonincx & de Boer, 2012).

The land use to produce one kilogram of insect protein is considerably smaller than other forms of livestock (de Vries & de Boer, 2010; Oonincx & de Boer, 2012) with production of one kilogram of mealworm protein requiring 43% and 10% of the land required to produce one kilogram of animal protein of dairy and beef respectively. This is due to the small size of the insect allowing for vertical farming, efficient conversion of feed, and their feed being derived from by-products rather than pasture (Oonincx & de Boer, 2012). Similarly to land use, water use is also much lower for insect production with beef requiring five times the amount of water to produce the equivalent unit of mealworm protein (112 L/g to 23 L/g respectively) (Miglietta et al., 2015). Again, the high feed efficiency of mealworms is attributed to this small water footprint.

However, there are challenges associated with the use and development of insect-based food production. Yen (2008) highlights the important role of conservation and traditional ecological knowledge in avoiding over exploitation should wild harvesting of insects increase in Australia.

There have been instances where the popularity of an insect, and loss of traditional ecological knowledge, has resulted in over-harvesting in the wild leading to localised extinction (Ramos-Elorduy, 2006). Wild harvesting can also be compounded by land use change, intensive agriculture and climate change (Yen, 2008) as shown in the case of the Australian edible insect, the bogong moth (*Agrotis infusa*) which migrates between lowland pastures and alpine regions (where it is harvested by First Nations people). Arsenic from agricultural sprays used in the low land areas is accumulated by the moth and transported to fragile alpine ecosystems (Green, 2008; Green et al., 2001) elevating the presence of arsenic in these areas.

Farming insects also carries challenges and risks. Whilst being a poikilotherm benefits feed conversion efficiency, the same trait requires that the farmer maintain an optimal rearing temperature for the insect. This can result in higher energy use than livestock such as dairy, poultry and pork which, depending on the energy source used, can increase GHG emissions (Oonincx & de Boer, 2012). There is ample opportunity to reduce this energy use through careful selection of the species (such as those adapted to the ambient environment), optimising the rearing environment (e.g., through insulation), and using renewable or waste energy (e.g., geothermal, waste heat from industrial processes). Other risks can come from chemical contaminants from the insect feed being bioaccumulated: heavy metals (Diener et al., 2015; Lindqvist & Block, 1995; Purschke et al., 2017; van der Fels-Klerx et al., 2016), residues of dioxins, polychlorinated biphenyls and polyaromatic hydrocarbons (Charlton et al., 2015) and pesticides can also be present (Purschke et al., 2017). Insects can also be mechanical vectors of biological contaminants such as bacteria (Klunder et al., 2012; Stoops et al., 2016), parasites and prions (Müller et al., 2019; Post et al., 1999). These risks, by no means unique to insects, highlight the importance of quality assurance throughout the supply chain, from selection of feeds and substrates when rearing insects through to post-harvest treatment and storage (van der Fels-Klerx et al., 2018) and existing risk-mitigations can be adapted from the food industry.

There are numerous start-ups around the globe specialising in rearing edible insects, and 500,000 tonnes of insect protein per annum is predicted to be produced by 2030 (de Jong & Nikolik, 2021). However, it is difficult to approximate the size of the current edible insect market as many early-stage companies are reticent to share details of their production capacity. An example of the scale of commercial production can be seen in Thailand where around 20,000 cricket farms supply 7,500 tonnes per annum and are the primary source of income for many farmers (Hanboonsong et al., 2013). Several companies in Thailand also export to overseas markets, including New Zealand.

A growing interest in eating insects in the west is contrasted against a perceived historic dislike of insects as food in most Western cultures. Several studies have investigated Western consumers' perceptions of insects and suggested various strategies to increase market uptake of insects as food (See reviews by: Alhujaili et al., 2023; Ardoin & Prinyawiwatkul, 2021). There are, however, isolated examples of insects having been consumed in a traditional setting in Western countries. For example, cockchafer beetles in France and Germany were made into a soup; it was proposed that rocky mountain locusts be eaten to control their numbers in the United States of America; and a traditional delicacy, *casu marzu*, is a cheese consumed in regions including and surrounding Sardinia that contain the larvae of the dipteran, *Piophila casei* (Lockwood, 2004; van Huis, 2013, 2020; van Huis & Tomberlin, 2016).

Cerambycids are used as a food source in many cultures around the world (Chakravorty et al., 2011; Gahukar, 2011; Mitsuhashi, 2003; Pond, 1994; Schrader et al., 2016; Turpin & Si, 2017) with the larvae and, in some cases, the adult life stage being consumed. In all cases cerambycids are derived from wild harvest when seasonally available. This poses a constraint on consumption which limits uptake and risks exploitation of wild populations. Aotearoa is host to a well-known edible prionine cerambycid, the huhu beetle *Prionoplus reticularis* White 1843 (Coleoptera: Cerambycidae).

P. reticularis is saproxylic, feeding on a range of native and exotic timber and can reach high densities in stands of *Pinus radiata* (Edwards, 1956, 1959, 1961c). Much of what is known about the biology of *P. reticularis* is shaped by its role as a pest of untreated timber and efforts to stop it (Cross, 1994; Dentener et al., 1999, 2001; Hedley et al., 1979; Hood & Gardner, 2004; G. Hosking, 2007; Lester et al., 2000; Murray, 1973; Pawson et al., 2020; Pawson & Sky, 2009; Pawson & Watt, 2009). The larvae are relatively slow growing, taking between two and three years feeding on decaying wood prior to making a pupal chamber and maturing into the non-feeding adult stage (Rogers et al., 2002). Despite feeding on a fairly nutrient poor substrate, the adult beetle can accrue substantial energy reserves, a feat likely due to the diverse range of rare microbial phylotypes inhabiting its digestive system (Reid et al., 2011). Upon eclosion, the adult beetle spends roughly two weeks searching for mates and ovipositioning sites, whilst surviving on energy stores accrued as a larvae (Edwards, 1961c). These energy stores are reflected in the nutritional profile of *P. reticularis*, which contains relatively high levels of lipids (~58 % by dry weight in the pupae), with protein extracts being of good nutritional quality, and abundant in manganese, magnesium, phosphorus, iron, copper, and zinc (Hutchins & Christmas, 1973; Kavle et al., 2022b, 2022a, 2023). Cadmium and lead is also detectable, however at moderate levels of consumption, *P. reticularis* are safe to eat (Kavle et al., 2022a). *P. reticularis* is a well-known and culturally significant species that historically has been regarded as a food item, eaten raw or roasted (Best, 1902; Miller, 1952). The larvae are known to vary in flavour depending on the wood they have been feeding on (Pond, 1994), indicating that the compounds in different timber influence the chemical profile of the larvae.

Whilst the huhu beetle is the most well-known edible insect, several insect species have been used as a traditional food source and a contemporary industry appears to be forming around a variety of native and exotic edible insect species. Despite this nascent industry, there is little scientific literature around which species of insects have been traditionally consumed and their

basic biology and life histories. This knowledge gap underscores the need for comprehensive research to not only identify insect species but also understand their fundamental biological attributes and life cycles. Such insights are essential for the sustainable development and informed promotion of the edible insect industry in New Zealand.

Thesis Outline

This thesis is guided by an emphasis on the identified need for multi- and trans-disciplinary approaches to advance the edible insect industry (van Huis, 2017). In this thesis, I aim to understand the social licence of edible insects in this country by investigating (1) the diversity and history of use of edible insect species in Aotearoa New Zealand and their place within our culture, and (2) the uses and perceptions of New Zealanders towards consuming insects. I then focus on the biology of a well-known, but little researched, endemic edible insect species, the huhu grub *P. reticularis* to: (1) develop and assess the potential of metabolomic techniques to identify the provenance of edible insect species, using *P. reticularis* as a case study, (2) investigate the mating system of *P. reticularis* using static allometry and flight mills to assess the importance of traits associated with fitness in a scramble competition mating system, and (3) describe the sexually dimorphic antennal ultrastructure of *P. reticularis* to shed light on the importance of chemical ecology on this mating system.

In Chapter 2, I conduct a comprehensive review of the use of insects in Aotearoa New Zealand. For this, I reviewed the literature, grey literature, newspaper articles, existing legislation, companies register, and websites of businesses selling insects for food, to (a) catalogue known species of insects used for food, past and present, (b) review what is currently known about potential native insect food species to identify promising candidates for rearing, (c) investigate the legislative and social landscape companies operate in, and (d) catalogue companies operating in this sector over the last 10 years, and how they market edible insects to the public.

In Chapter 3, using an online questionnaire, I explore the use and perceptions of the public towards insects as food in Aotearoa. The objective of this questionnaire was to identify the acceptability of insects as food, the traits associated with a willingness to eat insects, existing knowledge of edible insects, and the sentiments people exhibit towards insects as food. I analyse the data using a variety of techniques, including machine learning and sentiment analysis. I discuss my findings in the context of seemingly contradictory findings in Chapter 2.

After exploring the social licence within which the edible insect industry operates, I move to the biology of a charismatic edible insect species, that is well known to the public, the huhu beetle. Chapter 4 assesses the potential of metabolomic fingerprinting as a tool for determining the provenance of an edible insect species. To achieve this, I sample the larvae of *P. reticularis* along a natural latitudinal gradient the length of Aotearoa. I compare two different derivatisation techniques using GC-MS to create a metabolomic profile of beetles from different locations. I then use a variety of statistical techniques to describe the variance in metabolic traits and train and test a random forest classification model to predict which location a larvae came from.

Chapter 5 focuses on unravelling the intricacies of *P. reticularis'* scramble competition mating system, characterised by intense male competition for access to females. The objective of this chapter is to discern the extent of sexual dimorphism and patterns of allometry on traits that are important for male fitness in scramble competition: sensory and locomotory traits. In this chapter, I design and build a flight mill to assess how wing size and body size influence flight performance. This chapter identifies tension between sexual selection and stabilising selection, contrasting *P. reticularis* with other species exhibiting scramble competition mating systems.

Chapter 6 builds on the findings of Chapter 5, by using scanning electron microscopy to describe in detail the sensory ultrastructures and dimorphism found in the antennae of *P. reticularis*. This chapter reveals unique structures only present on the male antennae and I discuss possible implications for the sensory ecology of this species.

Chapter 2 A review of insects as food and the edible insect industry in Aotearoa New Zealand

Abstract

I conducted a review of the edible insect landscape in Aotearoa New Zealand. Despite evidence indicating the receptiveness of New Zealanders to insect consumption, little is known of the country's edible insect species, the cultural context of insect consumption, and the viability of native or endemic species for farming. To address these gaps, I compiled a comprehensive species catalogue encompassing a diverse array of endemic, native, and exotic edible insect species that have historical and/or contemporary uses in Aotearoa. Additionally, I examined edible insect companies that have operated in Aotearoa since 2015, assessing their current status, advertising strategies, and the promotional tactics employed within the nascent industry. This study contributes valuable insights into the edible insect landscape in Aotearoa, providing insight into the available species, cultural nuances, and the evolving dynamics of the industry. I hope this research provides a foundation for further research and the development of sustainable practices within the sector.

Introduction

The world's rapidly growing population, expected to reach over 9.7 billion by 2050 (United Nations, 2019), along with an increase in the wealth of developing countries, will increase the demand for animal proteins for food (Boland et al., 2013) and raises concerns for food security (Godfray et al., 2010). Insects have been highlighted by the United Nations Food and Agriculture Organisation (FAO) (van Huis et al., 2013) as an important source of dietary protein

and, due to key aspects of their biology, some insect species are a more sustainable option than sources of protein from livestock, poultry or seafood. Insects are poikilothermic (Byrd & Castner, 2002) which means energy gained from food is allocated to growth rather than maintaining body temperature (Bartley et al., 1980). This allows for a feed conversion efficiency higher than poultry and much higher than mammalian livestock (Nakagaki & Defoliart, 1991; Rumpold & Schlüter, 2013). Generally, most insects produce lower levels of greenhouse gasses than mammals and some species have the potential to convert organic waste into valuable products. Internationally, around 92% of edible insect species are wild harvested with 6% being semi-domesticated (i.e. not isolated from wild input) and 2% of species are farmed (Yen, 2015).

Most current research attention is directed towards farming a small number of cosmopolitan species, however, the insect kingdom in incredibly diverse and I would be shortsighted not to investigate other insects that are used as food around the world. The Pacific is often overlooked in discussions of insects as food despite having several wild harvested cerambycid species that are consumed, including *Agrianome fairmairei, Oletherius* spp., and *Prionoplus reticularis* which are consumed in New Caledonia, Fiji, Futuna, Sāmoa, Tonga and New Zealand (Pond, 1994; Schintu & Fasquel, 2019). In New Zealand, there are several edible insect species that have been recorded in the literature: the aforementioned *P. reticularis*, *Pyronota festiva* (Coleoptera: Scarabaeidae), *Aenetus virescens* (Lepidoptera: Hepialidae) (Jongema, 2017; Meyer-Rochow & Changkija, 1997).

As a nation in which the majority of the population are of European descent (Stats NZ Tatauranga Aotearoa, 2018), consumption of insects in Aotearoa New Zealand is not perceived to be a common practice. Some insects, of which the huhu beetle larvae (*P. reticularis*) is best known, were consumed prior to European arrival. There is, however, an increasing interest in entomophagy in Aotearoa New Zealand (O'Connor et al., 2023; P. Payne et al., 2023). However, with a small number of exceptions, most insect food products in Aotearoa New Zealand are

imported exotic species, characteristics which make it difficult to assess the sustainability and environmental impact of production.

Given this increasing interest in entomophagy amongst consumers, I believe that this is a worthwhile time to review the current state of entomophagy in Aotearoa New Zealand both within the market and current research into insect food production. I reviewed the literature, grey literature, newspaper articles, existing legislation, companies register, and websites of businesses selling insects for food, to (a) catalogue known species of insects used for food, past and present, and (b) review what is currently known about potential endemic food species to identify promising candidates for rearing, and (c) investigate the legislative and social landscape companies operate in, and (d) catalogue companies operating in this sector, and how they market edible insects to the public.

Definitions

Under the Food Standards Australia New Zealand Act 1991, "food" broadly refers to substances for human consumption, including ingredients, additives and substances used in preparation. It excludes goods with a therapeutic use i.e., medicines. Delineating between insects eaten entirely for nutrition rather than therapeutic good can be difficult especially when considering historical consumption (see Meyer-Rochow & Changkija, 1997) so I will include insects in this review that may have had dual purposes. For the purposes of this review, edible insects and entomophagy will only include the consumption of insects themselves and exclude products made by insects (e.g., honey). Definitions for te reo Māori are included in Table 1.

Table 1. Definitions for te reo Māori terms. These are derived from Te Aka Māori Dictionary however many of these terms have deeper philosophical nuances that cannot be adequately captured here.

Term	Definition
Iwi	Extended kinship group, tribe, nation, people, nationality, race - often refers to a large group of people descended from a common ancestor and associated with a distinct territory
Kaitiaki	Trustee, minder, guard, custodian, guardian, caregiver, keeper, steward
Kaupapa Māori	Māori approach, Māori topic, Māori customary practice, Māori institution, Māori agenda, Māori principles, Māori ideology - a philosophical doctrine, incorporating the knowledge, skills, attitudes, and values of Māori society
Kōrero	Speech, narrative, story, news, account, discussion, conversation, discourse, statement, information.
Mātauranga Māori	Māori knowledge - the body of knowledge originating from Māori ancestors, including the Māori world view and perspectives, Māori creativity and cultural practices
Rohe	Boundary, district, region, territory, area, border (of land)
Tangata whenua	Local people, hosts, indigenous people - people born of the whenua, i.e., of the placenta and of the land where the people's ancestors have lived and where their placenta are buried.
Taonga	Treasure, anything prized - applied to anything considered to be of value including socially or culturally valuable objects, resources, phenomenon, ideas, and techniques
Te ao Māori	The Māori worldview
Te reo Māori	The Māori language
Te Tiriti o Waitangi	The treaty of Waitangi; the document signed in 1840 which guides the relationship between the Crown and Māori
Waitangi Tribunal	The Waitangi Tribunal is a standing commission of inquiry. It makes recommendations on claims brought by Māori relating to legislation, policies, actions, or omissions of the Crown that are alleged to breach the promises made in the Treaty of Waitangi
Waka	Canoe or vessel. Allied kinship groups descended from the crew of a canoe which migrated to New Zealand and occupying a set territory
Whakapapa	Genealogy, genealogical table, lineage, descent

Species catalogue

The species catalogue was composed of insects, endemic, native, and exotic, that are used in New Zealand for human consumption at present and historically. This was collated from the literature, grey literature, newspaper articles and businesses selling insects for food. Only a small number of endemic insect species appear in written records as being a food source and it is likely that knowledge relating to the harvesting and preparation of insects as food is held in oral histories or has been lost through colonisation. It should be stressed that the species presented that may have had a traditional use as food have not been confirmed with mana whenua and future research would greatly benefit from this (See O'Connor et al., 2023).

The grey literature shows there was often confusion with names in Te Reo Māori, variation in terms between different iwi, misidentification of species and substantial taxonomic revision since original publication (Best, 1902; Ehau-Taumaunu et al., 2020; Miller, 1952). Settler accounts of insects being used as food should be viewed in the context of colonisation. The eating of insects has been used as evidence that a culture is "primitive" as opposed to "civilised" which has been used as a justification for colonisation as noted by Kosonen (2022), "so-called "primitive" foodways like entomophagy have been instrumentalized in the colonial differentiations between civilization and primitivism". Further, an ethnographer's interest may bias their interpretation of their observations (Mercier et al., 2022). For instance Drummond (1927a) states "as civilisation fosters fastidiousness, insects as food are repugnant to most civilised peoples". Yet, in Europe, the collection of the agricultural pest, *Melontha sp.,* was practiced by French and Germans and the soup produced from it was considered a delicacy well into the 1900's (J. J. Schneider, 1844; van Huis, 2020). In a similar fashion, some caterpillars were noted as pests of Māori but also served as food, medicine and artistic ingredients (Mercier et al., 2022).

It would be naive to believe that the available literature accurately encompasses all pre-European uses of insects by Māori. While the anthropological record has identified species which were "traditionally" consumed, it is not possible to extrapolate how frequent and how widespread these practices were. An incalculable amount of knowledge of te ao Māori remains within oral histories and future studies would greatly benefit from a kaupapa Māori-led approach.

Consumption of most endemic/native species of insects has been largely replaced by exotic species imported from overseas (Table 3). Of the ten known insect species that may have been included in a pre-European diet, only the huhu beetle (*P. reticularis*) is the widely known and used, frequently appearing in food festivals, popular culture and, briefly, a vodka brand (Gordon, 2015; Modlik & Johnston, 2017; Schulz, 2019; Ward, 2013). The larvae of the huhu beetle are perhaps the most well-known of the insects in New Zealand that have been eaten as food. Wild harvest is

currently the sole means of production of this insect for food and whilst there has been little research attention with the aim of commercial production of the species, there has been some research on their biology and ecology due to their potential as a pest in the timber products industry (e.g., Alma & van Boven, 1975; Cross, 1994; Dentener et al., 1999, 2001; G. Hosking, 2007; G. P. Hosking, 1979; Lester et al., 2000; Murray, 1973; Pawson et al., 2020; Pawson & Sky, 2009; Pawson & Watt, 2009; Reid et al., 2011; Romo et al., 2018).

A catalogue of insect species used as food in New Zealand, both past and present, is presented in Table 2. Overall, at least twenty-one species of insects from seven insect orders are, or were, consumed in New Zealand. Of the 2,111 species of insects in Jongema's (2017) collated list of edible insects (but see Van Itterbeeck & Pelozuelo, 2022) from around the world, only three species are listed for New Zealand: the huhu beetle, *P. reticularis*; pūriri moth, *Aenetus virescens* ; and Mānuka beetle, *Pyronota festiva*. From our findings, I can add a further seven endemic or native species that have traditional and/or contemporary use: grass grubs/tūtaeruru (*Costelytra zealandica* and/or *Odontria zealandica)*, migratory locust (*Locusta migratoria*), black field cricket (*Teleogryllus commodus*), kumara moth/hīhue (*Agrius convolvuli*), kihikihi kai (*Kikihia muta*), *tīkopa*/leaf rollers (Tortricidae family - species unknown) and puene (possibly *Archichauliodes diversus*).

Table 2. A catalogue of past and present edible insects recorded in Aotearoa New Zealand.

Taxa	Species	Common Names	Biostatus	Traditional use	Harvest Method	Production	Consumption	Products	Lifestage	Source
Coleoptera	*Prionoplus reticularis*	Huhu beetle, pepe tunga, haircutter	Endemic	Yes	Wild	Domestic	Domestic	Whole insect	Larvae	(Andersen, 1933; Anonymous, 1922; Anteater, 2019; Best, 1902; Bodenheimer, 1951; Drummond, 1927a, 1927b, 1933; Fiso et al., 2020; Magister, 1909; Meyer-Rochow & Changkija, 1997; Miller, 1952; Murihiku, 1931; Pond, 1994; Pycroft, 1929)
Coleoptera	*Costelytra zealandica* and/or *Odontria zealandica*	Tūtaeruru, grass grubs	Endemic	Yes	Wild	Unknown	Domestic	Whole insect	Adult	(Best, 1902; Miller, 1952)
Coleoptera	*Pyronota festiva*	Kerewai, kekerewai or reporepowa, mānuka beetle	Native	Yes	Wild	Unknown	Domestic	Whole insect	Adult	(Best, 1898, 1902; Drummond, 1927a; Meyer-Rochow & Changkija, 1997; Miller, 1952)
Coleoptera	*Tenebrio molitor*	Mealworm, darkling beetle	Non-native	No	Farmed	Domestic/international	Domestic	Whole insect	Larvae	(Eat Crawlers, 2019)
Coleoptera	*Zophobas morio*	Mealworm, superworm, superworms, king worms, morio worms	Non-native	No	Farmed	International	Domestic	Whole insect	Larvae	(Eat Crawlers, 2019)
Hemiptera	*Kikihia muta*	Variable cicada, kihikihi kai	Native	Yes	Wild	Unknown	Unknown	Whole insect	Unknown	(Meyer-Rochow & Changkija, 1997; Miller, 1952; Stevens, 1930)
Hymenoptera	*Polyrhachis* sp.	Black ants	Non-native	No	Unknown	International	Domestic	Whole insect	Adult	(Eat Crawlers, 2019)
Hymenoptera	*Vespula* sp.	German wasp, common wasp	Non-native	No	Wild	Domestic	International	Whole insect	Larvae, pupae	(Matsuura, 1999; McLean, 2018)
Hymenoptera	unknown	New Zealand lemongrass ant	Unknown	No	Wild	Domestic	Domestic	Whole insect	Adult	(Anteater, 2019)

Lepidoptera	*Aenetus virescens*	Pūriri moth, pepetuna, ghost moth	Endemic	Yes	Wild	Unknown	Unknown	Whole insect	Larvae	(Meyer-Rochow & Changkija, 1997; Miller, 1952)
Lepidoptera	*Agrius convolvuli*	Hīhue, wenewene, kōwenewene, kumara moth	Native	Possibly	Wild	Domestic	Domestic	Whole insect	Larvae	Investigated as a food at PFR (Pers. Comms.)
Lepidoptera	*Tortricidae Family,* sp. Unknown	Moka, tīkopa, leaf rollers	Native/endemic	Yes	Wild	Unknown	Unknown	Whole insect	Larvae	(Best, 1902; Miller, 1952)
Lepidoptera	*Bombyx mori*	Silkworm, beondegi	Non-native	No	Farmed	International	Domestic	Whole insect	Pupae	(Tan, 2017)
Orthoptera	*Locusta migratoria*	Locust, migratory locust, kapakapa, rangataua	Native	No	Farmed	domestic/international	Domestic	Whole insect	Adult, nymph	(Anteater, 2019)
Orthoptera	*Teleogryllus commodus*	Black field cricket	Native	No	Farmed	Domestic	Domestic	Meal	Adult, nymph	(H. King, 2018; Mulligan, 2019)
Orthoptera	*Anostostomatidae family*, sp. unknown	Wēta	Native	Doubtful	Wild	Unknown	Unknown	Traditional use seems unlikely, unconfirmed by any primary sources	Adult, nymph	(Crowe, 2002; O'Connor et al., 2023; Tucker, 2013)
Orthoptera	*Nomadacris succincta*	Bombay locust	Non-native	No	Unknown	International	Domestic	Whole insect	Adult, nymph	(Eat Crawlers, 2019)
Orthoptera	*Gryllus bimaculatus*	African field cricket, mediterranean field cricket	Non-native	No	Farmed	International	Domestic	Whole insect	Adult, nymph	(Eat Crawlers, 2019)
Orthoptera	*Acheta domestica*	House cricket	Non-native	No	Farmed	International	Domestic	Whole insect	Adult, nymph	(Eat Crawlers, 2019)
Lepidoptera	*Wiseana sp., Aoraia enysii*	Porina moth, forest ghost moth	Native	Possibly	Wild	Domestic	Domestic	Possibly referring to the "vegetable caterpillar" which is an entomopathogenic fungus	Larvae	(Crowe, 2002; Relph, 1991)

								which had several uses and is described as tasting nutty.		
An aquatic insect: Ephemeroptera, Megaloptera or, Plecoptera	Sp. unknown, possibly *Archichauliodes diversus*	Puene	Unknown	Possibly	Wild	Domestic	Domestic	In the Whakatane River: a little creature having six legs and eaten by children.	Larvae	(Best, 1902; Miller, 1952)

Huhu beetle – *Prionoplus reticularis*

P. reticularis is mentioned as being a prized food item eaten raw or roasted after removal from rotting logs (Best, 1902, 1908; Miller, 1952). When eaten raw, they were "eaten alive like an oyster, only the horny black head is bitten off and thrown away" (Anonymous, 1922) or roasted till brown and eaten as a delicacy (Andersen, 1933). Best (1902) mentions that two stages in the life cycle of *P. reticularis* are consumed, the larval stage (tunga rākau) and the final instar after feeding ceases and it undergoes a final moult in the pupal chamber (tātaka). The larvae feeds on several tree species found across New Zealand. It has been suggested that larvae extracted from different tree species have different flavour profiles for instance larvae found in matai timber are described as "distinctly sweet in flavour", whilst larvae from pine or macrocarpa have an "aromatic taste not at all unpleasant" (Anonymous, 1922). It is also recorded that Māori are familiar with the different flavours imparted on grubs by the timber it comes from, with miro being particularly prized (Pond, 1994). *P. reticularis* was frequently mentioned as a traditional food or delicacy in early newspaper articles and letters to the editor where they were described as being fat and greasy in texture (Drummond, 1933), "palatable and of a rich nut-like flavour" (Pycroft, 1929), and "tastes of hazelnut" (Andersen, 1933).

Pūriri moth – *Aenetus virescens*

A. virecens is a large moth whose larvae have been eaten as food. During part of the larval lifecycle, the insect forms a "7"-shaped burrow in the trunk of a host tree and spins a silk cover at the mouth of the burrow (Grehan, 1987b, 1987a). To harvest the larvae, water would be poured into the burrow causing the larvae to exit the tunnel (Best, 1902). Best (1902) also mentions "a grub called mokoroa, which is found in the houhi and kai-weta trees, was eaten", likely referring to *A. virescens*, which is known to produce burrows in houhi (*Hoheria populnea*) and kaiwētā (*Carpodetus serratus*) trees (after the larvae leaves, these burrows become home to the wētā mentioned in the name kaiwētā (Wehi et al., 2020)).

Kēkerewai – *Pyronota festiva* & Tūtaeruru – *Costelytra giveni*

P. festiva and *Costelytra spp./Odontria spp.* are scarabid beetles in the Melolonthinae subfamily. The adults of *P. festiva* are commonly found feeding on mānuka and kānuka whilst *Costelytra and Odontria spp.* are generalists. The larval forms feed on the roots of trees and pasture grasses and many species are considered agricultural pests.

It is generally *P. festiva* (kerewai, kekerewai) recorded as being used as food (Best, 1902; Drummond, 1927a; Miller, 1952). Best (1902) originally indicates that both kekerewai and tūtaeruru are a small green beetle (indicating *P. festiva*) but clarifies that tūtaeruru is a separate beetle that is also used as food. Miller (1952) suggests that tūtaeruru could be *Odontria zealandica* (now *Costelytra giveni*) due to its description by Best (1902) as flying in the evening with a distinctive booming noise. The beetles would be harvested in large quantities then ground up with the pollen of raupō and then steamed in a small basket and eaten (Best, 1902). It is interesting to note that cockchafer soup, a dish consumed in France and Germany, featured another beetle from the Melolonthinae family which was also an agricultural pest reaching high population densities (J. J. Schneider, 1844; van Huis, 2013).

Kihikihi kai – *Kikihia muta*

The variable cicada K. muta is a common cicada found from November to May on Carex sp. and Phormium sp. which are considered to be its hosts (Cumber, 1952; Logan, 2006). There are sparse records of its use as food with some references referring to it being mashed into a paste and used as food (Meyer-Rochow & Changkija, 1997; Miller, 1952; Williams, 1957).

Tīkopa/moka – Tortricidae family

Tortricidae is a family of Lepidoptera known as leaf roller moths. Best (1902) describes a caterpillar (moka), which was eaten, that draws leaves of plants closed around itself with silk matching the description of leaf rollers. Miller (1952) also mentions the edible moka and tīkopa

(similar to moka but larger) as the larvae of the leaf-tying family Tineidae, however, I must presume that Tortricidae was meant as Tineidae are better known for eating fungus and fabrics.

Puene – An unknown aquatic insect

Best (1902) describes a small, aquatic, six-legged creature called puene which is eaten by children. Miller (1952) advises that the term puene applies to larva of stoneflies, mayflies and the fish fly, A*rchichauliodes diversus*.

Wētā – Anostostomatidae family

I found only one reference to wētā being consumed in Crowe (2002). With the wētā being such an iconic insect in Aotearoa its consumption would be expected to have been recorded in some way in the grey literature. However, wētā were named as food by five participants in a recent survey of Māori (O'Connor et al., 2023). There has been contemporary use of wētā as food at the Wild Food Festival, where they have been served on crostini (Tucker, 2013). The name wētā can also refer to the Rhaphididophoridae, however there was no reference to this groups use in a traditional or contemporary setting.

Wiseana spp., *Aoraia* spp., *Dumbletonius* spp.

Porina moth (*Wiseana spp.*) is stated in Crowe (2002) as being eaten in the same way as huhu grubs. There were no references to this in other sources, however, some other moths in the Hepialidae family can become infected with *Ophiocordyceps robertsii* to become what was commonly called the "vegetable caterpillar" or āwheto/āwhato which was charred in a kiln and used as a dye for tā moko (Best, 1908; Pycroft, 1931). Some sources state that fresh vegetable caterpillars would be eaten and have a nutty taste (Crowe, 2002; Relph, 1991) whilst other sources stated that the vegetable caterpillar was not eaten (Pycroft, 1931).

Hīhue – *Agrius convolvuli*

A. convolvuli is a large moth in the Sphingidae family. The larvae are pests of kumara plantations, eating the leaves of the crop. The Te Aka Māori Dictionary lists anuhe, hīhue, āwheto and

mūwharu as Te Reo names for *A. convolvuli* (Moorfield, 2023). Miller (1952) mentions that a grub called mūwharu was eaten and suggests that this is the larvae of the sphinx moth. A traditional pest control method for this species was to remove the insects and burn them in a fire as well as use smoke from burning leaves and karakia (Ehau-Taumaunu et al., 2020).

Recently, *A. convolvuli* has been investigated as an edible insect by Plant and Food Research Limited, a Crown Research Institute with food safety and nutritional analyses undertaken as well as taste tests (Pers. Comms.).

Locusta migratoria

Locusts, *L. migratoria*, are found in many parts of the country and have been farmed in New Zealand for human consumption by Malcolm Diack of Otago Locusts (Nadkarni, 2022). The locusts are fed on freshly cut grass and reared inside shipping containers. Otago Locusts provides locusts for both pet and zoo animals and is certified with Ministry of Primary Industries to sell locusts to restaurants.

Teleogryllus commodus

The introduced black field cricket, *T. commodus*, is well known for their noisy night-time calls throughout New Zealand. They have been farmed in the Wairarapa by John Hart to produce cricket flour for making wraps (Mulligan, 2019; Skerrett, 2019; Walters, 2020). The crickets are grown in a controlled environment inside a shipping container with feed and oviposition material protected as intellectual property, Hart has also received Callaghan Innovation funding to investigate chitin extraction and health benefits from consuming cricket flour (*Cricket Farming*, 2020).

Vespula spp.

Perhaps one of New Zealand's more unusual exports were the excavated nests containing the larvae and pupae of *Vespula* wasps sent to Japan (Matsuura, 1999). New Zealand no longer

appears to export *Vespula* however although there does appear to still be interest from Japan (Pers. Comms.).

New Zealand Lemongrass Ant – species unknown

The former edible insect company, Anteater, marketed an ant found in New Zealand that has the taste of "lemongrass & kaffir lime with a mild blue cheese aftertaste". The ant was foraged from the Canterbury region and sold to restaurants at a price point of $0.50 - $1.50 per plate (Anteater, 2019). Unfortunately, due to difficulties scaling up and getting products certified for export Anteater have ceased trading (De Prospo, 2019).

Imported Insect Products

There are several insect products that are available for purchase in New Zealand, usually as shelf-stable, processed goods. The products are often dried, processed into flour or included in novelty items such as chocolates and lollipops. Species included are mealworms (larvae of *Tenebrio molitor*), superworms (larvae of *Zophobas morio)*; adult black ants (*Polyrhachis sp.*), African field cricket (*Gryllus bimaculatus*), house cricket (*Acheta domestica*) and, Bombay locust (*Nomadacris succincta*) (*Eat Crawlers*, 2019).

The Korean dish beondegi contains silkworm pupae and can be found canned in Korean supermarkets as well as in some Korean restaurants in Auckland. The dish is served as a stew in a stone pot with spring onions and chilli peppers (Tan, 2017).

Viable species for farming and other opportunities

Industrial scale production of insects was defined at the Expert Consultation Meeting organised by the FAO and Wageningen University in January 2012 as meeting a minimum reach of one tonne of per day of fresh weight insects (Vantomme et al., 2012). At the meeting, characteristics of a species that would make it suitable for industrial production were suggested to be "a high intrinsic rate of increase (r_m) (short development cycle, high survival of immatures and high

oviposition rate); a high potential of biomass increase/day (weight gain/day); a high conversion rate (kg biomass gain/kg feedstock) the ability to live in high densities (kg biomass/m^2); and low vulnerability to diseases (resistance)" (Vantomme et al., 2012). Other factors that are suggested to be considered are whether the species is amenable to process automation, if the species can be contained in non-native areas and whether there any ecological impacts if they were to be introduced outside of their natural range, potential for the species to be genetically improved through selective breeding and quantification of the ecological footprint and water requirements of the species (Vantomme et al., 2012). Within a New Zealand context, I would add that the cultural rights of indigenous peoples should be considered prior to industrial scale production being attempted.

Of the species that are native or adventive to New Zealand that could meet these criteria, *Locusta migratoria*, *Teleogryllus commodus* and *Tenebrio molitor* are the most suitable. *L. migratoria* and *T. commodus* are farmed at various scales overseas already and both are currently being farmed in New Zealand (H. King, 2018; Mulligan, 2019; Nadkarni, 2022). Other New Zealand species may also be able to be farmed on a smaller scale (which can still be financially viable) but they are unlikely candidates for industrial production due to various aspects of their biology. Examples of these difficulties can be seen in the pūriri moth (*A. virescens*). *A. virescens* larvae have two distinct dietary phases, mycophagy and then phytophagy and a long life cycle of 3 – 4 years (Grehan, 1987b; Grehan & Care, 2018) which poses challenges around diet formulation and the inherent low intrinsic rate of increase with such a lengthy life cycle.

In contrast to other possible endemic food species, there is a greater understanding of the biology of *P. reticularis*, a consequence of being a pest of forestry products (Alma & van Boven, 1975; Brockerhoff et al., 2006; Dentener et al., 1999, 2001; Hedley et al., 1979; Hood & Gardner, 2004; G. P. Hosking, 1979; Lester et al., 2000; Pawson et al., 2020; Pawson & Sky, 2009; Pawson & Watt, 2009, 2009). In the 1960's, John Edwards investigated the reproductive, larval, pupal,

and adult biology as well as their host range and biogeography (Edwards, 1959, 1961b, 1961c, 1961d, 1961a, 1965). Later, work by Rogers et al. (2002) investigated rearing larvae in the lab on an artificial diet and different temperature regimes. This reduced the larval life-stage from two years in the wild to c. 250 days in the lab, using a diet of *Pinus radiata* sawdust. While this research was originally conducted with the intention of better understanding a forestry pest, it has also formed a basis for further research into the viability of the species as a food source.

Characteristics of the New Zealand Edible Insect Marketplace

Cultural context

In western cultures, the concept of eating insects frequently elicits disgust (Deroy et al., 2015). Disgust towards eating insects appears to be a learned behaviour derived during childhood and seems to be particularly prevalent in Western consumers. These feelings of revulsion are generally attributed to differences in culture, that is, the prevalent practices, values and perceptions of a given time or place. In Aotearoa New Zealand, it has been suggested that for Western cultures, entomophagy and nose-to-tail eating practices (i.e. utilising all parts of the animal) carry connotations of low cultural status and subsistence lifestyles (Hoskins, 2007; Tucker, 2013). There is also some evidence to suggest that consumers from a Western background are more likely to perceive insects as vectors of disease (Curtis & Biran, 2001; G. C. L. Davey et al., 1998; Prokop & Fančovičová, 2010).

The cultural values of individuals will be further influenced by dietary environment and the physical characteristics of the food in question (Hartmann & Siegrist, 2016). Specifically, the extent to which insects are commonly consumed and the range of species available. Insects are an unfamiliar food source for most European consumers and unfamiliar or rarely consumed food sources can alienate consumers that are less adventurous (food neophobic) (Jaeger et al., 2017; Pliner & Hobden, 1992).

In Aotearoa New Zealand's case, Prescott et al. (2002) found that consumers have significant concern for the "sensory appeal" of food which, combined with the absence of regularly consumed insects, may exacerbate the neophobia that consumers feel. Indeed, Tucker (2013) found in a small pilot study of young adults, that participants still reported feelings of revulsion at the idea of eating insects even though they could acknowledge the environmental benefits. This challenge of overcoming revulsion remains a significant perceived factor for those trying to introduce insects to Western diets.

A commonly held view (P. Payne & Ryan, 2019) is that the more processed an insect is the more acceptable it is (i.e. flours, dyes etc.). However, at the Insects to Feed the World 2018 conference, a keynote presentation by Aspire Food Group indicated that the sale of whole, unprocessed insects has been surprisingly popular, which challenges this common view (Ashour, 2018). Hartmann and Siegrist (2016) found that people are willing to eat processed insect products and that people who have consumed processed insect products (in the form of cookies incorporating cricket flour) are more likely to consume unprocessed insects.

Deroy et al. (2015) suggest that sensorially-driven strategies (such as name changes, focussing on texture, taste and visual presentation) combined with researchers teaming up with chefs and gastronomic experts will improve uptake by the public. This is an approach that was championed by the New Zealand start-up 'Anteater' which sourced insect ingredients from around New Zealand and supplied insects only to chefs to ensure that the publics first experience with insects was a positive one. However, as discussed in the next section, this sensory focus is not one shared by the majority of New Zealand retailers of insects which appeal to consumers ideas of sustainability and the environment.

WAI262 - Ko Aotearoa Tēnei

While *P. reticularis* may represent a wedge facilitating the entry of insects into a mainstream diet, its status as a traditional food will most likely dictate the growth of that particular food.

Indigenous consumption of huhu grubs is an inextricable element of their marketing. In recent years huhu grubs have been included in the menu at the Wellington-based *Hiakai* restaurant, run by chef Monique Fiso, that champion Māori cooking and ingredients (Fiso et al., 2020) and cooked huhu grubs are also regular features at the *Kawhia kai* and the *Hokitika Wild Food* festivals.

The continued relationship between Māori and huhu beetles will require that any resulting industry is based on, at least, partnership with Māori if not Māori leadership. Of particular consideration for the commercialisation of any endemic insects in New Zealand is the WAI 262 report, Ko Aotearoa Tēnei (Waitangi Tribunal, 2011). WAI 262 was lodged by six iwi claimants to the Waitangi Tribunal, a standing commission of inquiry relating to alleged breaches of Te Tiriti o Waitangi by the Crown, in 1991. Often called the flora and fauna claim due to its particular focus on kaitiaki relationships with flora and fauna, it also encompasses kaitiaki relationships with the environment, protection, and transmission of mātauranga Māori, taonga works and, intellectual property (IP) relating to taonga species. As the first whole-of-government claim, its complexity is reflected in the extensive timeline with the hearings completed in 2007 and the report being issued in 2011, twenty years after the initial claim.

The recommendations in the report are far ranging, many of which may need to be considered if commercialising a taonga species as food. Defining a taonga species is challenging. The language of the tribunal report limits this designation to species of flora or fauna in which an iwi, hapū, or whānau says it has a kaitiaki relationship, evidenced through a wide body of mātauranga Māori, whakapapa and kōrero. Meanwhile, for many Māori, "a 'taonga species' is simply any species which co-exists in their rohe, known or unknown" and the requirement to demonstrate a kaitiaki relationship is prohibitive (Potter & Māngai, 2022).

Of particular interest to the commercialisation of insects, the chapter on "the genetic and biological resources of taonga species" contains recommendations relating to bioprospecting,

genetic modification, and intellectual property. Start-up companies often rely on the protection of intellectual property to attract investment. This can be achieved in several ways: copyright for creative works, trademarks for protecting brand identity, patents to protect an invention meeting specific requirements, plant variety rights (PVRs) to give sole rights to the dissemination of a new variety of plant, and trade secrets that protect something a company considers to be commercially valuable.

While Ko Aotearoa tēnei is not legally binding, in 2019, The Crown announced that it was developing a whole-of-government approach to the report. To date, this approach has included the commencement of the Plant Variety Rights Act 2022 to replace the previous one (PVR 1987). One of the purposes of the new act is "to protect kaitiaki relationships with taonga species and mātauranga Māori in the plant variety rights system" (Plant Variety Rights Act, 2022). In this situation, "indigenous" (a native plant that occurs naturally, or has arrived naturally, in New Zealand) and "non-indigenous" plant species of significance (plant species brought to New Zealand before 1769 on waka from other parts of the pacific and are listed in schedule two of the legislation e.g., Kuru, *Artocarpus altilis*) are afforded additional procedures to recognise and protect kaitiaki relationships. The act creates a Māori Plant Varieties Committee to administer these procedures and enables the cancellation of PVRs that have adverse effects on kaitiaki relationships.

One of the recommendations in Ko Aotearoa Tēnei is the establishment of a Māori Advisory Committee (MAC) within the Intellectual Property Office of New Zealand (IPONZ) and this was included in the Patents Act 2013. Upon request, the MAC provides advice to the commissioner on whether an invention is derived from Māori traditional knowledge and, if so, whether commercialisation of that invention would be contrary to Māori values. However, the role is purely advisory and there is no requirement for the commissioner to action the advice (Patents Act, 2013).

The WAI262 report will have wide ranging implications for research and commercialisation however the full extent of this is not yet known. How The Crown gives effect to the recommendations made in the report remains to be seen, however, I suggest that it presents an opportunity to engage authentically with iwi to the benefit of all, whilst exploring developing insects as food outside of a traditional commercialisation model.

Food Sovereignty

The definition of food sovereignty has a complex history that is still not fully resolved (refer Patel, 2009). For our purposes, it can be understood as a precondition to food security, where food sovereignty is the right of peoples to determine their own food and agriculture systems and policies to provide good quality, healthy and culturally appropriate food. Indigenising food sovereignty is progressing this concept from an emphasis on rights to an emphasis on the cultural responsibility and relationships that indigenous communities have with their environment (Coté, 2016). The replacing of indigenous food systems with a globalised system of multinational companies has also resulted in a decrease in food sovereignty for indigenous cultures and an increase in diet related non-communicable diseases (Coté, 2016; Raschke & Cheema, 2008; Shirley, 2013). The importance of food sovereignty has been recognised by indigenous peoples as a means of decolonising land, reclaiming cultural identity and knowledge and securing health (Coté, 2016; Grey & Patel, 2015; Sweet Grass Consulting LLC, 2018).

Edible insects have been (and still are) an important resource for many indigenous peoples globally and there can be a deep understanding of insect species' life cycles, host plants and harvesting techniques embedded in traditional knowledge (Ramos-Elorduy, 2006). Colonial and neo-colonial forces have resulted in the loss of traditional knowledge and food habits (Raschke & Cheema, 2008) and customs around edible insects are no exception to this.

The food sovereignty of Māori has been adversely affected by British colonisation, land confiscation and urbanisation (Shirley, 2013) and regaining sovereignty of food production in a

culturally appropriate way could lead to greater health outcomes and community resilience. In a Whakatāne pilot study, the importance of food sovereignty and security was highlighted. All participants interviewed highlighted the importance of access to food gardens, kaimoana and food from the bush, the revival of the practice of "growing, preparing and obtaining Māori kai" and using this as a way of passing on traditional knowledge and practices (Moeke-Pickering et al., 2015).

The reclaiming of edible insects that are of cultural significance would contribute to the food sovereignty (and security) of Māori by (1) providing a source of animal protein and fat that complements other sources of kai, (2) fostering the retention of practices and knowledge involved in the collection of these species. This could be through wild harvesting of species, or in some cases, developing methods to domesticate or semi-domesticate a species. Farming is of interest to at least one iwi, with Ngāi Tahu having collaborated on a grant proposal with the now defunct company 'Anteater' and Crown Research Institute (CRI) Manaaki Whenua – Landcare Research to develop a huhu grub farm (Newth, 2018).

Outline of NZ food standards and legislation

One of the major challenges faced by any prospective edible insect industries in the developed world is the absence of a clear and broad legislation which regulates manufacture and sale (van Huis et al., 2013). Food standards for both New Zealand and Australia are developed by Food Standards Australia New Zealand (FSANZ), a statutory authority in the Australian Government Health portfolio, which operates under the Food Standards Australian New Zealand Act 1991. The object of the Act is "to ensure a high standard of public health protection" through the FSANZ which aims to achieve, amongst other goals, a "high degree of consumer confidence in the quality and safety of food produced, processed, sold or exported from Australia and New Zealand (Food Standards Australia New Zealand Act 1991, pt 1 s3). This is primarily achieved through the review and development of standards for food available in Australia and New

Zealand which are set out in the Australia New Zealand Food Standards Code. In New Zealand, the Australia New Zealand Food Standards Code is enforced by the Ministry for Primary Industries (MPI) and public health units (Food Standards Australia New Zealand, 2019).

Insects as food (as opposed to insect-produced foods such as honey or shellac) do not fit comfortably into the Food Standards Australia New Zealand Act, or the Australia New Zealand Food Standards Code. Consequently, legislation regarding food safety for edible insects covers a small number of disparate products which fall under a range of headings including "farmed animals", "wild catch" and "extracts and additives". Approved colouring includes carmine/cochineal extract (INS number 120) which can be derived from scale insects or produced synthetically and can be included in food "at GMP" (Australia New Zealand Food Standards Code – Schedule 16, S16—3), indicating "Good Manufacturing Practice" in which the amount of substance is limited to the "lowest possible level necessary to accomplish the desired effect" while also preparing and handling in the same manner as food ingredients (Australia New Zealand Food Standards Code – Standard 1.1.2 – Definitions used throughout the Code Section 1.1.2—2).

Meanwhile, while standards for farmed animals are governed by the Food Standards Code which is applied across both New Zealand and Australia, the management of wild catch is managed by individual territories (of which one is New Zealand). Under the former, definitions of meat do include animals which have been explicitly "permitted for human consumption" and consist of skeletal muscle, fat, connective tissue, animal rind etc. (Australia New Zealand Food Standards Code - Meat and meat products – Standard 2.2.1—2). Insects are not explicitly listed as a "meat", but the written definition certainly applies to them. In New Zealand, most insects listed as "farmed meat" would be imported, most notably from Thailand. By contrast there are clear provisions for the trade of wild harvested insects in New Zealand which are listed under the

Animal Products Act (APA) 1999 which requires certification for the hunter or person supplying the meat.

FSANZ recently released the recommendations from the Advisory Committee on Novel Foods (ACNF) on a small number of insects (super mealworm, *Zophobas morio*; house crickets, *Acheta domestica*; mealworm beetle, *Tenebrio molitor*) deeming them both "non-traditional" with no history of human consumption in New Zealand or Australia, and "non-novel", with no safety concerns identified. An amendment was later added regarding recent evidence around allergenicity of crickets when consumed by a person with a crustacean allergy (Food Standards Australia New Zealand, 2019). As non-novel foods, they are not subject to limits on consumption or import (Reverberi, 2017). While the concept of "novel foods" are a tool by which regulatory provisions may be made for individual species, it may be a cumbersome process for small-scale producers (van Huis et al., 2013).

Cumulatively, this suggests that while there are precedents of farmed and wild caught insects being allowed for consumption and import, the approval process seems to be relatively ad hoc and approval for wild caught meat is likely to be a much easier prospect for potential businesses than farming.

New Zealand edible insect retailers and marketing practices

To assess how insects have been marketed in New Zealand, I collected publicly available text from edible insect companies' websites to identify the words which occurred most frequently in marketing. The text I used was taken preferentially from descriptions of the company and their mission statement. If the company only retailed one edible insect product amongst other food products, I used text from the product information. In one case, the company did not have a website and I collected text a profile written about the company in a local newspaper.

I used R and the tidytext package (Silge & Robinson, 2016) to prepare the text for analysis. A custom list of 599 stop words was compiled to remove words not relevant to insects or insect products (e.g., pronouns, articles, numbers). I made all nouns singular (so “insects” became “insect”) and made abstract nouns and adjectives uniform (e.g., "environment", "environmental", "environmentally" became “environment”). I then quantified the top twenty most frequently used words and the top words used by all companies (Figure 1 A & B).

I identified eight (8) New Zealand based suppliers of edible insect products for human consumption that have been active within the last ten years (Table 3). Of these, five companies imported insect products/ingredients from overseas, two companies sourced insects locally, and one company both imported and sourced local ingredients.

Cricket powder or flour was the most common product, retailed by six of the eight companies. For five of the companies, cricket powder or flour was their only edible insect product, sometimes sold alongside other food and health products. Of these five, two companies modified imported cricket flour to produce protein bars, nacho chips, wraps and protein powder for retail. When the origins of imported ingredients were available Canada, South-East Asia and New Zealand were most frequent, and farmed was the primary method of production. The large number of companies selling cricket flour or food enriched with cricket flour appears to coincide with the increased availability of cricket flour being produced globally from 2013 onwards (Reverberi, 2020). By 2023, however, only two of the eight companies are still actively selling insect products suggesting a lack of commercial success. This could be due to several reasons including, market size and saturation, price and availability of raw materials (particularly during the pandemic), low demand with consumers, or difficulties marketing insect products to the New Zealand public.

Table 3. Companies selling insect products in New Zealand since 2013. Name of company, trading as, date registered, and date removed are sourced from the companies register. Status of products, website status, products and origin are sourced from the companies' website.

Name of Company	Trading As	Date Registered	Date Removed	Status of Products	Website Status	Products	Origin	Notes	Source
Otago Locusts Limited	Otago Locusts	11-May-16		Active	Active (2023)	Locusts - Whole Insect	Local (Otago)	Company is currently active and trading via Facebook	(*Otago Locusts*, 2023)
True Beginnings Limited	Eat Crawlers	8-Jun-20		Active	Active (2023)	Whole and Processed Insects and Arthropods	Imported (Asia)	Company is currently active and trading via website. Originally founded in 2013, sold to True Beginnings Limited in June, 2020.	(*Eat Crawlers*, 2019)
Primal Future Limited	Primal Future	18-May-16		Unknown	No longer active	Cricket Flour Cricket Flour Nacho Chips Cricket and Hemp Protein Powder	Imported (Unknown)	Website is currently down. Company still registered.	(*Primal Future*, 2023)
Champed Limited	NZ Naturally	8-May-17		No longer selling insect based products	Active (2023)	Cricket Flour	Imported (Unknown)	Company is still active but no longer selling insect products	(*NZ Naturally*, 2018)
Breadcraft Waiarapa Limited	Rebel Bakehouse	8-Dec-42		No longer selling insect based products	Active (2023)	Cricket Flour Wraps	Imported (Canada) and Local (Wairarapa)	Company is still active but no longer selling insect products	(*Rebel Bakehouse*, 2019)
Live Longer Limited	Live Longer	1-Oct-15		No longer trading	No longer active	Cricket Flour Cricket Flour Protein Bars	Imported (Canada)	Company is still registered (2023) however no longer trading insect products. Website forwards to a website design company.	(*Live Longer*, 2021)

Anteater Limited	Anteater	17-Jul-16	26-Jun-19	No longer trading	No longer active	Whole insects Wild Harvested NZ Lemongrass Ants NZ Native Grass-Fed Locusts New Zealand Huhu Grubs	Local (Various, New Zealand)	Company is no longer trading as Anteater	(Anteater, 2019)
Good Pantry Limited	Good Pantry	28-Jun-17	25-Jan-21	No longer trading	No longer active	Cricket Flour	Imported (Unknown)	Company is no longer trading	(*Good Pantry*, 2020)

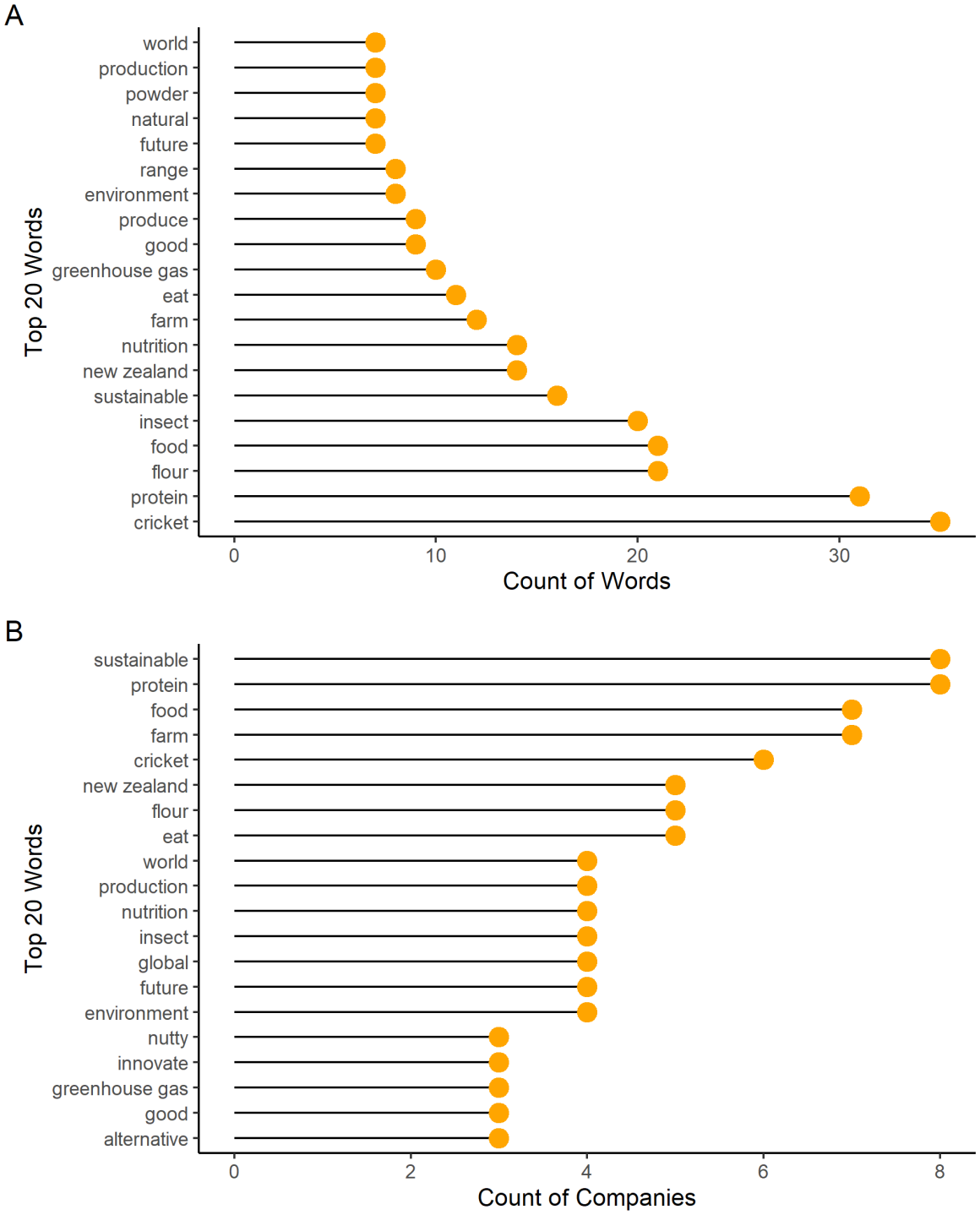

Figure 1. (A) The top 20 most frequently used words by New Zealand companies when marketing edible insect products. (B) The number of companies using a particular word. The words "sustainable" and "protein" were used by all eight companies.

In Figure 1A, we see that the most frequently used words were descriptive of the product "cricket", "protein", "flour", "food" and "insect". The next most frequent words were "nutrition" and "sustainable", with the word "sustainable" being used by every company analysed (Figure 1 (B)). The heavy emphasis on words such as "sustainable", "environment", "greenhouse gas" suggests that the companies and products are marketed towards environmentally minded consumers. The environmental benefits of insect farming over conventional livestock are highlighted in marketing. However, cricket flour often originates from overseas farms (e.g., Canada, Thailand) and long-distance shipping could impact the environmental footprint of these products and cause claims of sustainability to be misleading.

It has also been suggested that highlighting the environmental benefits of edible insects is not enough to persuade people to buy (Deroy et al., 2015; van Huis & Rumpold, 2023). Tastiness and familiarity are important for consumers, particularly for a first encounter with an insect so incorporating into a familiar dish prepared by a professional chef may assist (Marquis et al., 2020; van Huis & Rumpold, 2023). This was particularly important to one of the companies, who would only sell their products to chefs to ensure the first experience of a consumer would be a positive one.

Concluding remarks

New Zealand, like many temperate regions, is often regarded as having a depauperate number of native/endemic species that are consumed. However, I have found that this is not the case, with at least eleven endemic or native species with a history of consumption. This is likely to be a conservative estimate as colonial and neo-colonial influence has possibly resulted in the loss of oral histories that contained knowledge of species used as food. These unique insects could be an exciting addition to our cuisine providing any commercial development is done collaboratively with, or led by, mana whenua. Wild harvest is likely to be the primary method of obtaining native insects, due to the long and/or complex life cycle of many of our species.

However, further research may lead to opportunities in optimising the rearing of *P. reticularis* which could allow for farming.

Marketing of edible insects as environmentally sustainable alternatives to other animal proteins would benefit from a life cycle analysis to ensure claims are accurate. It is likely that use of imported ingredients reduces the environmental benefits that could otherwise be obtained by using these products. Developing a domestic supply of cricket flour, with New Zealand's ready access to renewable energy and agricultural by-products, could also assist in meeting the environmental benefits promised by edible insects. However, the small market size could be the most restrictive element to the development of an edible insect industry. Producing insects for use as pet and livestock feed whilst developing the market for insects as food may be a way for companies to stay financially viable.

Chapter 3 The use, perception, and existing knowledge of insects as food in Aotearoa New Zealand.

Abstract

The global population's rapid growth, expected to exceed 9.7 billion by 2050, raises concerns about food security. One growing opportunity for the sustainable provision of animal protein, and recognised by the United Nations, is an increase in the human consumption of insects. However, consumer perceptions and acceptance of edible insects vary worldwide. While Western consumers generally exhibit negative sentiments toward edible insects, nuanced variations exist within and between cultures. I investigated the use and perceptions of insects as food in New Zealand through the use of an online questionnaire (n = 620). I found 498 participants were able to name one or more insect species used as food. Prior experience eating insects and existing knowledge of insect species along with a low food neophobia score, were found to be predictors of future likelihood of eating insects. Despite participants being open to the use of insects as food, I found that words used to describe insect ingredients exhibited on average a more negative sentiment than that of plant or conventional meat ingredients. I suggest that future development of edible insects in New Zealand should build on the existing awareness of cricket flour and huhu grubs.

Introduction

The world's rapidly growing population, expected to reach over 9.7 billion by 2050 (United Nations, 2019), will increase the demand for animal proteins for food but also raises concerns for food security. Insects have been highlighted by the United Nations' Food and Agriculture

Organisation (FAO) as an important source of dietary protein (van Huis et al., 2013) and, due to aspects of their biology, some insect species are a more sustainable option than sources of protein from livestock, poultry or seafood (van Huis & Oonincx, 2017). In Western countries, this has prompted investment in business start-ups seeking to farm insects to meet a perceived demand for insect protein and there is great potential for an increase in the use of insect protein globally.

Whilst over 1600 species of insects are used in the cuisine of many cultures around the world (van Huis et al., 2022; Van Itterbeeck & Pelozuelo, 2022), consumption of insects is perceived to be relatively novel amongst many consumers from Western countries. However, novel does not necessarily refer to the invention of a technology but to its introduction to a market (Siegrist & Hartmann, 2020). As the end users, and therefore paying customers of a product, consumers and their perceptions have an important role in the success of novel food technologies such as edible insects (Dunphy & Herbig, 1995; Verkerk et al., 2007).

Current research on consumer perceptions and acceptance of edible insects predominantly concentrates on northern hemisphere countries. Studies have consistently identified gender, food neophobia, disgust sensitivity, and prior insect consumption as general predictors of individuals' attitudes towards insects as a food source (Hartmann & Siegrist, 2016; Lensvelt & Steenbekkers, 2014; Orsi et al., 2019; Schlup & Brunner, 2018; Verbeke, 2015). Research has also shown that an existing awareness of edible insects positively influences consumer acceptance of insects as food (Barsics et al., 2017; Kostecka et al., 2017; Verbeke, 2015). Individuals who have tried insects in the past tend to exhibit a significantly higher positive attitude towards insects as food than those who have not (Wilkinson et al., 2018).

Generally, Western consumers are often associated with harbouring negative sentiments towards edible insects, although this perception can vary based on whether the insects are consumed whole, processed, or integrated into familiar food items. However, recent reviews caution against

treating Western cultures as a homogeneous entity due to subtle and nuanced differences between various cultures and countries (Ardoin & Prinyawiwatkul, 2021; Mancini et al., 2019). For instance, Central European and French Quebecers share similar views on edible insects but differ from those of English speaking North Americans (Hénault-Ethier et al., 2020), and early-adopting Northern European countries like Finland and Sweden exhibit a more positive attitude toward insect consumption compared to Central European countries such as Germany and the Czech Republic (Piha et al., 2018). Countries with strong traditions of insect eating, such as Mexico, Peru and Thailand, are more willing to consume products containing insect powder, suggesting that cultures that already incorporate insects into cuisine are more accepting of novel insect products (Castro & Chambers IV, 2019; Hartmann et al., 2015).

Studies investigating consumer acceptance in the Australasia region focus primarily on Australia (Bogueva & Schmidinger, 2019; Castro & Chambers IV, 2019; Chan, 2019; Lensvelt & Steenbekkers, 2014; G. Myers & Pettigrew, 2018; Sogari et al., 2019; Wilkinson et al., 2018) with two studies from New Zealand (O'Connor et al., 2023; P. Payne et al., 2023) and no studies from other Pacific Island nations. Australia and New Zealand present an interesting comparison as both are predominantly English speaking settler countries, share a legislative framework governing the use of novel food items including insects, a fledgling insect farming industry, and have Indigenous traditions of consuming insects (Food Standards Australia New Zealand, 2019; Lähteenmäki-Uutela et al., 2021; O'Connor et al., 2023; P. Payne et al., 2023).

Barriers impacting the acceptance of insects as a food source for Australian consumers include feelings of disgust, food neophobia, and concerns related to disease, unnaturalness, and threats to traditional perceptions of masculinity (Bogueva & Schmidinger, 2019; Castro & Chambers IV, 2019; Lensvelt & Steenbekkers, 2014; G. Myers & Pettigrew, 2018; Sogari et al., 2019; Wilkinson et al., 2018). Whilst the threat to masculinity experienced by male Australian participants is

outside of the scope of this article, this has been explored in studies of plant based meat alternatives (Leary et al., 2023).

Disgust is a potent and universal emotion, prevalent in human societies. However, the specific objects or behaviours that trigger disgust are often culturally influenced, rooted in societal norms and moral values (Curtis & Biran, 2001). Food neophobia, closely related to disgust, represents the aversion to unfamiliar foods and has been identified as a key predictor of the acceptance of insects as a novel food source and it is also influenced by cultural factors (Hartmann et al., 2015; La Barbera et al., 2018; Verbeke, 2015). These traits, the evolutionary remnants of a defence mechanism, have historically helped reduce the risk of consuming poisonous or spoiled food (Curtis & Biran, 2001) and prevented the transmission of infectious diseases (Oaten et al., 2009). In Western societies, insects are generally perceived negatively, often evoking feelings of disgust and fear (Kellert, 1993; Looy et al., 2014). Overcoming these deeply ingrained perceptions poses a considerable challenge to the acceptance of edible insect products.

To address these barriers, various strategies have been proposed. These strategies include normalising the consumption of edible insects by gradually increasing their visibility in everyday life, a process akin to 'public exposure therapy' (Koch et al., 2021). Additionally, emphasising the multisensory qualities of insects as food, often through collaborations with chefs and innovative culinary experiences like bug banquets, has shown promise (Deroy et al., 2015; La Barbera et al., 2018; Looy & Wood, 2006; Padulo et al., 2022). Incorporating insect-derived ingredients into familiar products, such as cricket flour in tortilla chips, has also demonstrated potential (Hamerman, 2016; Hartmann & Siegrist, 2016; Wilkinson et al., 2018).

A study of older Australian adults showed high levels of disgust with eating insects viewed as not being compatible with their cultural beliefs and values (G. Myers & Pettigrew, 2018). However, the strategies highlighted by participants to increase uptake are remarkably similar to those suggested in the literature. Strategies suggested include: disguising insects (concealing

"animalness" by processing into a powder), ensuring safety through guarantees, inclusion on restaurant menus, instructions on cooking at home and utilising mass media to increase knowledge of the benefits of insects as food (G. Myers & Pettigrew, 2018). It has been demonstrated that being unwilling to eat insects does not necessarily mean being unsupportive of the research and development of an insects as a food industry (Ruby et al., 2015).

In New Zealand, texture, taste and disgust are perceived as barriers to insect consumption and the preferred way of consuming an insect was as a powder followed by frying the insect (P. Payne et al., 2023) which suggests that presenting the food in a culturally relevant context, such as incorporated into an existing food item (e.g. breads or pasta) or deep frying to avoid a texture that is not perceived to be pleasant (e.g., a "squish"). Demographic attributes that were found to impact willingness to consume insects included age and gender with participants in the age bracket 56 and above less willing to eat insects and males were significantly more willing to eat insects than females (O'Connor et al., 2023; P. Payne et al., 2023).

Several insect species have been used as traditional and contemporary sources of food in New Zealand, such as the huhu grub (*Prionoplus reticularis*), black field cricket (*Teleogryllus commodus*) and locust (*Locusta migratoria*). Current use of these insects relies on wild harvest in the case of the huhu beetle or farming in the case of black field crickets and locusts. There is occasional use of insects as ingredients in restaurants and in products available in supermarkets as well as features in the media on insects as food. Given the traditional use of insects as food and the consumer availability of insect-based products, it could be assumed that knowledge of insects as food is reasonably widespread and uncontroversial throughout Aotearoa New Zealand, however, this has not been quantified.

Understanding the drivers of consumer acceptance as well as consumer perceptions, knowledge, and use of insects in Aotearoa New Zealand is important to assist in the development of this sector as it grows. Using an online questionnaire and a mixed methods approach, I set out to

quantify existing public knowledge of edible insect species, the beliefs and attitudes associated with insects as food, understand drivers influencing the likelihood of eating an insect in the future, and the sentiment attached to insects when seen as ingredients on a menu. I hypothesised (1) prior experience of consuming insects and a greater knowledge of insects as food will increase the likelihood of being willing to eat insects in the future, (2) that negative words would be attributed to insects more than other food types.

Methods

Participants

Participants were recruited via organic (shared by users of social media) and paid for (targeted adverts) social media posts. An anonymous, online questionnaire was designed using the online survey platform Qualtrics (v. August 2021, Qualtrics, Provo, UT). The questionnaire comprised five sections, (1) categorisation of participants; (2) knowledge and perceptions of edible insects; (3) experience of eating insects; (4) likelihood of eating an insect or insect containing product in the future, and (5) demographic questions. The questionnaire used branch logic to conditionally present questions based on a participant's previous answers.

I received n = 735 responses to the questionnaire. Any responses that were indicated as spam (n = 3) or did not make it to the end of the questionnaire (n = 113) were discarded giving a total number of n = 620 for summary statistics. For the machine learning workflow, I removed any respondents who answered "prefer not to say" for any demographic questions (n = 60), leaving n = 560 for the logistic regression analysis. The median time taken to complete the questionnaire was 11.72 minutes (lower quartile = 8.88, upper quartile = 17.20).

Demographics

The demographic data collected were dietary preference, age, gender, ethnicity, country of birth, region of New Zealand, education, and income. For ethnicity data, answers were categorised into Level One groupings (European, Māori, Pacific Peoples, Asian, Middle Eastern/Latin

American/African (MELAA), and Other Ethnicity). Where multiple categories were selected, I used a prioritised output (Māori, Pacific peoples, Asian, other groups except NZ European; and NZ European) (Ministry of Health, 2004). For dietary preference participants could write freely and these were then coded into six categories (omnivore, pescetarian, flexitarian, vegetarian, vegan, allergy/medical).

Existing knowledge

To investigate existing knowledge of edible insects, I asked participants "Can you list any insects that you know are used as food in Aotearoa New Zealand?". I quantified how many insects or insect products each participant knew as a count. Answers were grouped to a single common name to make consistent (e.g., huhu beetle, huhu bug, huhu grub and huhu larvae were grouped into huhu grubs). I categorised answers by insect order (e.g., Hymenoptera for ants, coleoptera) and if a response referred to an organism or a product of an insect (e.g., insect flour, honey, cochineal) and presented in a table.

Likert Scales

Likert scales were used to measure the impact of COVID-19 on the willingness to eat novel food items, future likelihood of eating an insect, food neophobia, specific beliefs and attitudes, and encouragement. A likert scale is a technique for the measurement of attitudes where a participant ranks how strongly they agree or disagree with a statement on an ordinal scale consisting of a 5 or 7 points (Likert, 1932; Sullivan & Artino, 2013). Where a scale was used in a previous study, I kept the original number of scale choices to enable comparisons.

Impact of COVID-19 on willingness to eat novel food items

To assess whether the Covid-19 pandemic impacted willingness to try an unfamiliar food ingredient, I used a 7-point likely-unlikely scale and asked the question "Imagine you are visiting a new restaurant, compared to pre-COVID-19, how likely are you to try a dish containing:" and

presented with three items; (1) an unfamiliar ingredient, (2) an insect, (3) and an unfamiliar animal protein.

Future likelihood of eating insects

I asked all participants what their future likelihood of eating an insect was, creating two groups "likely" and "unlikely". Depending on the responses, I asked the reasons for this. For the unlikely group, the options were: allergic to shellfish/insects, religious reasons, dietary reasons, disgusted by insects, fear of insects, other (please specify). For the likely group, the options were: sustainability/environmental benefits, price, health benefits, curiosity, cultural reasons, taste/flavour, other (please specify).

Food neophobia, specific beliefs and attitudes, and encouragement

Participants were categorised using the following scales: a food neophobia score (FNS) (ten questions with a likert scale from 1 to 7, then each score was added to give a value out of 70, higher scores indicate a higher degree of neophobia; adapted from Pliner and Hobden (1992)), specific beliefs and attitudes towards insects as food (nine questions with a likert scale of 1 to 5, covering themes of disgust, risk, benefits, ethics and naturalness (Ruby et al., 2015)), and attitudes towards the research and promotion of eating insects (two questions with a likert scale of 1 to 7 (Ruby et al., 2015)).

Word association

Word association is a widely used qualitative sensory technique in food science used to study consumer needs, beliefs and perceptions (Gambaro, 2018). It can allow access to emotions and motivations that may not be picked up using a quantitative scale. I used a word association exercise which asked, "You are reading a menu when you see the following ingredient in a dish, what are the first words or phrases that you think of?". One ingredient was presented at a time and in a random order. The ingredients were: lamb, locust, tofu, mealworm, beef, silkworm pupae, jackfruit, grasshopper, chicken, huhu grub, kumara, cricket flour, shrimp, and sky prawn

(an alternative name for a locust). These ingredients fell into three broad categories of "conventional" animal, plant, and insect ingredients with the exception of sky prawn and silkworm pupae which were included to examine how participants respond to branding of an edible insect (sky prawn) and to the inclusion of an unfamiliar edible insect protein (silkworm pupae). The data were cleaned (e.g., removing punctuation, consolidating different spelling) using OpenRefine (v. 3.7.0), resulting in 18,808 responses consisting of 7405 unique words or phrases for analysis. Each word or phrase was attributed a sentiment (positive = 1, negative = -1, or neutral = 0).

Topics/Themes

To extract important themes from the word association data, I used term frequency-inverse document frequency (TF-IDF) (Salton & Buckley, 1988). TF-IDF is a technique used in text mining to suggest the topic of an individual document within a corpus (a collection of documents). In our context, a document was a specific ingredient in a menu item and the corpus was composed of all of the specific ingredients (see word association section for list of ingredients). TF-IDF is comprised of two parts, the term frequency (TF) and the inverse document frequency (IDF). Firstly, the term frequency is calculated within a document by counting the number of times a word or phrase appears within the document. Secondly, the inverse document frequency is calculated by dividing the number of documents by the number of documents containing a term and then taking the log of the output. This leads to terms that are unique to specific documents, indicating predictive power, being ranked highly, whilst terms common to all documents are given a lower ranking.

Thematic analysis

Thematic analysis was used to interpret the word association data following (Clarke & Braun, Virginia, 2021). This will be addressed in a separate paper.

Data Cleaning

The data were cleaned using R and Open Refine. Statistical analyses were conducted using R and R Studio (R Core Team, 2022; RStudio Team, 2022).

Statistical Analyses

Sentiment Analysis

Sentiment analysis and term frequency-inverse document frequency (TF-IDF) was conducted using the tidytext package (Silge & Robinson, 2016). To assess whether there was a difference in sentiment between ingredient groups (animal, plant, and insects) and ANOVA was conducted with a post-hoc Tukey's Honest Significant Difference test used to assess differences between groups.

Machine Learning Model Selection

I used machine learning to empirically compare different models and select the best model to predict the outcome. The tidymodels metapackage is an ecosystem of packages that facilitate the construction and comparison of multiple models (Kuhn & Wickham, 2020). The R packages contained in this ecosystem were used for the machine learning analysis of whether likelihood of eating an insect in the future (likely/unlikely) is predicted by prior experience (yes/no), existing knowledge (number of edible insect species known), Food Neophobia Score (a score between 10 and 70), demographic data (education level, gender, and income). I removed participants who answered "Prefer not to say". I split the data set (n = 560) into training (n = 419) and test (n = 141) sets with the test set only used to assess the final model. Both the training and test sets were stratified by gender to minimise sampling bias arising from the imbalance between male and female. From the training data set, I used bootstrap resampling to create 25 new training and testing sets with which to evaluate the models. As the outcome variable was imbalanced (I had 3.25x as many likely to unlikely), I used the synthetic minority over-sampling technique (SMOTE) (Chawla et al., 2002) to create synthetic data for the minority outcome class (unlikely)

in the training dataset, allowing us to train the model efficiently without altering the testing dataset and reducing the risk of overfitting.

I compared a logistic regression, random forest, and a Bayesian logistic regression model and selected the model using the confusion matrix, the area under the receiving operating characteristic (ROC) curve (AUC), specificity, and sensitivity. A confusion matrix contrasts false positives and negatives with true positives and negatives predicted by the models. Specificity measures how well the model correctly classifies actual positives a percentage, whilst sensitivity is a percentage of how well the model correctly classifies actual negatives. A ROC graph plots the true positive rate on the Y-axis and the false positive rate (1 – specificity) on the X-axis. I found the logistic regression model to perform best on the training data and used this as the final model.

Ethics

Ethics approval was received from the University of Auckland Human Participants Ethics Committee on 10 March 2020 for three years (ref: 024336).

Results

There were 620 total responses to the questionnaire. Most respondents were Female (n = 422), European (n = 474), from Auckland (n = 267), in the 25 – 34 age bracket (n = 222), with a post-graduate university education (n = 264), an individual income of between $50,001 - $70,000 (n = 121) and had an omnivorous diet (n= 473). In response to the question "have you eaten insects before?", 367 (59.2%) participants responded 'Yes'. The demographic breakdown of participants is presented in Table 4. The sample is not representative of the New Zealand population for the characteristics of gender, ethnicity, age and education and so generalisations for the whole of New Zealand should not be made when interpreting the results.

Table 4. Demographic parameters of questionnaire respondents

Demographic Parameter	Category	n	%
Total Sample Size		620	100
Age	16 – 18	9	1.45
	19 – 24	98	15.81
	25 – 34	222	35.81
	35 – 44	143	23.06
	45 – 54	84	13.55
	55 – 64	49	7.9
	65 – 74	14	2.26
	Prefer not to say	1	0.16
Education	High school	73	11.77
	Diploma or Certification	89	14.35
	Undergraduate University	182	29.35
	Postgraduate University	264	42.58
	Prefer not to say	12	1.94
Gender	Male	161	25.97
	Female	422	68.06
	Gender diverse	30	4.84
	Prefer not to say	7	1.13
Income	Under $10,000	95	15.32
	$10,001 - $30,000	90	14.52
	$30,001 - $50,000	90	14.52
	$50,001 - $70,000	121	19.52
	$70,001 - $100,000	119	19.19
	Above $100,001	96	15.48
	Prefer not to say	9	1.45
Ethnicity	European	474	76.45
	Asian	62	10
	Māori	44	7.1
	Middle Eastern / Latin American / African (MELAA)	8	1.29
	Other Ethnicity	8	1.29
	Pacific Peoples	8	1.29
	Prefer not to say	16	2.58
Region	Northland	15	2.42
	Auckland	267	43.06
	Waikato	57	9.19
	Bay of Plenty	15	2.42
	Gisborne	1	0.16
	Hawke's Bay	15	2.42
	Taranaki	6	0.97
	Manawatu-Wanganui	32	5.16
	Wellington	79	12.74
	Tasman	4	0.65
	Nelson	11	1.77
	Marlborough	3	0.48

	West Coast	3	0.48
	Canterbury	73	11.77
	Otago	32	5.16
	Southland	6	0.97
	Prefer not to say	1	0.16
Diet	Omnivore	473	76.29
	Vegetarian	69	11.13
	Vegan	25	4.03
	Pescetarian	21	3.39
	Allergy/medical	17	2.74
	Flexitarian	15	2.42

Existing Knowledge

The median number of edible insects that could be listed was 1 (n = 223) followed by 2 (n = 168) and 0 (n = 122) (Table 6). The edible insect species and products identified by respondents are presented in Table 6. The most common species listed were huhu grubs (n = 333), followed by crickets (n = 298).

Table 5. The frequency of the number of edible insect species that could be named by participants.

Number of Insects Named	Frequency
0	122
1	223
2	168
3	60
4	28
5	11
6	6
7	1
8	1

Table 6. The diversity of edible insects and edible insect products that participants could name.

Type	Order	Insect	n	% of Participants (n = 620)
Organism	Blattodea	Cockroaches	1	0.16
		Termites	1	0.16
	Coleoptera	Huhu grubs	333	53.71
		Kekerewai (Pyronota spp.)	1	0.16
		Mealworms (yellow)	71	11.45
		Mealworms (lesser)	1	0.16
		Mealworms (superworms)	1	0.16
		Beetles	4	0.65
	Diptera	Black soldier fly	4	0.65
	Hemiptera	Cicada	10	1.61
		Scale insects	2	0.32
	Hymenoptera	Ants	48	7.74
		Ants (darwin's)	1	0.16
		Ants (green)	1	0.16
		Honey bee	2	0.32
		Honey bee larvae	1	0.16
	Lepidoptera	Agave worms	2	0.32
		Anuhe caterpillars	1	0.16
		Silkworm pupae	1	0.16
	Orthoptera	Black field cricket	2	0.32
		Crickets	298	48.06
		Grasshoppers	36	5.81
		Locusts	32	5.16
		Weta	7	1.13
	Phasmatodea	Stick insects	1	0.16
Insect Product/Processed Insects	Coleoptera	Mealworm flour	1	0.16
	Hemiptera	Cochineal	15	2.42
		Shellac	3	0.48
	Hymenoptera	Honey	1	0.16
	Orthoptera	Cricket flour	22	3.55

COVID-19 Impact on Trying Unfamiliar Ingredients (Figure 2)

Participants were asked "Imagine you are visiting a new restaurant, compared to pre-COVID-19, how likely are you to try a dish containing:" and then presented the following options "an unfamiliar ingredient", "An insect", "An unfamiliar animal protein". The majority of respondents reported that they were more likely to try an unfamiliar ingredient, insect or unfamiliar animal protein than compared to pre-COVID-19 (64%, 47% and 46% respectively)

with the minority being less likely to try these items (10%, 27% and 28% respectively). With all options 26% of respondents stated they were neither more or less likely to try an unfamiliar ingredient, insect, or unfamiliar animal protein.

When responses were grouped based on those who had eaten an insect ('yes' group, n = 367) and those who had not eaten an insect ('no' group, n = 253), I found the 'Yes' group were much more likely to try an unfamiliar ingredient (70%, 58% and 54% vs. 55%, 35% and 32%) than not (4%, 17% and 19% vs. 18%, 41% and 41%) the neither more nor less likely category was largely the same between both groups (ranging from 24% - 27%).

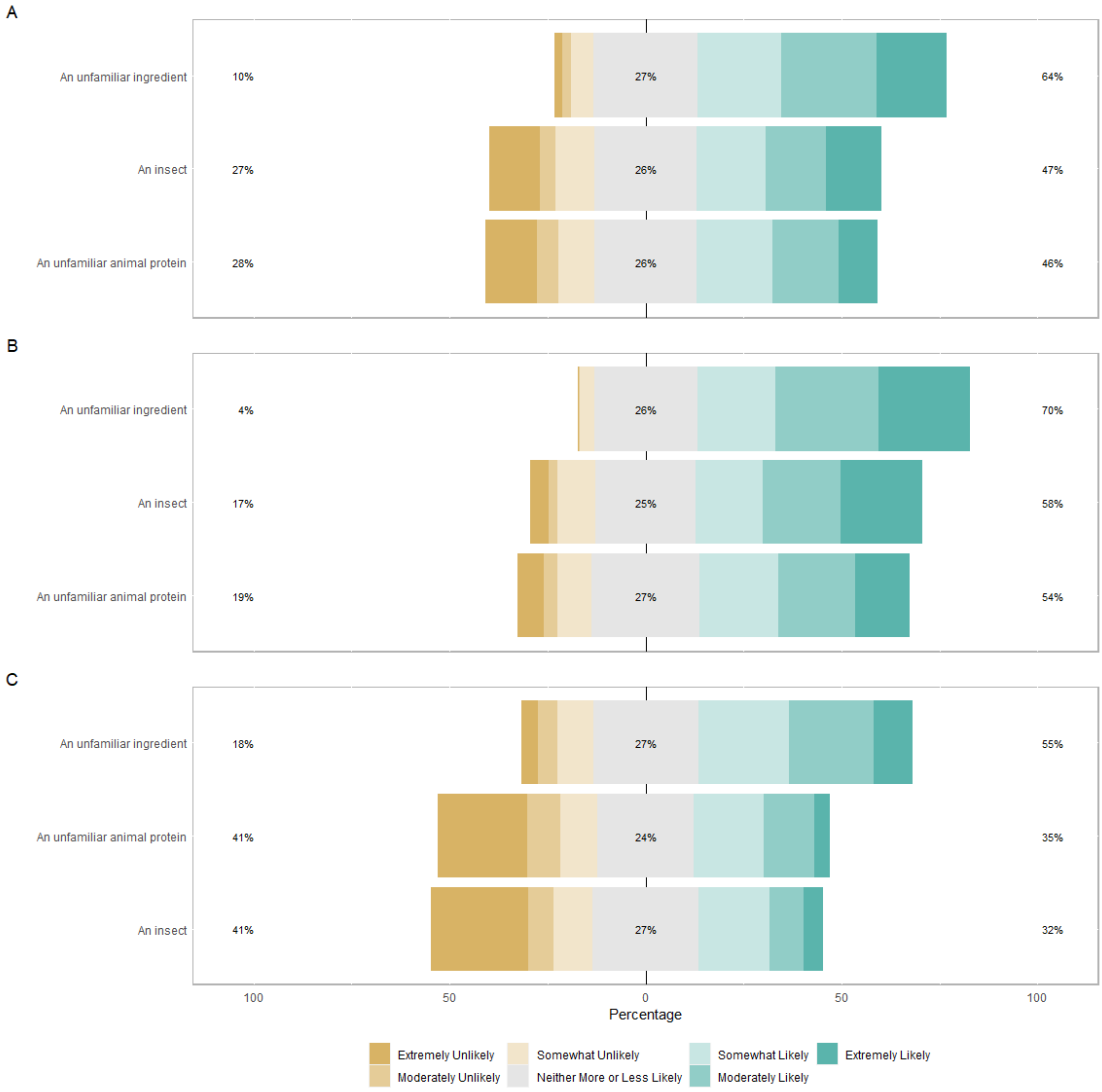

Figure 2. Impact of COVID-19 on respondents' willingness to try unfamiliar food items measured on a 7-point likert scale. (A) All participants, (B) participants who have eaten an insect in the past, (C) participants who have not eaten an insect in the past.

Specific Beliefs/Attitudes Towards Insects (Figure 3)

The participants' specific beliefs and attitudes relating to the consumption of insects is shown in Figure 3 with disgust, risk, benefits, ethics and naturalness measured using nine items. Participants were grouped by those who have eaten an insect before ("Yes" group) and those who have not eaten an insect before ("No" group). Disgust was measured with the statement "eating insects is disgusting". The yes group tended to disagree (66%) with this statement more than the no group (31%). Participants' perceptions of risks associated with eating insects was measured with three statements "eating insects will increase risk of infectious disease", "insects contain harmful toxins" and, "insects carry harmful microbes". Both groups showed similar patterns in response to these statements with a majority of participants selecting neither agree or disagree in response to the toxins and microbes statements. In the statement which specifically mentions the consumption of insects ("eating insects will increase risk of infectious disease"), the majority of participants disagreed with this statement (71% & 63%). Statements investigating benefits of eating insects ("insects are highly nutritious" and "eating insects is good for the environment") received majority agreement from both groups (92% vs. 75%; 77% vs. 58%), however there was a greater degree of uncertainty found in the "No" group (7% vs. 20%; 20% vs. 31%). Ethics statements included "insects are capable of feeling pain" and "killing insects is immoral", the majority of both groups agreed that insects feel pain (52% vs. 56%) but disagreed that killing insects is immoral (67% vs. 55%). Finally, for the statement "it is not natural for humans to eat insects" the majority of participants in both groups disagreed (84% vs. 70%) although there was a difference between groups in those who agreed with the statement (5% vs. 15%).

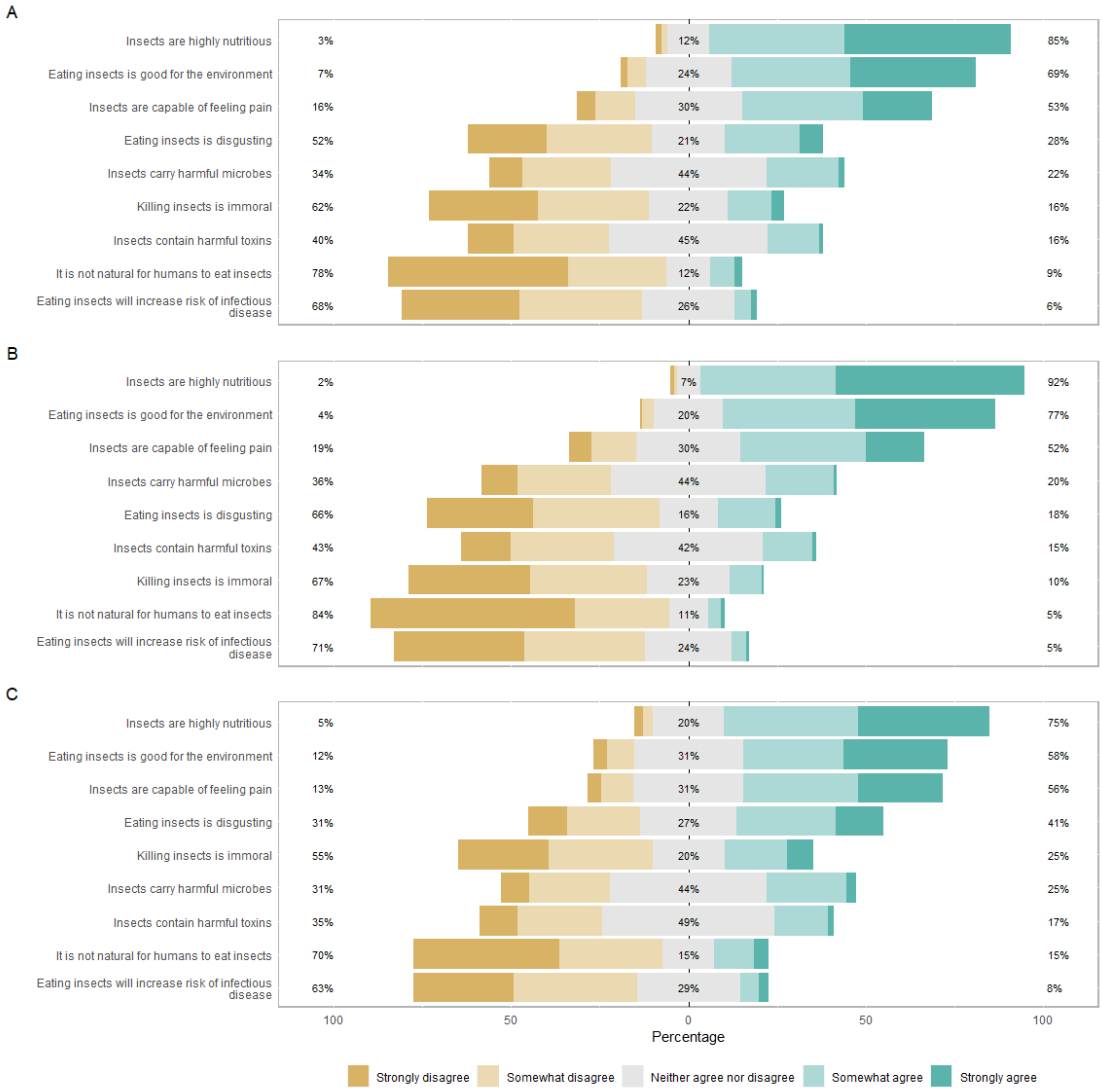

Figure 3. Specific beliefs and attitudes towards eating insects, measured on a 5-point likert scale. (A) All participants, (B) participants who have eaten an insect in the past, (C) participants who have not eaten an insect in the past.

Encouragement of Insects as Food (Figure 4)

In response to the question "We should encourage research on insects as food", the majority of participants agreed (89%). When responses were grouped based on those who had eaten an insect, this rose to 94% and when grouped by those who had not eaten an insect it decreased to 82%. In response to the question "We should support programs designed to encourage people to consume insects", the majority of participants agreed (78%). When responses were grouped based on those who had eaten an insect, this rose to 85% and when grouped by those who had not eaten an insect it decreased to 67%.

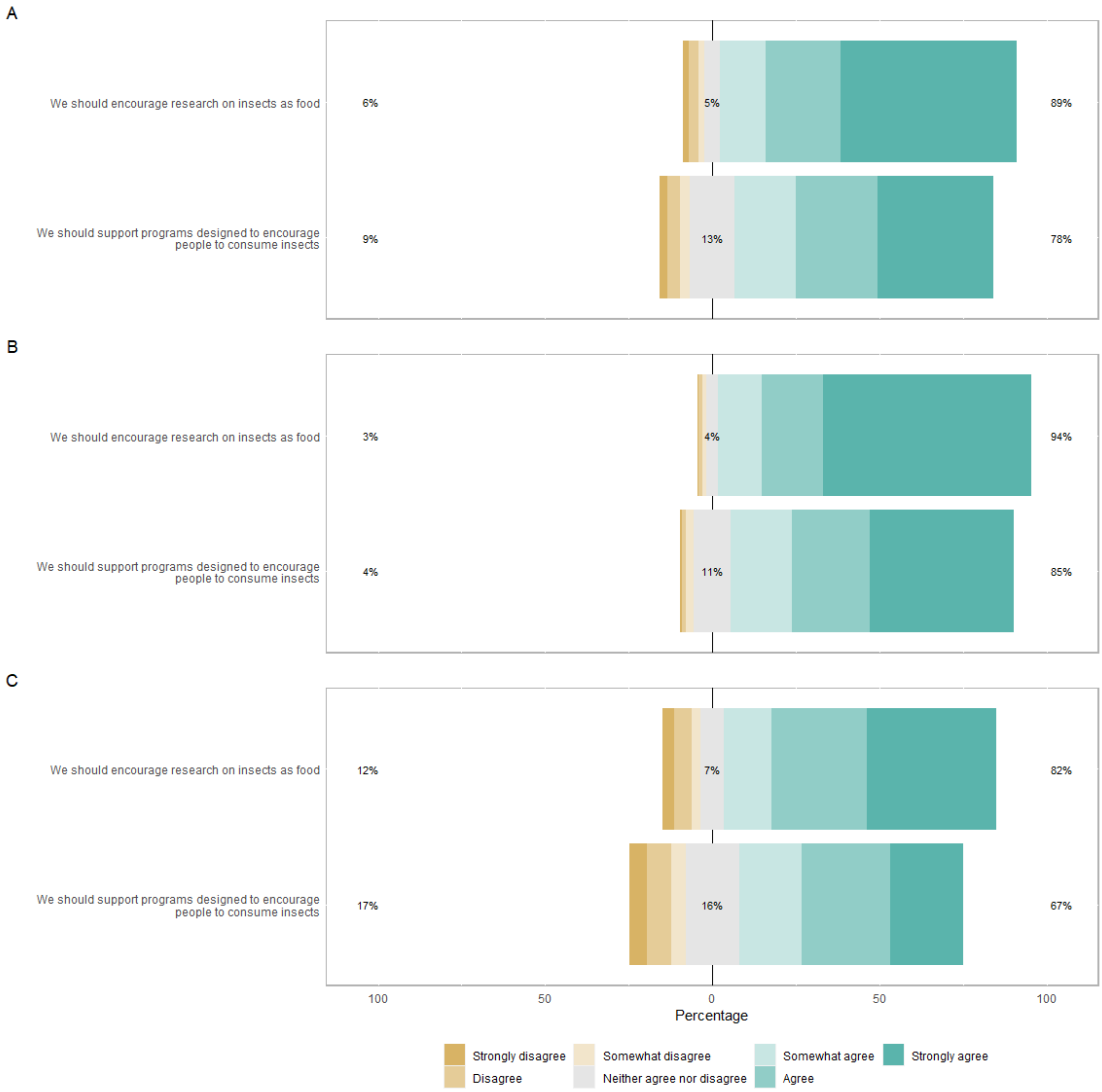

Figure 4. The majority of participants were supportive of policies encouraging research and consumption of insects as food. Participants responses on a 7-point likert scale to policies encouraging the eating of insects or research into eating insects. (A) All participants, (B) participants who have eaten an insect in the past, (C) participants who have not eaten an insect in the past.

Likelihood of eating an insect in the future (Figure 5)

Overall, the majority of participants were likely to eat an insect in the future (76%). When grouped by whether they had eaten an insect in the past, the majority of the 'Yes' and 'No' groups were likely to be willing to eat an insect in the future. The 'Yes' group, had a higher proportion of people who were likely to eat insects (88% vs. 60% in 'No'), and within the likely group, a larger proportion of participants indicated they were 'Extremely Likely' to try insects in the future. In contrast, in the 'No' group, there were a larger proportion of people who were unlikely to try insects (40% vs. 12% in 'Yes') and within this 'Unlikely Group' a larger proportion indicated 'Extremely Unlikely' to eat an insect.

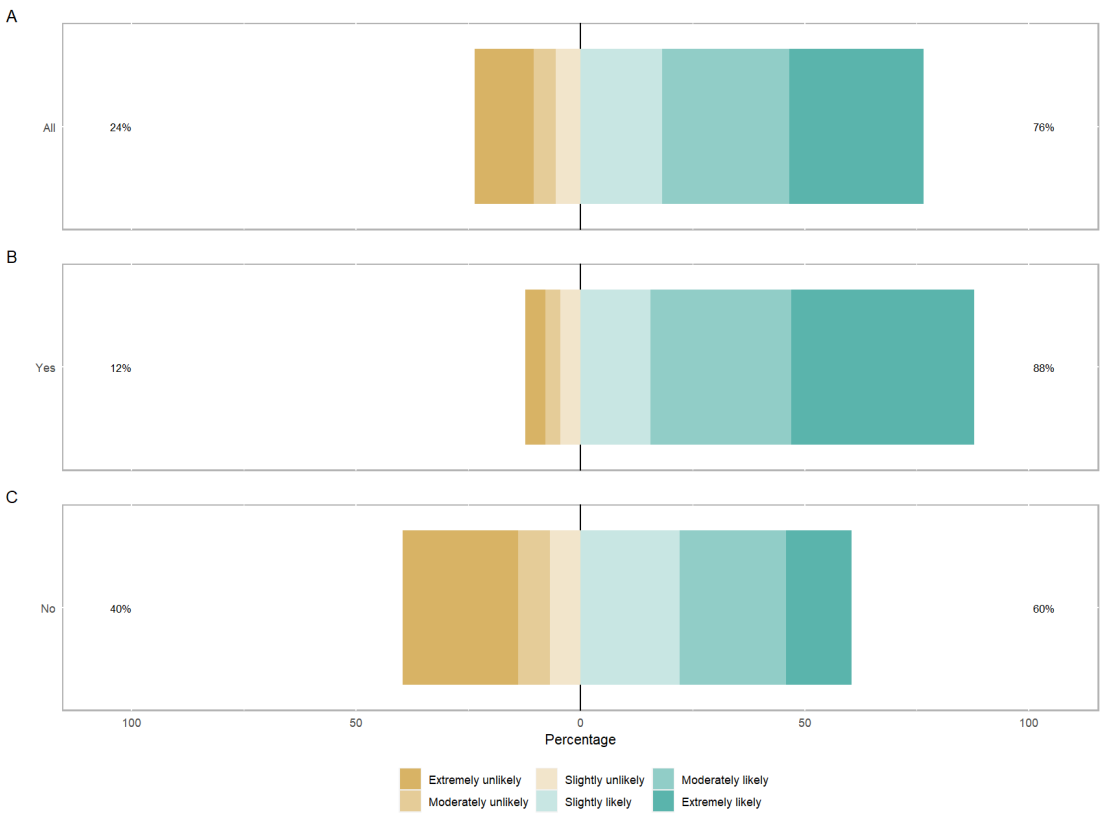

Figure 5. Likelihood of eating an insect in the future, measured on a 6-point likert scale. (A) All participants, (B) participants who have eaten an insect in the past, (C) participants who have not eaten an insect in the past.

Logistic Regression Model

I fitted a logistic model (estimated using ML) to predict future likelihood of eating an insect with prior experience, education, income, gender, food neophobia score and existing knowledge (Figure 6, Table 7). The model's explanatory power is moderate (Tjur's $R^2 = 0.23$). The model's intercept, corresponding to prior experience = no, education = diploma or certification, income = $10,001 - $30,000, gender = gender diverse, food neophobia score = 0 and existing knowledge = 0, is at 4.44 (95% CI [2.82, 6.28], $p < .001$).

The likelihood of eating an insect in the future was positively associated with having eaten an insect in the past (prior experience = yes; OR = 2.422, 95% CI [1.48, 3.99], $p < .001$) and existing knowledge (OR = 1.360, 95% CI [1.09,1.71], $p = .007$). The effect of food neophobia score is statistically significant and negative (OR = 0.912, 95% CI [0.88, 0.93], $p < .001$). All other terms were not statistically significant (Table 7).

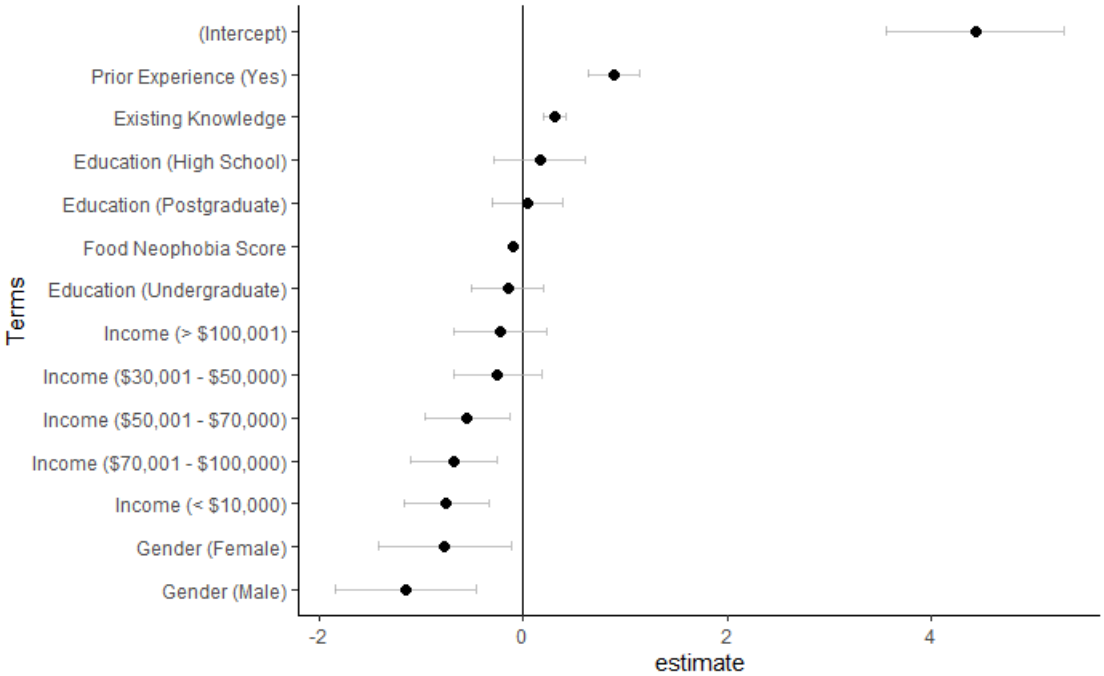

Figure 6. Coefficient plot of the logistic regression model factors associated with influencing future likelihood of eating an insect.

Table 7. Logistic regression analysis of factors associated with influencing future likelihood of eating an insect.

Effect	Estimate	SE	OR	Z	95% CI		P
					LL	UL	
(Intercept)	4.442	.873	84.95	5.088	16.718	531.921	< .001
Prior Experience (Yes)	0.885	.252	2.422	3.505	1.481	3.991	< .001
Education (High School)	0.160	.450	1.174	0.356	0.488	2.866	.721
Education (Postgraduate)	0.044	.348	1.045	0.126	0.521	2.050	.900
Education (Undergraduate)	-0.150	.356	0.861	-0.422	0.423	1.714	.673
Income ($30,001 - $50,000)	-0.252	.429	0.777	-0.586	0.332	1.800	.558
Income ($50,001 - $70,000)	-0.550	.411	0.577	-1.338	0.254	1.280	.181
Income ($70,001 - $100,000)	-0.683	.418	0.505	-1.633	0.219	1.134	.103
Income (> $100,001)	-0.226	.450	0.797	-0.503	0.327	1.925	.615
Income (< $10,000)	-0.757	.416	0.469	-1.821	0.205	1.051	.069
Gender (Female)	-0.769	.657	0.463	-1.172	0.109	1.502	.241
Gender (Male)	-1.155	.693	0.315	-1.665	0.070	1.109	.096
Food Neophobia Score	-0.092	.014	0.912	-6.709	0.887	0.936	< .001
Existing Knowledge	0.308	.114	1.360	2.707	1.097	1.712	.007

Note. OR = odds ratio; CI = confidence interval of the OR; LL = lower limit; UL = upper limit

Sentiment Analysis

When asked for the first words or phrases that come to mind when seeing an ingredient on a menu, all item groups received a similar number of mean responses per food item (animal = 1383.25, plant = 1376.33 and, insect = 1306.43). Neutral responses were the most common type of response across all groups. I found that food items in the animal and plant groups had more positive responses than negative, however this was reversed in the insect food item group (Figure 7).

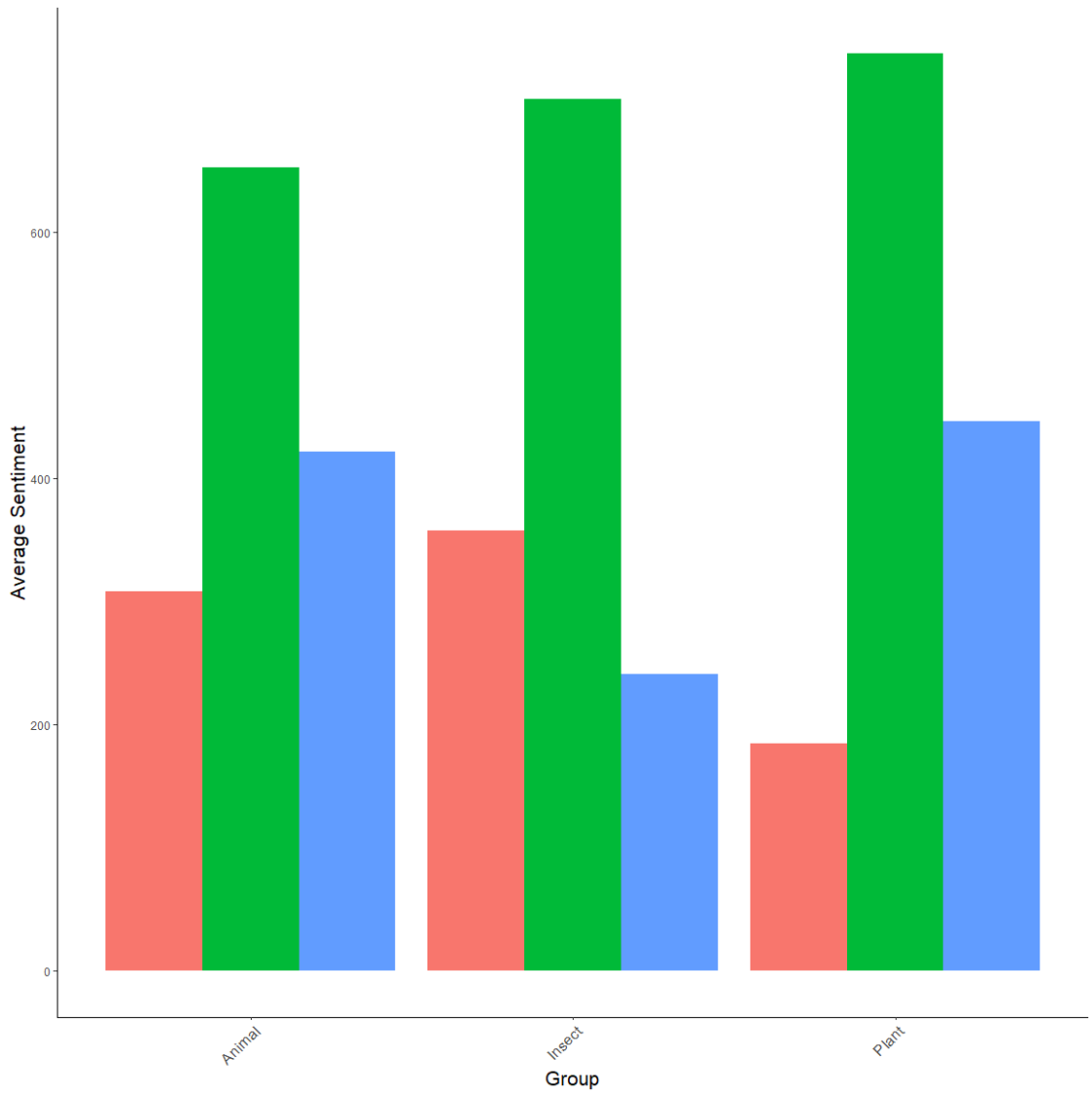

Figure 7. Positive = blue, negative = red, and neutral = green. Plant and animal ingredients received more positive words than negative words, whilst insect ingredients received more negative words than positive words. Words/phrases provided by participants for each food item were assigned a score: -1 for a negative sentiment, 0 for a neutral sentiment and 1 for a positive sentiment. These were totalled across each food group and divided by

the number of items within each food group to provide the mean number of positive, negative, or neutral words by food group.

Insect ingredients were associated with a lower average sentiment than animal and plant ingredients (Figure 8A). I performed a one-way ANOVA to compare the effect of ingredient type (animal, plant or insect) on sentiment revealing a statistically significant difference in sentiment between at least two groups ($F(2, 1850) = 85.48$, $P<.001$). I then performed a post-hoc Tukey's HSD Test for multiple comparisons which found that the mean value of sentiment was significantly different between insect and animal ($P< .001$, 95% C.I. [-0.49, -0.27]), plant-animal ($P< .001$, 95% C.I. [0.12, 0.35]) and plant-insect ($P< .001$, 95% C.I. [0.5, 0.73]).

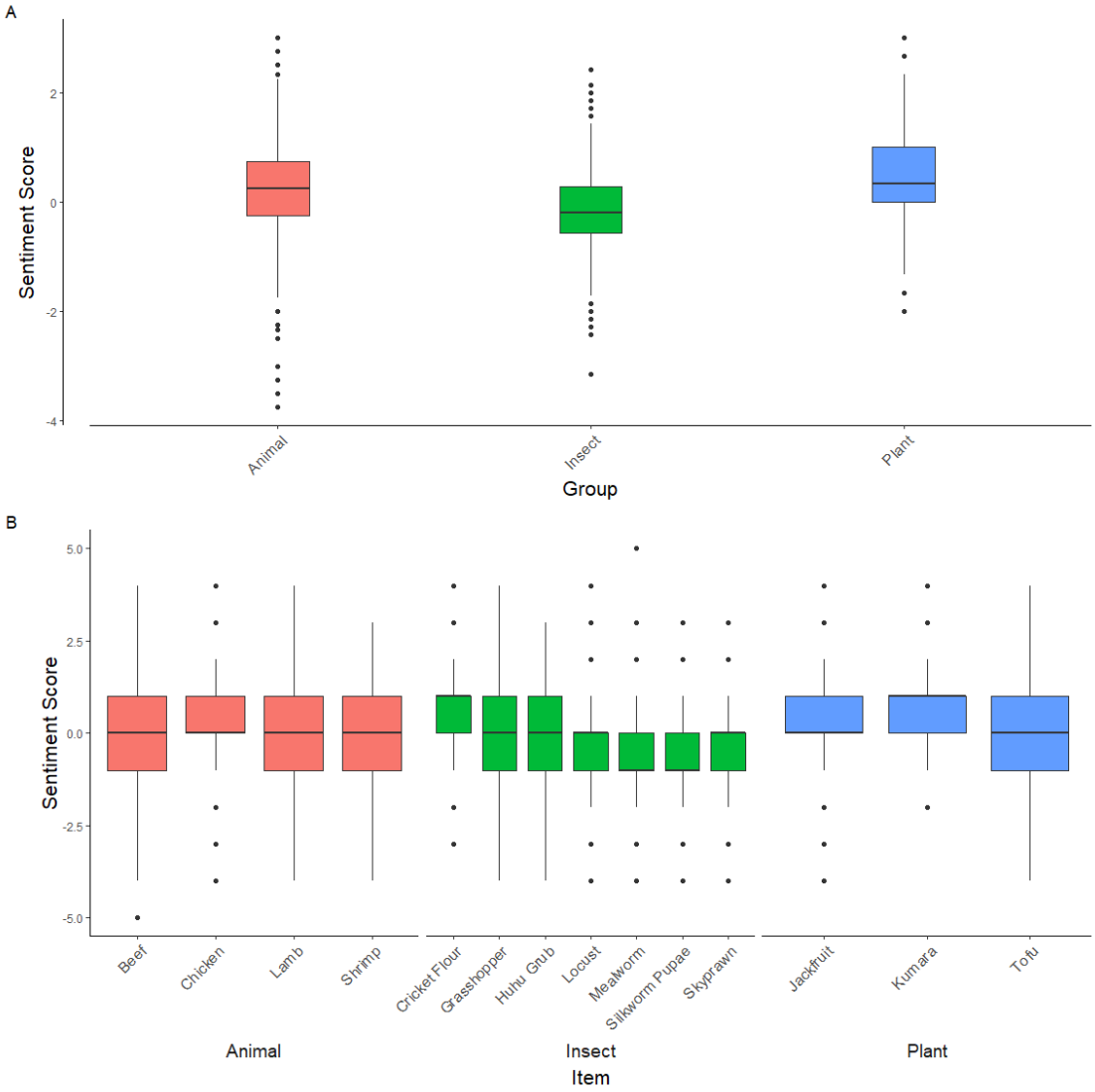

Figure 8. Insect ingredients have a lower average sentiment score than animal or plant ingredients. Words/phrases provided by participants for each food item were assigned a score: -1 for a negative sentiment, 0 for a neutral sentiment and 1 for a positive sentiment. (A) shows the average overall sentiment of food groups and (B) shows the average sentiment of individual food items.

The distribution of sentiment scores for individual ingredients are presented in Figure 8B. Within the insect group, cricket flour had the highest median sentiment score indicating a positive sentiment is associated with this ingredient. Both grasshoppers and huhu grubs had a neutral association with a median sentiment score of 0 and an interquartile range spread evenly either side of the mean, locusts and sky prawns also had a neutral median sentiment however the interquartile range is skewed towards the 25th percentile. Mealworms and silkworm pupae feature a negative association with a median score of -1 but with a skew towards the 75th percentile.

Words or Phrases Associated With Insect Products

In Figure 9, words that have the greatest discriminatory power are plotted for each insect ingredient. Different ingredients have clear differences in the words used to describe them, corresponding to the differences in sentiment seen in Figure 8B. When compared to animal and plant ingredients (Supplementary Figure 10 & Figure 11), I can see that insect ingredients tend to be less associated with food, and focus more on perceived physical properties of insects such as their movement or texture.

Words used to describe grasshoppers and locusts focused on physical characteristics ("green", "legs", "crunchy"). Grasshoppers do appear to have more positive associations ("cute", "jiminy cricket") than locusts and their possible role in food was mentioned ("flour"). Locusts were frequently described in negative historical terms ("swarm", "plague", "pest", "biblical", "Egypt") suggesting they are associated with their impacts on human society and agriculture through history.

Huhu grubs were described by perceived flavour profile and texture ("peanut butter", "gooey", "creamy", "slimy"), their ecology ("native", "bush", "beetle"), their cultural significance ("Māori"). Broadly, the words used to describe huhu grubs were neutral or positive and fairly specific, reflecting their unique and culturally significant status as a food source in New Zealand.

Both mealworms and silkworm pupae had a median negative sentiment, and this is reflected in the words participants associated with them. Descriptive words, describing perceived movement or textural qualities featured heavily and were similar for both ingredients ("wriggly", "squishy", "crunchy", "slimy", "worm", "maggot"). There was also a focus on the human utility of these insects with the use of silkworms in textiles highlighted ("silk", "cocoon", "thread", "clothing") and the use of mealworms in the pet industry ("pet food", "fish food", "bird food", "chicken food", "frog food", "lizards") however, neither ingredient was associated with human food with any frequency.

Sky prawns, a name used in the marketing of locusts, were largely met with confusion and unfamiliarity amongst respondents. The most frequent phrases associated with this ingredient were variations of "what is a sky prawn?". There was an association with familiar foods ("seafood", "prawn", "flying prawn", "crustacean") and some respondents correctly identified the ingredient as an insect ("locust", "cricket") however, there seems to be a general lack of recognition and understanding of the term.

On the other hand, cricket flour, which had a positive median sentiment score, was described using words that suggest it is perceived in a positive way ("curious", "interesting") as an environmentally friendly ("green", "environmental", and "alternative") and nutritious ("high protein", "gluten free") alternative to traditional flour. There appears to be associations between cricket flour and traditional culinary applications ("bread", "pancake," and "tortilla") suggesting that respondents associate this ingredient with foods.

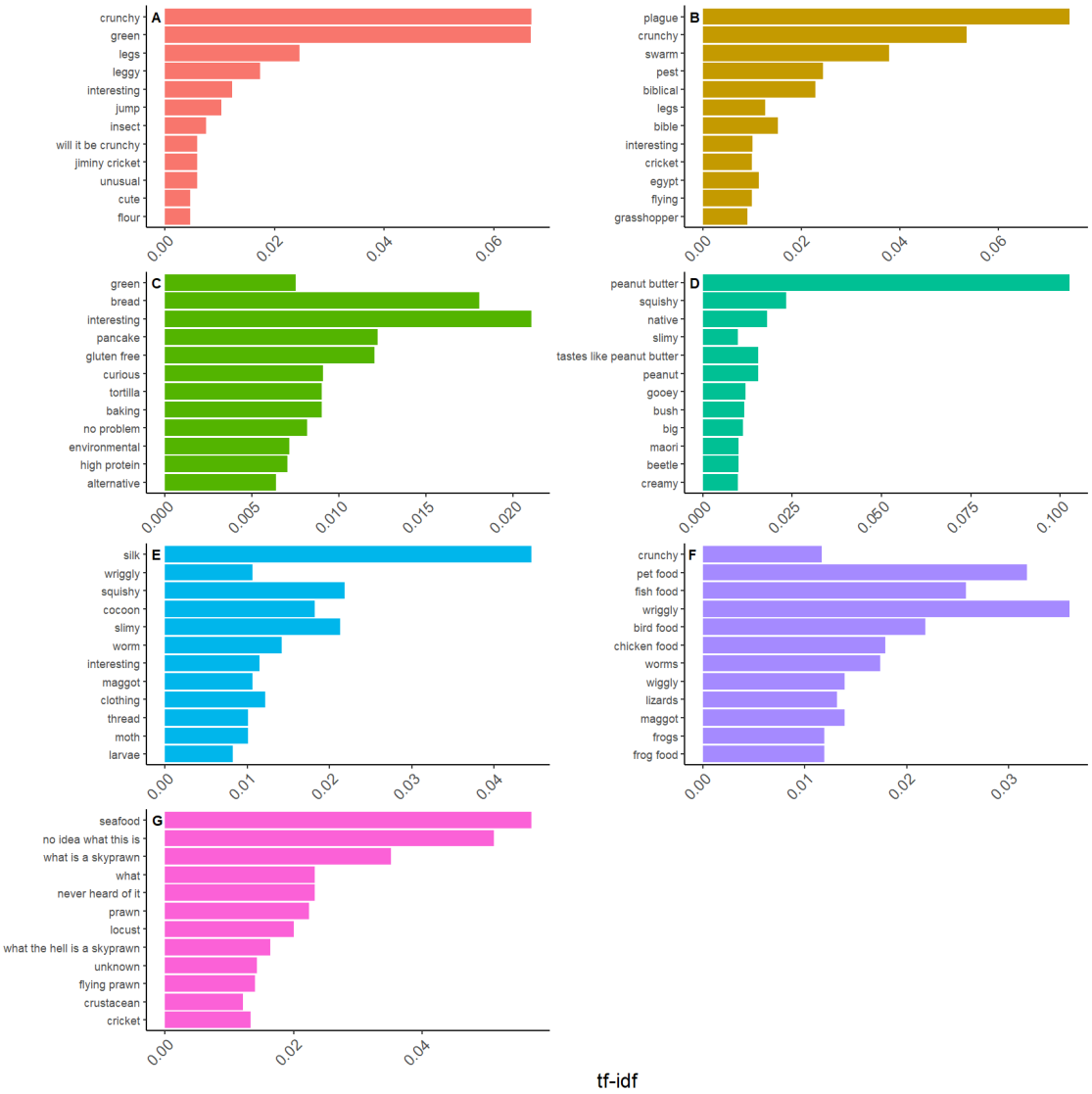

Figure 9. Term frequency – inverse document frequency (tf-idf) plots for insect ingredients, showing the words most frequently used to describe an ingredient. A) grasshopper, B) locust, C) cricket flour, D) huhu grub, E) silkworm pupae, F) mealworm, G) sky prawn.

Discussion

My research found strong support for insect consumption in New Zealand, in contrast to my findings on the viability of New Zealand edible insect businesses in the previous chapter. A majority of respondents had prior experience with insect consumption, expressing a willingness to continue eating insects in the future. Participants also possessed knowledge of edible insect species found in New Zealand, with huhu grubs and crickets being the most frequently mentioned species. Using a logistic regression model, several key predictors were influential in increasing the likelihood of future insect consumption, including prior experience with eating insects, familiarity with edible insect species, and a low food neophobia score (FNS). Factors such as gender, income, and education level did not appear to significantly influence individuals' likelihood of consuming insect-based foods. Despite this general openness to insect consumption, the analysis of the language used to describe insect ingredients reveals a contradiction; the average sentiment of the descriptors for insect-based ingredients was more negative than for plant or animal ingredients, with the exception of cricket flour which elicited more favourable associations.

Prior experience of eating insects was found to be an important predictor of future likelihood of eating insects, which was consistent with findings in the literature (Hartmann et al., 2015; Hartmann & Siegrist, 2016; Menozzi et al., 2017). Existing knowledge of edible insect species was also a significant predictor of future likelihood of eating insects. My findings suggest that New Zealanders are familiar with some edible insects, with huhu grubs and crickets both being frequently referenced by respondents. Familiarity has also been shown to be an important factor in food choice and it is commonly held that Western societies are unfamiliar with insects as food (Hoek et al., 2011; Verbeke, 2015). However, that does not appear to be the case in New Zealand with a large number of participants able to name one or more species of edible insect (n = 498). Huhu grubs in particular were recognised by participants with 54% (n = 333) of

respondents listing the huhu grub as an edible insect, reflecting the iconic status of this endemic species. Crickets were mentioned by 48% (n = 298) of respondents which is likely due to the high-profile crickets have received in both global and local media coverage of edible insects.

Unsurprisingly, a respondents' food neophobia score is negatively correlated with the likelihood of eating insects. It is likely that food neophobia is closely related to disgust (Hartmann et al., 2015), an emotion believed to have evolved to reduce the risk of disease, contaminants and poison (Oaten et al., 2009). It is difficult to overcome an emotional response using rational means although this can help in assuaging fears of sickness and disease (Jensen & Lieberoth, 2019). There is a significant cultural component to food neophobia which presents an opportunity to change people's perceptions towards insects as food over time through increasing public exposure (visibility of products in shops) and focussing on the sensory, gastronomic and marketing aspects of these products (Deroy et al., 2015; Koch et al., 2021; La Barbera et al., 2018). Frequently, insects as food are compared to caviar, lobsters, sushi and sashimi as foods which were once considered disgusting to many Western cultures but have since become popular (Koch et al., 2021; Ruby & Rozin, 2019), and it has been suggested that emulating the route-to-market of sushi in the West could be an option for insect products (Cf. House, 2018). Given the importance of prior experience and existing knowledge in influencing individuals' openness to eating insects, educational tastings are one way to increase exposure to, and acceptance of, insects as food (Sogari et al., 2017). Employing a trained professional to cook the insects in a culturally relevant way whilst educating on the history and benefits of insects as food enables the first experience to be a positive one.

Despite most participants stating their approval towards insects as food, the word association exercise revealed an interesting contradiction; insect ingredients on a menu received a higher number of words with a negative sentiment than animal or vegetable ingredients. This suggests that there may be unconscious bias around insect ingredients and insects as food. Investigating

the words people use when thinking about insects provides an opportunity to assess how people conceptualise insect ingredients and insects more broadly. By ranking words with the greatest discriminatory power for a food item (i.e., words that distinguished an insect ingredient from other ingredients), I found that insects evoke strong imagery with cultural, historical and biological contexts playing a clear role in shaping participants' perceptions of insects. For instance, locusts evoked Judeo-Christian references with: bible, plague, swarm, and Egypt; whilst references to children's movies (The Lion King and Pinocchio) appeared fairly often for huhu grubs and grasshoppers. Menu items that contained the term pupae or worm (silkworm pupae and mealworms) tended to evoke negative reactions. Also, responses were often not directly associated with food; instead being descriptive of the appearance, texture and movement of the insect (maggot, worms, larvae, moth, wriggly/wiggly, squishy, slimy), or their associated uses by humans (silk, clothing, thread, pet food, bird food, frog food etc.).

Cricket flour was the only ingredient with a mean positive sentiment score. The words that participants associated with cricket flour tended to match existing food concepts, for example; bread, pancake, tortilla, baking, gluten-free, and high protein. These are food items that either are associated with flour or are products containing cricket flour that have been marketed in New Zealand. This supports previous research finding that processing an insect into a meal or flour is preferred by New Zealand consumers (P. Payne et al., 2023). Other words used to describe cricket flour related to sustainability such as: environmental, alternative, and green. Sustainability over other livestock and environmental benefits are an area that has been marketed heavily by companies selling cricket flour as shown in Chapter 1. Other words used to describe cricket flour indicated generally positive sentiments and acceptance as a food ingredient: interesting, curious, and no problem. Overall, the words participants used to describe cricket flour highlights the effectiveness of the marketing and media attention this product has received.

However, associating an ingredient with food does not guarantee that it will be well received. In the case of sky prawn, a marketing term used for locusts in New Zealand and overseas, the overwhelming response was of confusion (words and phrases that occurred with frequency were: "what", "unknown", "no idea what this is", "never heard of it", "what is a skyprawn"). This indicates that the product has not achieved the same kind of public awareness as cricket flour and that attaching a word that is associated with food (i.e., prawn) is not enough to guarantee it will be well received. Providing more context would likely assist in alleviating feelings of distrust in this food product and it should be noted that the menu item was presented to survey participants without context. In a restaurant setting there would likely be access to further information (e.g., via the menu or waiter) and research has shown that being well informed reduces feelings of distrust (Schouteten et al., 2016).

Despite this, the use of "sky prawn" as a marketing device is a clever way of pointing out the morphological similarities between insects and crustaceans, which are already consumed, generally without issue. Indeed, at an evolutionary scale, insects are closely related to crustaceans, sitting within the clade Pancrustacea (Aleshin et al., 2009), and a recent review of the functional food properties of insects and shrimps, highlights that the shared phylogenetic history means that they should be viewed as terrestrial and marine counterparts with similar attributes and opportunities for exploration of novel functional food properties (Mishyna & Glumac, 2021).

Huhu grubs, which are a well-known edible insect in New Zealand (54% of participants identified huhu grubs as an edible insect), provide an interesting area to explore in the future. Participants had clear associations between huhu grubs and food with preconceived notions (positive or negative) of flavours and textures mentioned frequently (e.g., peanut butter, gooey, creamy, squishy, slimy). Participants also associated huhu grubs with Māori culture, bush (a New Zealand term for native forest) and native insects which suggests that there is a strong cultural association between huhu grubs and food in New Zealand.

A recent survey found huhu grubs are considered a culturally significant food by Māori and that Māori are open to eating insects particularly if they are harvested sustainably, have been used by Māori in the past, or incorporated rongoā rākau (medicinal plants) in the insects feed (O'Connor et al., 2023). Clearly there are opportunities for Māori-led businesses to develop and lead in this space and it would appear that there is a high level of acceptance of insects as food by Māori. Indigenous groups reclaiming and promoting culinary practices to promote insects and other indigenous ingredients in an authentic way whilst preserving and reviving traditional harvesting techniques and knowledge should be supported and encouraged (Bermúdez-Serrano, 2020; Reyes & Lessard, 2023). Restaurants such as Amazónico, in Colombia, and Hiakai, in New Zealand, are championing local culinary practices in contemporary dishes (Fiso et al., 2020; Taylor, 2023). At Hiakai, award winning chef Monique Fiso uses traditional ingredients, including huhu grubs, in her restaurant whilst also promoting the sustainable harvesting of ingredients according to tikanga (customs) (Fiso et al., 2020).

I measured participants specific beliefs and attitudes towards insects as food, finding that the majority of participants had positive attitudes towards insects as food, finding that the majority of participants agreed that insects were highly nutritious, good for the environment and are capable of feeling pain whilst disagreeing that eating insects is disgusting, unnatural or immoral and disagreeing that insects carry harmful microbes, toxins or increase the risk of disease. My findings contrast with those of Ruby et al. (2015) of American and Indian perceptions towards eating insects whose participants agreed that eating insects is disgusting, not natural for humans and that insects carry harmful microbes, whilst disagreeing that eating insects is good for the environment. This highlights there can be stark differences in perceptions between different cultures and lends support to avoid treating Western cultures as one homogenous entity (Ardoin & Prinyawiwatkul, 2021; Mancini et al., 2019).

Participants' agreement with the statement that insects are capable of feeling pain raises ethical considerations regarding the treatment of insects in farming and harvesting situations, an area that has received less research attention than other aspects of insect farming. Given a majority of participants agree (53%) or are uncertain (30%) that insects feel pain but disagree (67%) or are uncertain (22%) that it is immoral to kill them, it would be reasonable to assume that there is social licence to farm and kill insects but an expectation amongst the public that a duty of care towards the animal is taken into account when farming. Whether insects feel pain is an area still under debate and has implications for the development of welfare practices within the industry (van Huis, 2021). Similar discussions have occurred within the aquaculture industry regarding pain in fish, animal welfare and legislation and there may be opportunities to learn from these (Franks et al., 2021; Rose et al., 2014). However, farmers point out that applying codes of welfare from other sectors is unlikely to work directly with insects (Bear, 2019). There is difficulty in distinguishing nociception (the detection of noxious stimuli) and pain (the subjective experience of actual or perceived pain) empirically and so it would be best to approach the topic philosophically. The current landscape of insect welfare was recently summarised providing an excellent overview of the topic (van Huis, 2021), and a recent article investigated the complexities of care involved in insect rearing in New Zealand (Yee & Sharp, 2023).

Questionnaires as a research tool carries limitations which I have attempted to minimise but is still worth bearing in mind when interpreting the results. The sampling technique involved advertising on social media and snowballing which made it difficult to collect a representative sample of the New Zealand population and thus the results should not be generalised to the whole of New Zealand. Similar to a recent survey (P. Payne et al., 2023), this survey had a bias towards participants being European females with higher levels of education and higher income than would be expected from a representative sample of New Zealand. There are two potential sources of bias which are inherent in surveys: (1) social desirability bias where participants

answer questions in a way they feel will be viewed favourably, which I hope to have minimised by having an anonymous questionnaire, and (2) the value-action gap where participants intentions are different to their actions (e.g., saying will eat insects in future may not translate to carrying through with this).

The value-action gap is a well-known phenomenon in sustainability research (Barr, 2006), and has been shown to influence the purchase of sustainable food products (Vermeir & Verbeke, 2006). Participants in my study expressed a willingness to consume insects, but the overall sentiment when describing insects was negative. When viewed alongside the limited viability of companies specialising in insects as food (discussed in Chapter 1) I suggest this may indicate a disconnect between value and action and that future research should prioritise this area.

In summary, my findings suggest that participants are accepting of insects as food with many having prior experience and some existing knowledge, which, along with a low neophobia score, predicted likelihood of consuming in the future. Despite this approval, an analysis of the language used to describe insects as food uncovered a nuanced contradiction where the sentiment of descriptors for insect ingredients were, on average, more negative than traditional meat or vegetable ingredients. However, the notable exception to this was for cricket flour which demonstrates the effectiveness of the marketing used for this product and lends support to the idea of processing insects to disguise their appearance. The specific beliefs and attitudes of participants towards insects suggest differences to how other cultures perceive insects as food and indicate that future research should be directed towards the welfare and ethics of insect farming. I conclude by suggesting that future development of edible insects in New Zealand should build on the existing awareness of crickets and huhu grubs to leverage off existing acceptance and the unique history of these insects as food in New Zealand.

Supplementary Material

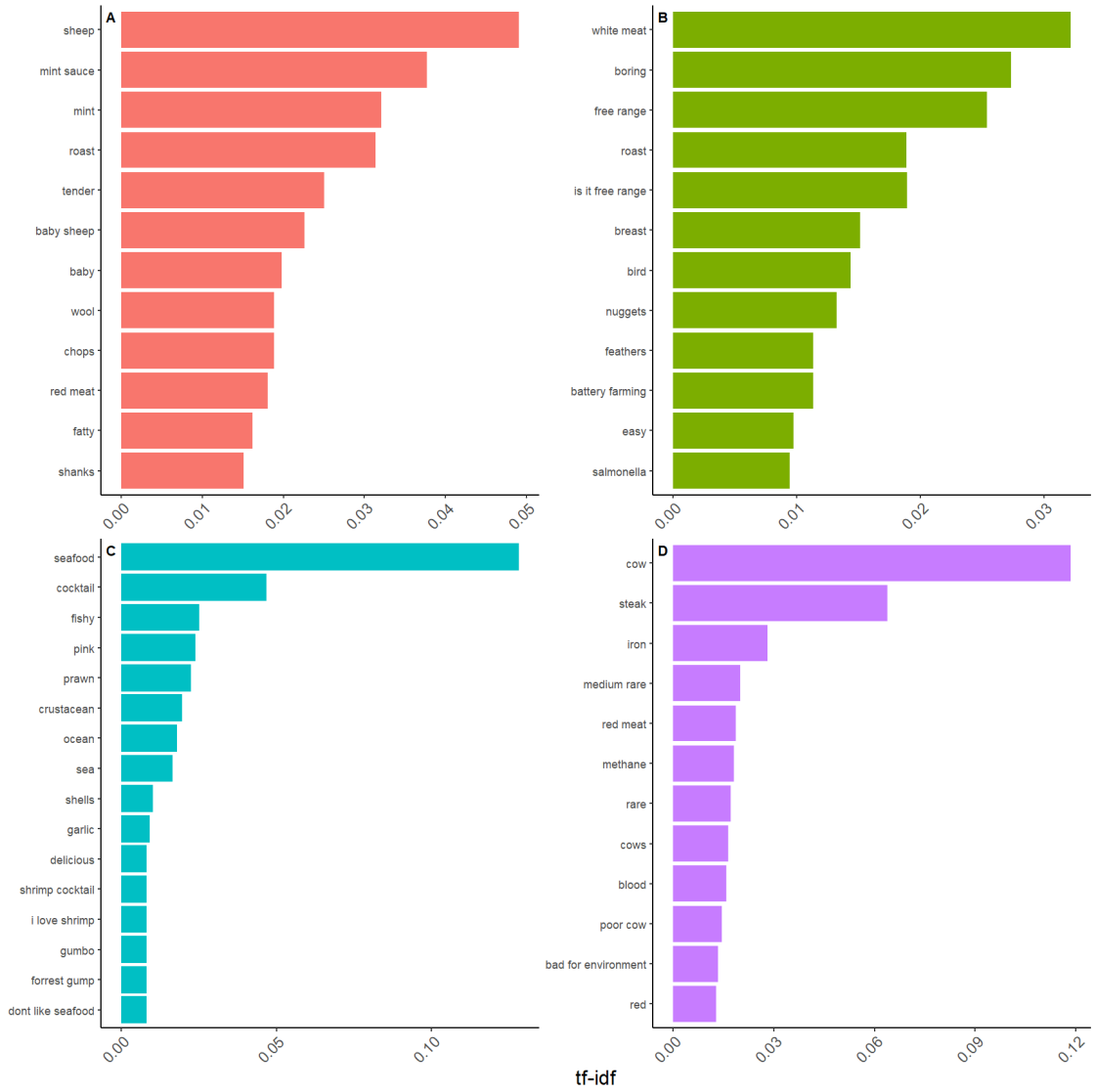

Figure 10. Term frequency – inverse document frequency (tf-idf) plots for animal meat ingredients, showing the words most frequently used to describe an ingredient. A) lamb, B) chicken, C) shrimp, D) beef

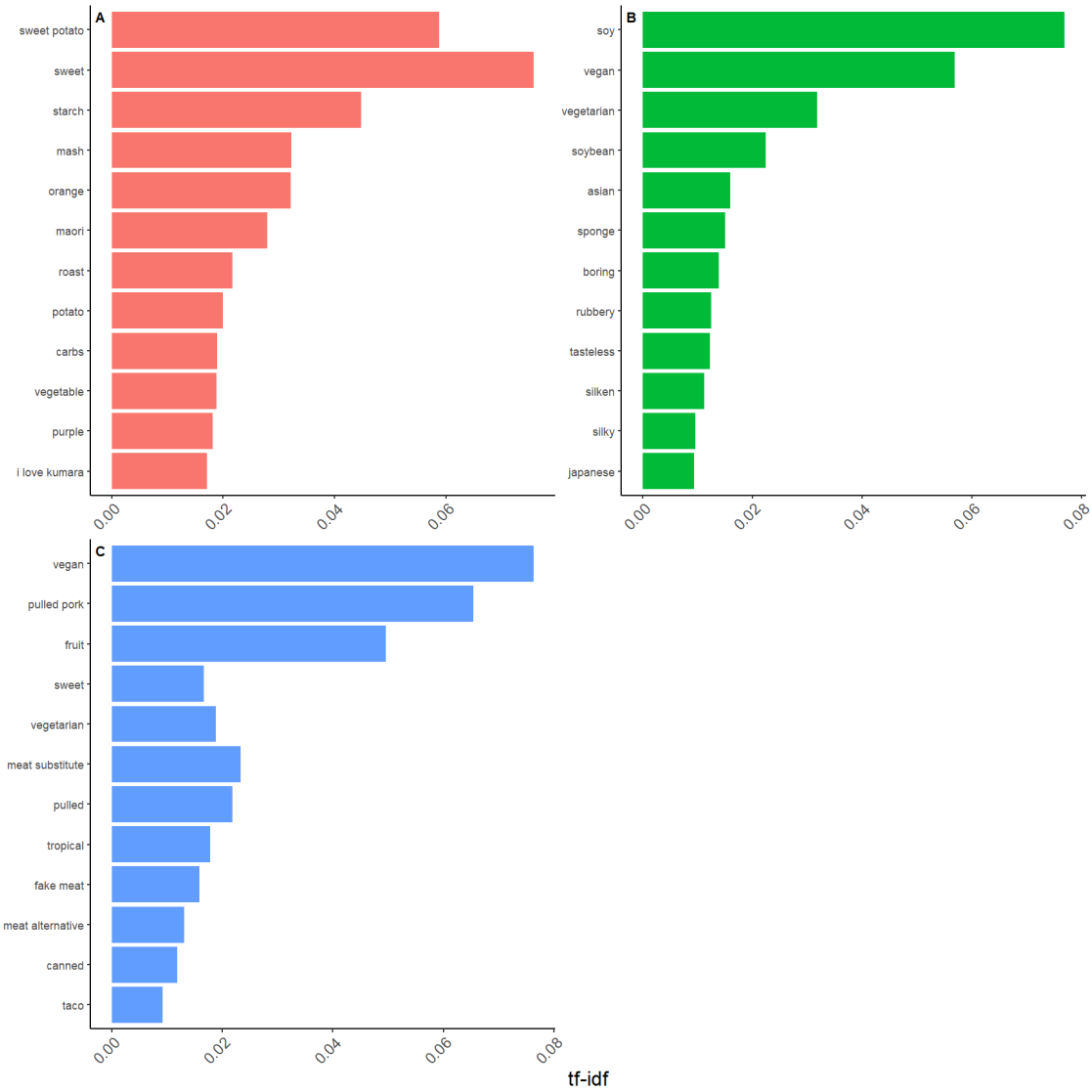

Figure 11. Term frequency - inverse document frequency (tf-idf) plots for plant ingredients, showing the words most frequently used to describe an ingredient. A) kumara, B) tofu, C) jackfruit

Chapter 4 The metabolomic fingerprint of an edible insect species, *Prionoplus reticularis* (Coleoptera: Cerambycidae), along a latitudinal gradient.

Abstract

Molecular fingerprinting of food items can provide information on the species, geographic location and quality of an organism making it a useful tool for identifying food fraud, provenance and assessing quality. Metabolomics is frequently used for this purpose due to its ability to identify and quantify metabolites at a high resolution. This study investigates the efficacy of untargeted GC-MS metabolic profiling using two different derivatisation techniques, MCF and TMS, to fingerprint an edible insect species - the huhu grub (*Prionoplus reticularis*) - along a latitudinal gradient. Huhu grubs, the larvae of Aotearoa New Zealand's largest longhorn beetle, are a culturally significant and commonly eaten insect throughout its range. I found that the accuracy of a random forest model trained on the TMS-derived metabolite profile was superior compared to MCF. I found several compounds, including arabinose and urea, that had a weak, linear correlation with latitude. Compounds with high relative abundance in this species were mannose and trehalose, which are known to be important for freeze tolerance in other species, and exhibited a surprising trend along the latitudinal gradient which warrants further research. Proline and alanine were the most abundant amino acids present, and these compounds are known to be important metabolites for flight in adult beetles. I suggest that future research should investigate the metabolomic signatures of temporal and dietary variations in this species, which would further validate the use of metabolomic fingerprinting in this species.

Introduction

The "fingerprinting" of food products has been used to protect consumers from food fraud, confirm the provenance of a food, and identify indicators of quality or functional properties of value (Capozzi & Bordoni, 2013; Ellis et al., 2016; Mialon et al., 2023). Fingerprinting involves characterising a food at the molecular level and recent advancements in high-throughput omics technologies and multivariate statistics has allowed this to be done at lower costs and greater accuracy (Capozzi & Bordoni, 2013). There are four major categories of omics: genomics, transcriptomics, proteomics, and metabolomics; of these, metabolomics is the most frequently used for fingerprinting. Metabolomics identifies and quantifies the metabolites within a biological sample, where the metabolome is strongly shaped by biological origin, biotic and abiotic conditions the sample is exposed to, and any treatments or processing experienced post-harvest (Sobolev et al., 2017).

A variety of metabolomic spectroscopy techniques have been applied to identify fraudulent products including: Fourier transform infrared (FT-IR) spectroscopy, near infrared (NIR) spectroscopy, Raman spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS) (Ellis et al., 2016). Mass spectrometry is a technique that detects and measures the mass-to-charge (m/z) ratio of charged ions of volatile or, following derivatization, semi-volatile metabolites (Dunn, 2008). The advantages of mass spectrometry are its incredible sensitivity, capable of detecting compounds in μM concentrations, and its ability to identify and detect known and unknown compounds based on mass spectra (Dunn, 2008). Frequently, mass spectrometry is coupled with gas or liquid chromatograms (GC & LC respectively) to further increase the sensitivity of the technique (Dunn, 2008). Metabolomics provides a useful technique to analyse a wind range of different characteristics of an insects biology (Poma et al., 2022; Snart et al., 2015). To date, fingerprinting research on edible insect species has focused on using

technologies such as polymerase chain reaction (PCR), proteomics, FTIR and MS, to differentiate between species (Belghit et al., 2019; Debode et al., 2017; Dhami et al., 2011; Francis et al., 2020; Marien et al., 2018; Mellado-Carretero et al., 2020; Tramuta et al., 2018; Zagon et al., 2018). In other non-insect contexts, due to the high precision and ability to distinguish a wide range of metabolites, metabolomics has been used in the detection of food fraud and geographical provenance (Capozzi & Bordoni, 2013; Mialon et al., 2023; Sobolev et al., 2017).

Food fraud is a broad term that refers to a food item being misrepresented for economic gain through deliberate and intentional substitution, addition, tampering, the misrepresentation of food, food ingredients or packaging (Spink & Moyer, 2011). The risk of food fraud increases when there is high demand for a product, the product is expensive and when there are complex supply chains involved (Black et al., 2016). Recent examples of this have propelled food fraud into global headlines, 24 % of dried oregano in the UK was found to be adulterated, olive oil has been mixed with lower quality oils, and the widespread sale of horsemeat as beef in the European market caused public outcry (Azadmard-Damirchi & Torbati, 2015; Black et al., 2016; Brooks et al., 2017; Tibola et al., 2018). Metabolomics has been used to distinguish wild vs. farmed sea bass, and Kopi Luwak (coffee that has been pre-digested by the Asian palm civet) from imposter coffees (Ellis et al., 2016; Jumhawan et al., 2013; Vidal et al., 2014). A variety of metabolomic techniques have been applied to insect products to ascertain their ability to detect between different insect species in powdered forms, which are an obvious candidate for food fraud (Mellado-Carretero et al., 2020; Tata et al., 2022).

Attempts to determine the geographical provenance of a food product have also become more prevalent with the introduction of protected geographical indication (PGI), protected designation of origin (PDO), and traditional specialities guaranteed (TSG) statuses by legislative bodies. Metabolomics presents a useful way of investigating the variability of metabolites found

within different geographical populations of an edible insect species as it is relatively cheap compared to genomic and proteomic analysis and has been used to successfully discriminate the geographical origin of tea, rice, hazelnuts, honey, mussels and snapper fish (Azevedo et al., 2017, 2017; Ch et al., 2021; Drivelos et al., 2021; Goode et al., 2020; Klockmann et al., 2016; Peng et al., 2023; Rochfort et al., 2013).

Increasing demand for a food product can lead to unintended outcomes such as placing pressure on wild food species through the unsustainable harvesting of wild organisms or the adulteration of food for commercial gain (Spink & Moyer, 2011; van Huis & Oonincx, 2017). This can pose a risk to human health, consumer trust and the population viability of wild organisms. Whilst edible insects are enjoyed in many countries around the world, demand for edible insects in Western nations has increased since the publication of a 2013 FAO report, proposing insects as a more sustainable alternative to livestock (van Huis et al., 2013). Generally, this demand has been satisfied through the farming of insects, such as crickets and mealworms. These are usually sourced from domestic or international production and legislative constraints on what can be sold as food in Western countries restricts the use of wild insects. There has been discussion around the flavour of wild harvested insects being superior to farmed insects (Evans et al., 2017, p. 179), and demand for wild insects for gourmet use is likely to increase over time.

In many countries around the world, insects are wild sourced and an increase in demand could place undue strain on populations already experiencing challenges with climate change, habitat loss and pollution (van Huis & Oonincx, 2017). This has occurred in some countries with an existing history of eating insects. Whilst insects have been sustainably harvested for generations by indigenous peoples with an intimate knowledge of the life cycle and ecology of the species, there is evidence that changes in environment, the impact of human development, urbanisation, along with growing demand for insects from outside of these communities, is putting insect populations at risk (van Huis & Oonincx, 2017). Mexico, a country with a rich history of insect

use, many of which are highly sought after, provides a useful case study. Ramos-Elorduy (2006) studied the use of 30 edible insect species over a 25 year period in the village of Tulancalco, Mexico. In this village, there have been changes in the population demographics over several generations where people have moved from villages to cities. There are still strong ties to the food culture, and this drives high prices for insects for sale in the city. This demand for insects is fulfilled by outsiders who are not familiar with the traditions and deep ecological knowledge held by villagers and now, of these 30 species, 14 are threatened and at risk of local extinction (Ramos-Elorduy, 2006).

There are other examples of wild populations of edible insects that are no longer sustainable to harvest due to a variety of pressures, including crickets belonging to the genus *Oxya* in Asia, aquatic hemipterans in Mexico and India and the larvae of Lepidoptera in Africa and Australia (Badillo-Camacho et al., 2015; Parsons, 2010; Ramos-Elorduy, 2006; Samom, 2016; van Huis & Oonincx, 2017). Whilst the causes of decline in these species are multi-faceted, it is possible to reverse population loss and restore the sustainability of wild harvest. For instance, an increase in pesticide use in farming has led to a decrease in the populations of *Oxya* spp. crickets in Korea and Japan which has resulted in a reduction in their use as food (C. L. R. Payne, 2015; Pemberton, 1994). However, when farmers in areas of Korea moved away from pesticide use, the *Oxya* spp. populations returned and their use as a food saw a revival (Pemberton, 1994). Given the suitability of harvesting an insect population can vary geographically, is there a way to ascertain if an edible insect comes from a specific geographic region within a country? This would allow consumers, legislators, or importers to confirm an insect comes from a region where it is sustainable to harvest from and thereby make an informed decision.

Metabolomics would appear to be an ideal candidate for this as the metabolome of an organism is intricately shaped by its biology and the environment it inhabits. Several New Zealand studies have explored using metabolomics to characterise the metabolomic profile of native and

endemic New Zealand species, showing that the geographical location and environmental factors experienced influence the metabolomic profile of the organism. In a study of scale insects in New Zealand, Dhami et al. (2011) found that the metabolite composition of honey dew varied between species, sites and host tree suggesting that geographic location alone is not the sole driver of differences between metabolite profiles. Goode et al. (2020) investigated the metabolome of snapper *Chrysophrys auratus* (prev. *Pagrus aurata*) from different harbours around New Zealand finding that differences in amino acids were attributable to turbidity of their environment, an aspect known to be a stressor resulting in suboptimal physiological responses. Temperature is another important environmental variable that is known to impact the metabolism of organisms. In New Zealand, metabolites have been identified that can be used to detect thermally stressed green-lipped mussels (*Perna canaliculus*).

The endemic cerambycid longhorn beetle, *Prionoplus reticularis*, better known as the edible huhu grub, is an ideal candidate to explore the effect of geography and latitude on variation of metabolomic profiles. The species is found throughout Aotearoa New Zealand from Northland to Stewart Island and the larvae infest rotten logs of several different host plant species, including *Pinus radiata* (radiata pine). *P. radiata* accounts for 90% of New Zealand's forestry stock and is found across New Zealand (Beets et al., 2012), which allows for an assessment of geographic variation in metabolites in huhu, while controlling for a consistent diet.

I used GC-MS (gas chromatography – mass spectrometry) to investigate the relative abundance of metabolites in the huhu grub (*Prionoplus reticularis*) collected from Pinus radiata, along a latitudinal gradient the length of New Zealand. I investigated (1) variation in metabolites and lipids of huhu between individuals and locations, (2) whether metabolites of huhu show a trend along a latitudinal gradient, and (3) if FAAs, FAs & sugar metabolites can be used to 'fingerprint' an individual to a geographic location.

Methods

Fieldwork

I sampled larvae (n = 144) of *P. reticularis* during a three-week period, from the 7th November to 29th November 2020, at six locations along a latitudinal gradient within New Zealand (Figure 12). At each site, logs of similar decomposition were selected. The diameter of the log was measured using a tape measure and temperature within the log was recorded with a thermocouple (TM-902C, Shenzhen Glory Line Co., Ltd.). The log was then split open, and any larvae of *P. reticularis* removed. Larvae were weighed on a portable jewellery scale (8028 Series, Guangzhou Juanjuan Electronic Technology Co., Ltd.), wrapped in aluminium foil and frozen in liquid nitrogen before placing in a -80 °C freezer upon return to the lab. At each location a sample size of 24 larvae were collected, except for Seabush (n = 23) and Mangaroa (n = 25).

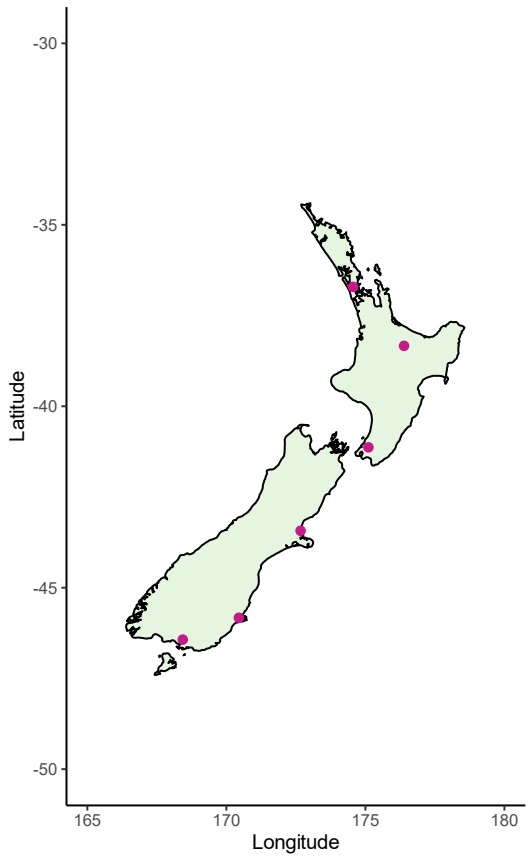

Figure 12. Sample locations from North to South; Riverhead (Auckland), Kaingaroa (Rotorua), Mangaroa (Wellington), Cheney's Forest (Christchurch), Flagstaff (Dunedin), and Seabush (Invercargill)

Metabolite extraction and derivatisation

Metabolite chemical extraction and derivatisation of *P. reticularis* larvae was achieved using methyl chloroformate (MCF) and trimethylsilyl (TMS) protocols developed at The University of Auckland (Smart et al., 2010; Villas-Bôas et al., 2011; Zarate et al., 2017). The methods are outlined briefly below.

Extraction of metabolites

Samples were placed in a freeze-drier for a 48-hour period then macerated with a glass rod. A 10 mg (±2 mg) amount was placed in a 1.5 ml Eppendorf tube and the remainder of the sample was stored in a -80 °C freezer. I added 500 μL of 50 % cold MeOH:H2O, resuspended the solute using a vortex and froze using dry ice. I then centrifuged at -9 °C at 3500 rpm for five minutes. The supernatant was transferred to 2 mL microcentrifuge tubes. The extraction process was then repeated on the supernatant with 500 μL of 80 % cold MeOH:H2O. The pellet from the 1.5 mL Eppendorf tube was retained for biomass measurement. For MCF, the internal standard was D4-alanine (20 μL, 10 mM) TMS, the internal standard was D-ribitol, (20 μL, 10 mM). The extracts were dried in a Speed-Vac Concentrator with a Refrigerated Vapor Trap (SC250EXPP2-115, Thermo Scientific, New Zealand) for 4 hours at 0.8 HPa, ramp 3-5, with no heating. Once dried, the samples were kept in a desiccator overnight prior to derivatisation.

Methyl chloroformate Derivatisation

I followed the modified MCF derivatisation method outlined in Smart et al. (2010). In brief, I added 400 μL of 1 M sodium hydroxide to the derivatisation blank, standard amino acid mix and each sample and then vortexed to resuspend the freeze-dried samples. 334 μL of methanol and 68 μL of pyridine were added to individual silanised tubes, the corresponding sample was then added. I added 40 μL of methyl chloroformate to each sample, vortexing for 30 seconds and then adding a further 40 μL and vortexing again. 400 μL chloroform was added and then the sample was gently vortexed for 10 seconds. 800 μL sodium bicarbonate solution (50 mM) was

added and then vortexed for 10 seconds. I then centrifuged the samples at 2500 rpm for 5 minutes. The aqueous phase was removed using a vacuum, and anhydrous sodium sulphate added to remove any residual water. The samples were then transferred to a glass GC-MS vial with glass insert and lid.

Each day I randomly assigned seventeen MCF derivatised samples to a GCMS run for a period of nine days. Each GCMS run also included a chloroform vial, an alkane mix vial, one blank vial, one standard mix containing reference amino acids at 0.5 mM and three quality controls (containing pooled samples).

Trimethylsilate Derivatisation

I derivatised sugar metabolites using the protocol outlined in Zarate et al. (2017). Samples were removed from the desiccator and 80 μL of methoxyamine hydrochloride in pyridine (2g.100 mL) was added before vortexing for 1 minute to dissolve the dried sample. Samples were then transferred to glass GCMS vials and incubated for 90 minutes at 28 °C. I then added 80 μL N-methyl -N-(trimethylsilyl trifluoroacetamide (MSTFA) and incubated for 30 minutes at 37 °C.

Each day I randomly assigned sixteen TMS derivatised samples to a GCMS run for a period of nine days. Each GCMS run also included a chloroform vial, an alkane mix vial, one blank vial, two standard mix containing reference amino acids at 0.5 mM and two quality controls (containing pooled samples).

GCMS

To analyse the metabolite profile, I followed the protocol outlined in Villa-Bôas et al. (2011) using an Agilent GC 7890A coupled to a MS 5975C (Agilent Technologies, Santa Clara, CA, USA) with a quadrupole mass selective detector (electron ionization) with a split/splitless inlet.

The GC oven was held at 45 °C for 2 minutes then increased at a rate 9 °C a minute to 180 °C where it was held for 5 minutes before increasing at 40 °C per minute to 220 °C and held for 5

minutes before increasing 40 °C per minute to 240 °C where it was held for 11.5 minutes before increasing at 40 °C per minute to 280 °C where it was held for 10 minutes. The mass spectrometer interface was set to 250 °C, the source was set at 230 °C and the quadrupole was set at 150 °C. 1 μL of sample was injected into the column using splitless mode and the carrier gas was ultra-high purity helium (BOC Ltd.). The mass spectrometer was set to scanning mode with a start time of 4.78 minutes and a mass range of 38 – 550 atomic mass units and the detection threshold of 50 ion counts.

Data Preparation

I used the MassOmics pipeline developed at the University of Auckland to process the raw spectra using Automated Mass Spectral Deconvolution and Identification System (AMDIS, v2.66) (Aggio et al., 2011). Agilent Chemstation was used to create a subset of the Wiley and NIST Libraries. Then the identification output was merged in the MassOmics R package to make a library which was converted to an AMDIS compatible format using the Lib2Nist converter. For the MCF analysis, I merged both the NIST library and in-house libraries. For the TMS analysis, I used the in-house library. I then used AMDIS to deconvolute and identify compounds in each sample with the output of this converted into a summary report in R. I curated the summary report using excel and R following the protocol outlined in Guo et al. (2017). Prior to analysis I normalised the data by sample biomass and the internal standard (MCF = D4-alanine; TMS = D-ribitol).

Data Analysis

I used MetaboAnalyst (Pang et al., 2021; v. 5.0) and R to analyse the data. The MCF data and TMS data were analysed independently of each other using the following procedure. First, any zero or missing values were imputed using 1/5 of the minimum positive value for each variable. The data were then normalised using log10 transformed and pareto scaled (mean centred and divided by the square root of standard deviation of each variable).

To assess variation in the metabolic profiles of individuals and locations, I used hierarchical clustering analysis to produce a dendrogram of clustered metabolites, with Euclidean distance as the similarity measure and Ward's linkage as the clustering algorithm and presented the results as a heat map.

I used the pattern hunter analysis in MetaboAnalyst to identify metabolites that correlated best with a latitudinal gradient running from north to south. Pearsons R was used for the correlation coefficient, and the top 25 metabolites that increased or decreased linearly with the latitudinal gradient are presented as a bar chart.

I conducted a principal component analysis (PCA) to assess the variance in the data set by reducing the dimensionality of the data by summarising into scores, a weighted average of the original variables. The output of this was presented as pairwise score plots for the first five principal components, as a two-dimensional plot of the first two principal components and as a three-dimensional plot visualising the first three principal components.

To confirm if the separation by location visible in the PCA plots were statistically significant, I used a type I permutation multivariate analysis of variance (PERMANOVA) on the Euclidean dissimilarity matrix of each data set (M. J. Anderson, 2001). The PERMANOVA was performed using the R package 'vegan' (Oksanen et al., 2022) with n = 10,000 permutations. I then performed pairwise comparisons on significant terms to compare between different groups. P values were adjusted using the Benjamini-Hochberg procedure.

To assess if I could classify a random huhu grub sample to the location it was sampled from, I used a random forest (RF) supervised machine learning algorithm to construct a classification model. RF is suited to high dimensional data, where there are a high number of features (metabolites/compounds) to samples (Acharjee et al., 2016). RF uses random feature selection from a bootstrap sample of each branch to create an ensemble of classification trees from which

class prediction calculated from a simple majority vote. As RF is prone to overfitting (Fawagreh et al., 2014), data were split into a training set of 2/3 and testing set of 1/3 to calculate out-of-bag (OOB) error and a confusion matrix. A variable importance measure was also calculated by measuring the increase in the OOB error when a metabolite is permuted whilst all others are left the same. I Implemented this through the 'randomForest' R package (Liaw & Wiener, 2002) via MetaboAnalyst.

Results

A total of 144 samples, representing six geographic locations along a latitudinal gradient, were extracted with two derivatisation methods. Using GCMS, I identified 146 compounds (including 39 'unknown' metabolites) comprising a variety of fatty, amino, and organic acids, and sugar and sugar alcohols and other compounds. Of the 146 compounds identified, 103 were derivatised using MCF and 43 were derivatised using TMS. As the two datasets contain relative abundances to different internal standards, I analysed each separately and present them individually.

Methyl chloroformate

I present the 10 most abundant compounds (using untransformed data) identified by MCF in Figure 13. The remainder of analyses presented use data that has been normalised.

Using a heatmap, I explored variation in the MCF derivatised metabolite profile of the edible insect *P. reticularis* between individuals and locations (Figure 14). There was a large amount of variation in the metabolite abundance between individuals, and no visually distinct patterns between locations.

I found that several metabolites exhibited a weak correlation along a latitudinal gradient. The top 25 metabolites that correlate with a latitudinal gradient are presented in Figure 15. Of these 25 metabolites, only two were significant after correcting using FDR: unknown compound 117

showed a weak positive correlation ($r = 0.30$, $p = 0.01$) and unknown compound 198 showed a weak negative correlation ($r = -0.27$, $p = 0.04$) with latitude (Figure 15).

I constructed an unsupervised PCA model for the MCF dataset which showed the general structure of the data (Figure 16). For the MCF dataset, the first two principal components cumulatively explain 64.8 % of the total variation (PC1 explains 53.9%, PC2 explains 10.9%). When visualising in two-dimensions (Figure 16 & Figure 17), I was able to see some grouping in relation to location although there was a lot of overlap between groups. However, when visualising this in three-dimensions (Figure 18), a greater degree of separation between locations was visible.

The PERMANOVA results (Table 8) showed that location played a significant role in the metabolite composition of *P. reticularis*, explaining 12.3 % of the variation observed in the data set ($p < 0.001$, $R^2 = 0.123$). Larval weight was also significant but only explained 5 % of the variation ($p < 0.001$, $R^2 = 0.05$). The log the larvae was sourced from and temperature of the log were also significant, explaining 9.3 % of the variation and 1.4 % of the variation respectively ($p = 0.03$, $R^2 = 0.093$; $p = 0.05$, $R^2 = 0.014$). I performed pairwise comparisons among locations of origin to understand which locations significantly differed from one another (Table 9).

The classification model (using random forest algorithm) had an overall out-of-bag (OOB) error rate of 0.319, meaning the model correctly identifies the location of a sample 68.1 % of the time. Kaingaroa had the greatest OOB error rate of all sample locations with 0.42 (Table 10, Figure 19). I also used a random forest model to determine metabolites that have the greatest impact on the accuracy of the model and could be used as biomarkers in the future (Figure 20). These 15 metabolites accounted for a mean decrease in accuracy of 16.7% when permuted in the model, with the top 5 metabolites accounting for a 7.2 % decrease in accuracy.

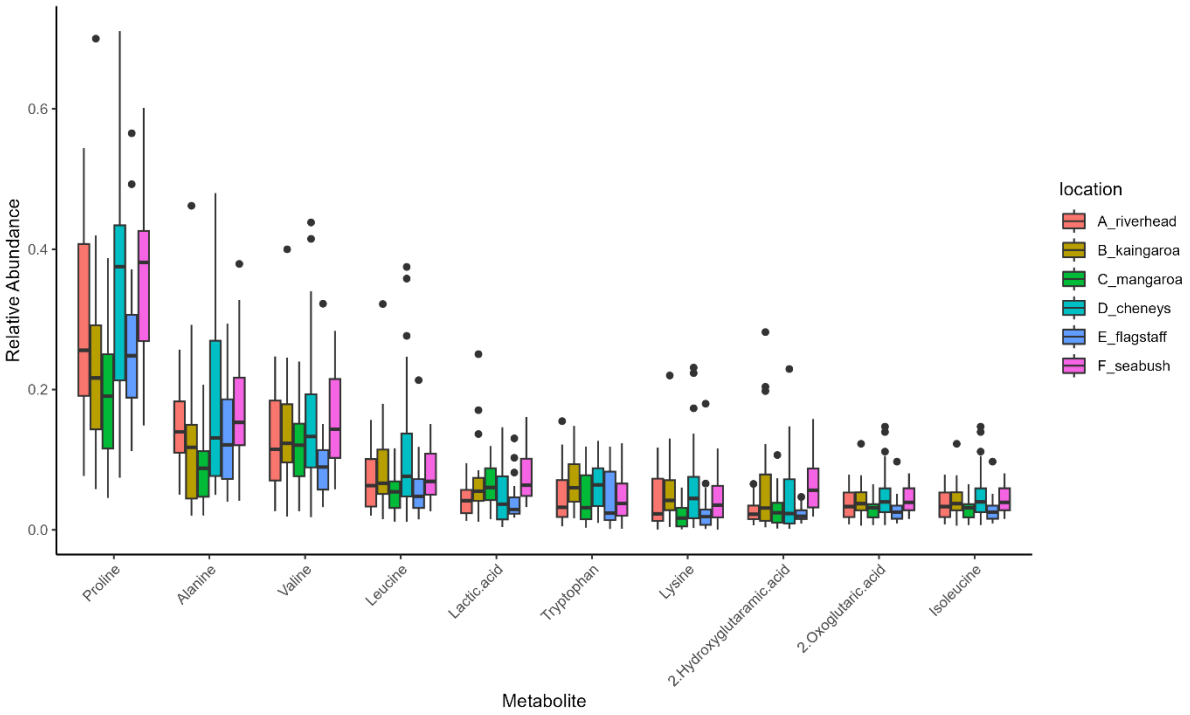

Figure 13. The top ten most abundant compounds derivatised by MCF using untransformed data, grouped by location.

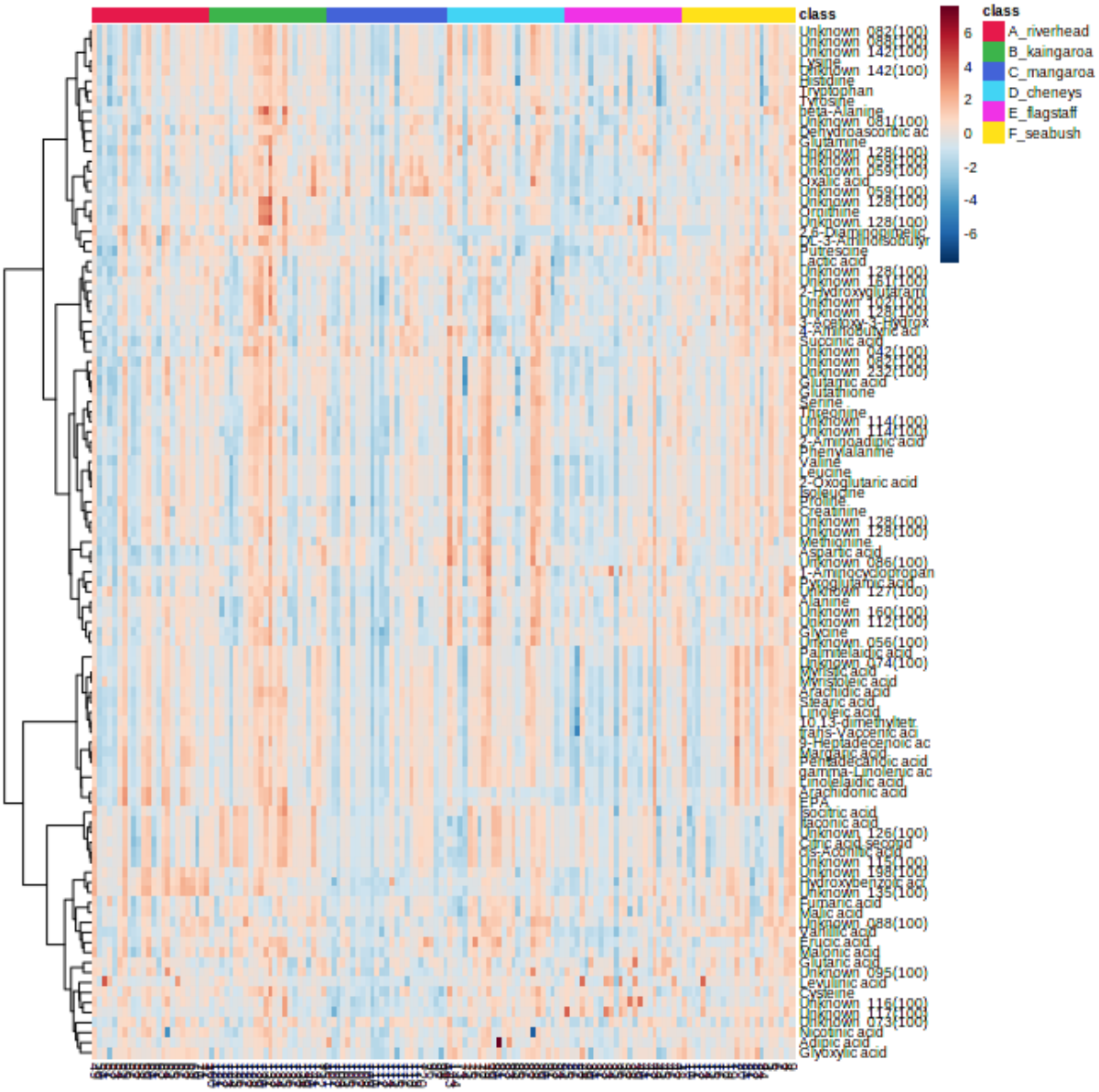

Figure 14. The heat map represents the metabolite profile of larvae collected from six different locations and derivatised using MCF. Clustering of metabolites was performed with a Euclidean distance measure and the Ward algorithm in MetaboAnalyst.

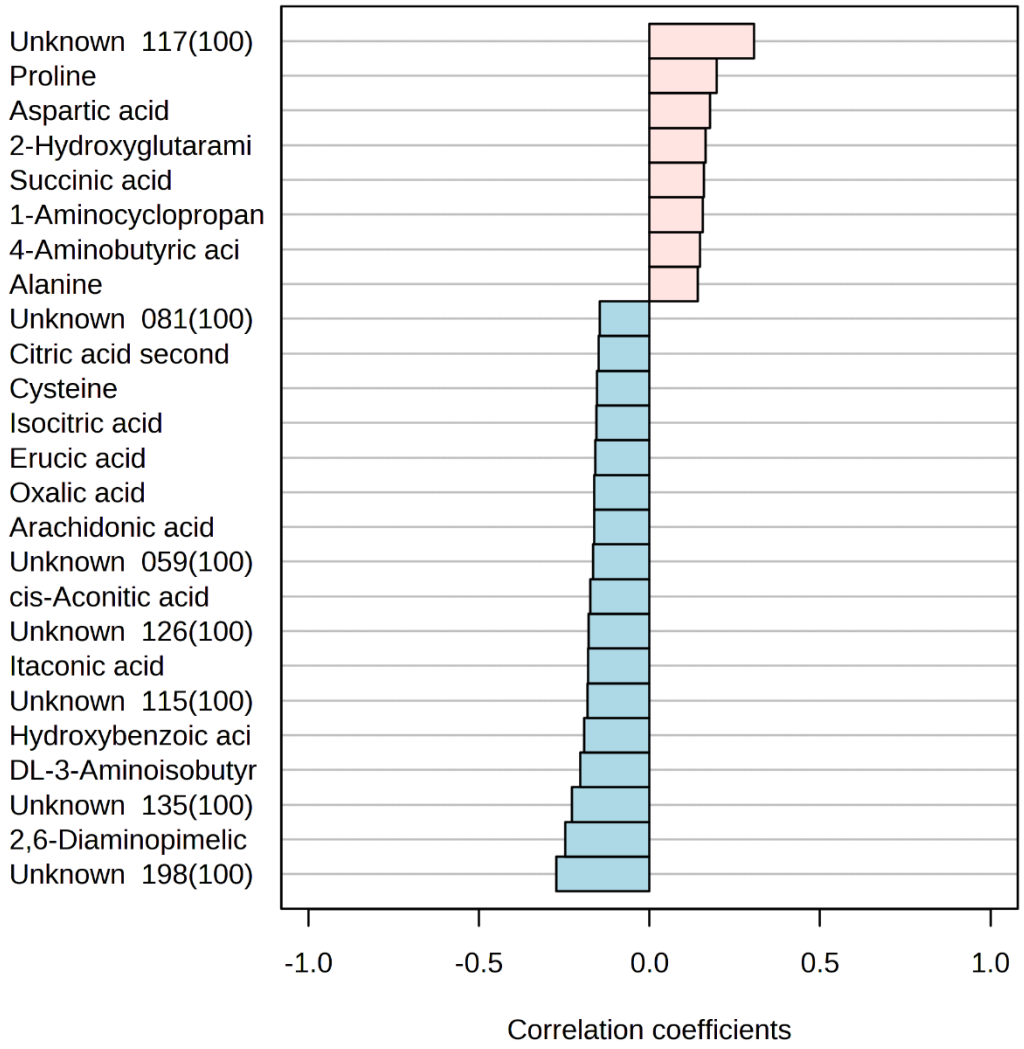

Figure 15. Top 25 compounds identified by MCF that are correlated with a north to south pattern. Pearson's r was used as the correlation coefficient.

Figure 16. Pairwise score plots between the first five MCF principal components. The variance explained by each principal component is presented in the corresponding diagonal cell. The first five principal components accounted for 77% of the explained variance.

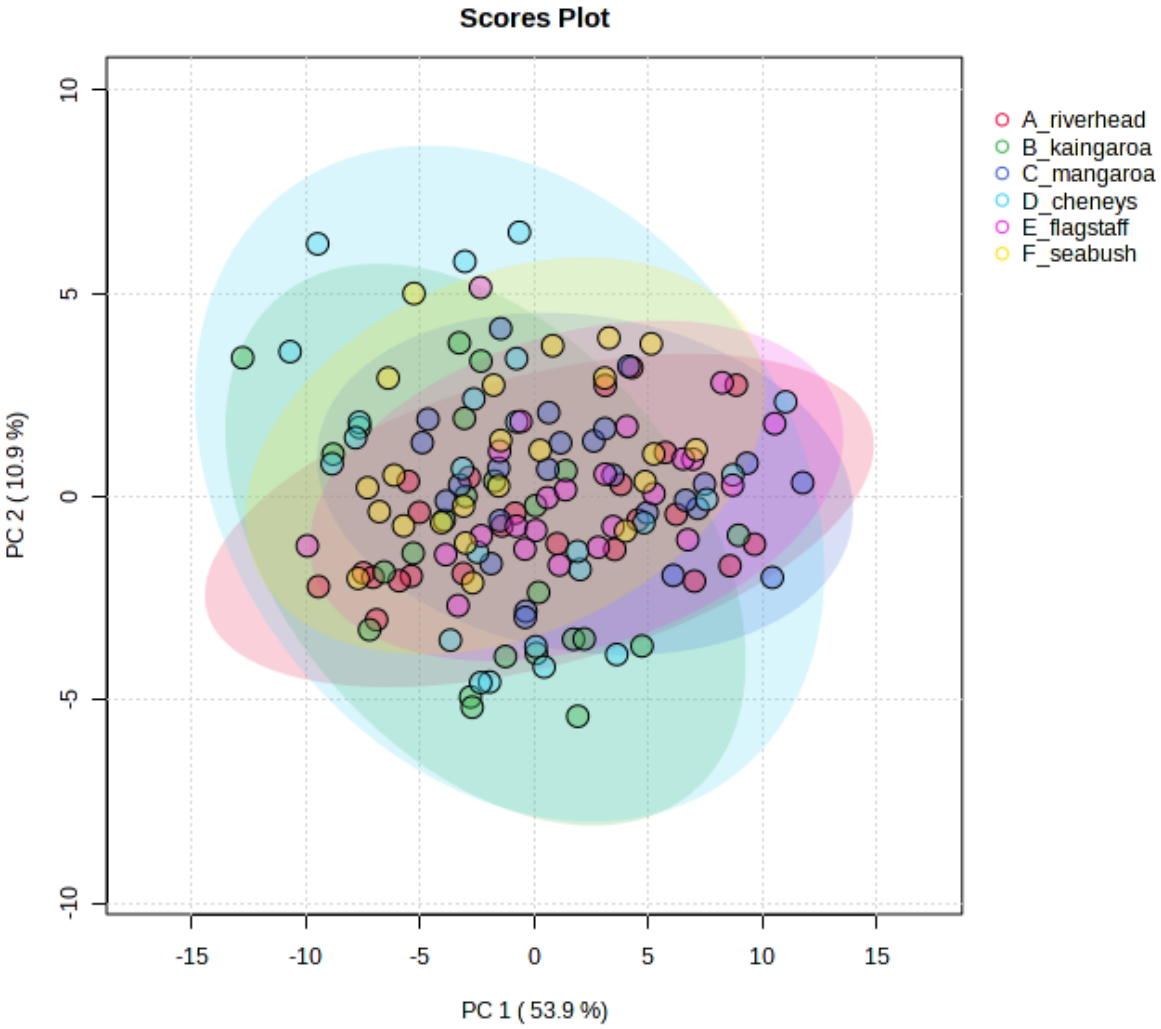

Figure 17. Scores plot between MCF principal component 1 and 2, with explained variances shown in brackets on respective axis.

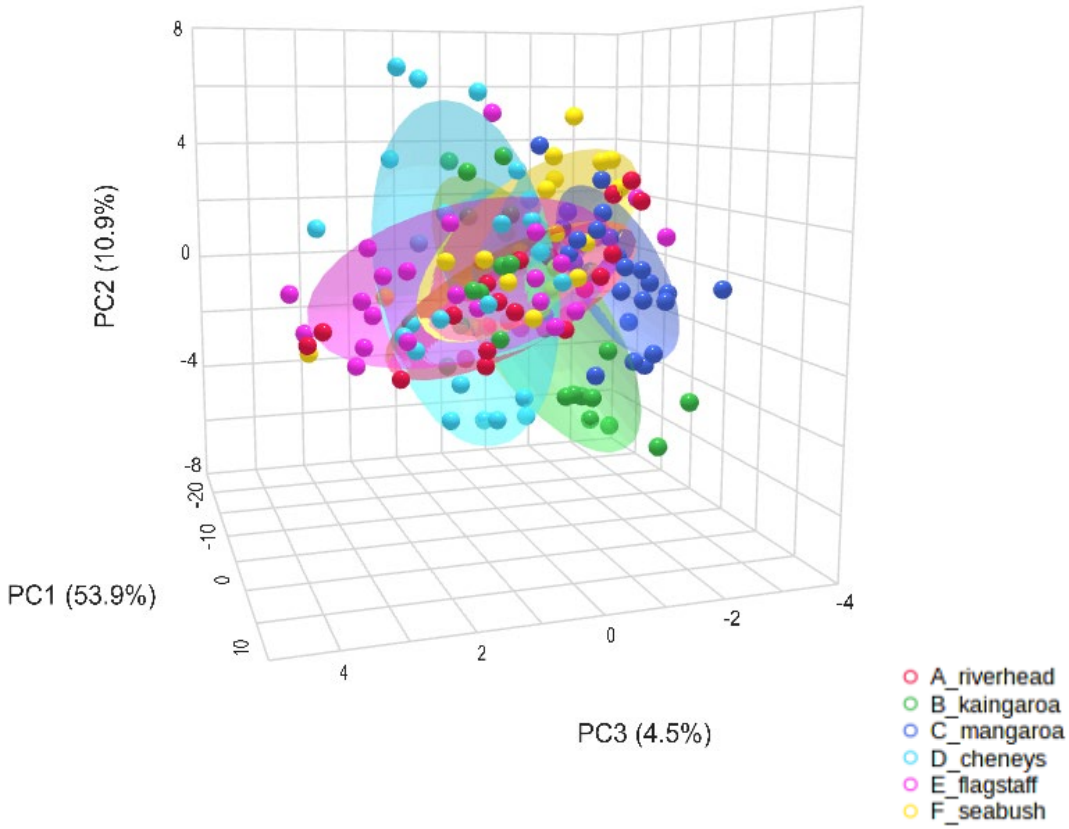

Figure 18. A 3D scores plot between the first three MCF principal components. The explained variances are shown in brackets. 95 % confidence intervals are shown.

Table 8. PERMANOVA results for the normalised MCF metabolites.

	Df	F	R^2	P
Location	5	4.430	0.123	< .001
Log temperature	1	2.489	0.014	0.05
Larval weight	1	8.972	0.050	< .001
Log	11	1.517	0.093	0.03
Log temperature:larval weight	1	1.798	0.010	0.12
Larval weight:log	13	1.313	0.095	0.09
Residuals	111		0.616	
Total	143		1	

Note: Df = Degrees of freedom, F = F statistic

Table 9. Pairwise comparisons between locations for MCF metabolites. All P values were corrected using the Benjamini-Hochberg correction.

	Riverhead	Kaingaroa	Mangaroa	Cheneys	Flagstaff
Kaingaroa	0.039				
Mangaroa	0.021	0.010			
Cheneys	0.065	0.082	0.020		
Flagstaff	0.082	0.010	0.042	0.042	
Seabush	0.082	0.039	0.010	0.129	0.021

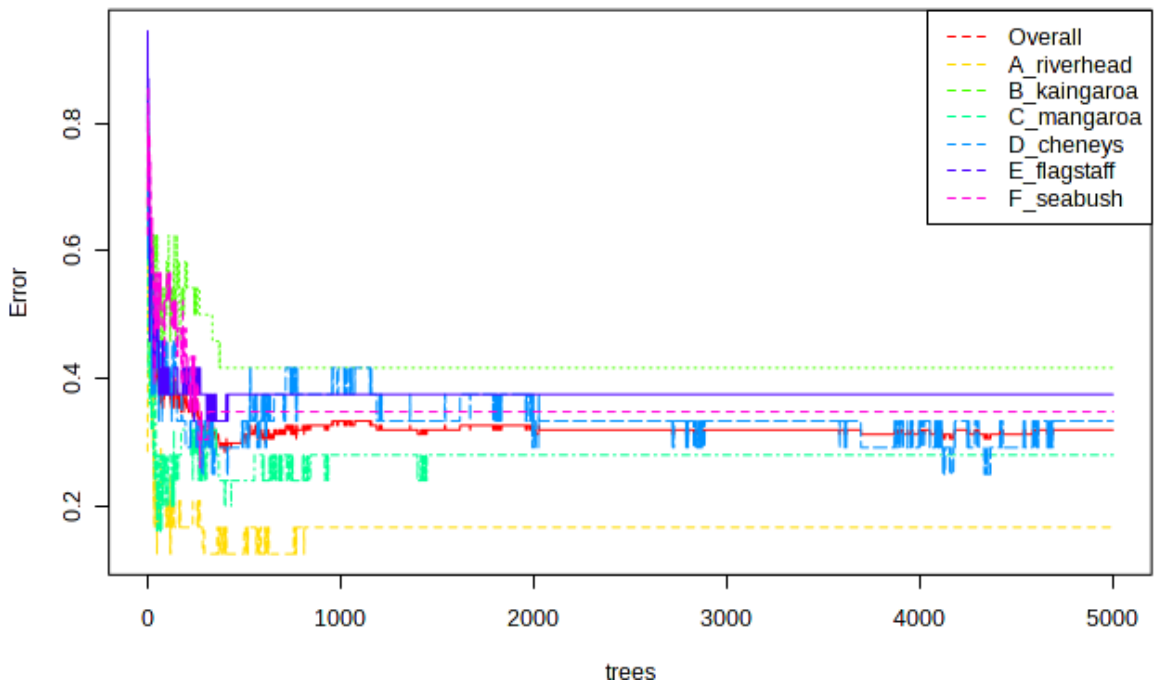

Figure 19. Cumulative error rates of the random forest classification model for metabolites identified by MCF over 5000 permutations.

Table 10. Random forest classification performance

	Riverhead	Kaingaroa	Mangaroa	Cheneys	Flagstaff	Seabush	Class Error
Riverhead	20	0	0	0	3	1	0.17
Kaingaroa	0	14	5	2	0	3	0.42
Mangaroa	1	5	18	0	0	1	0.28
Cheneys	2	0	1	16	5	0	0.33
Flagstaff	1	1	3	1	15	3	0.38
Seabush	2	2	0	0	4	15	0.35

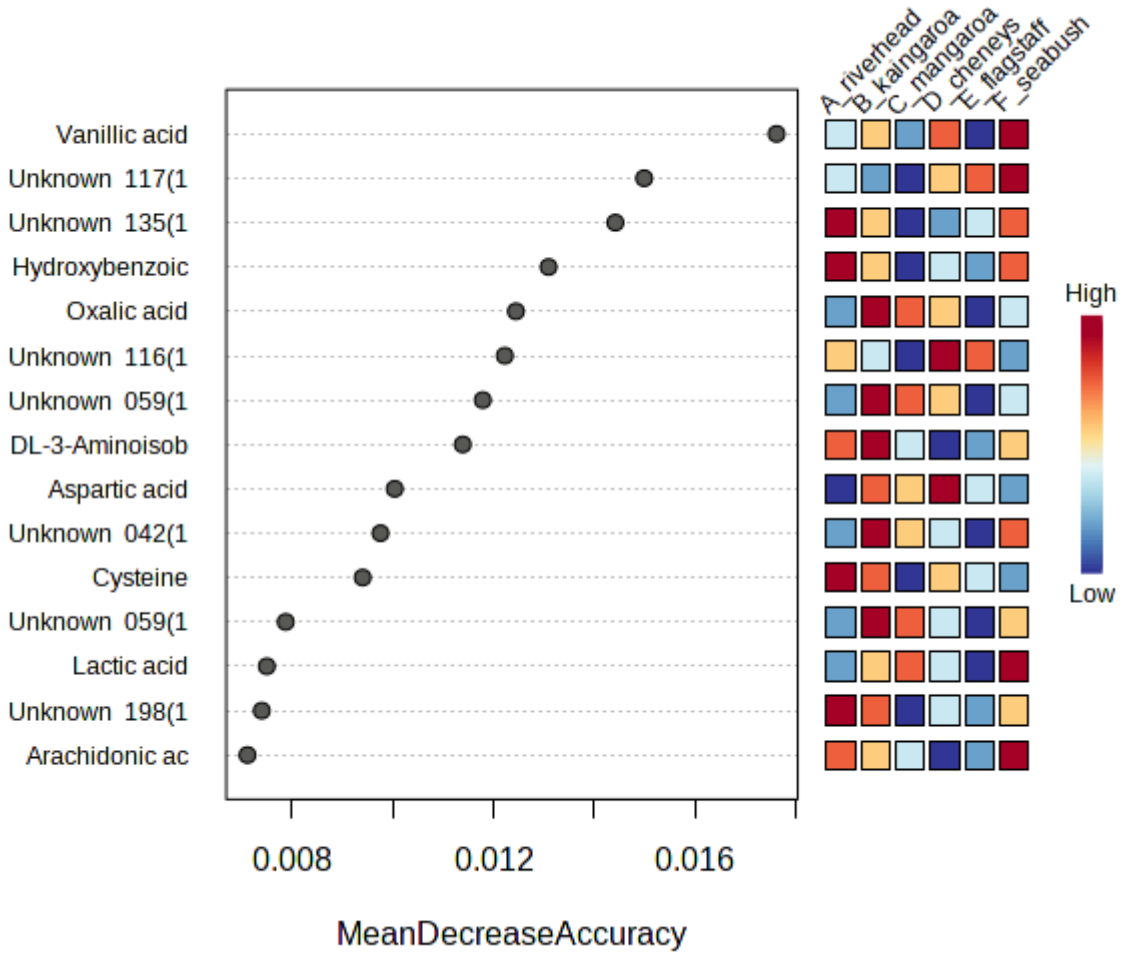

Figure 20. Top 15 significant features (MCF metabolites) identified by the random forest model. Removal of the identified compound results in a mean decrease in the accuracy of the model. The heat map on the right of the model shows the relative abundance of the compound by location. Top 15 account for a 16.7% reduction in model accuracy

Trimethylsilyl dataset

I present the 10 most abundant compounds (using untransformed data) identified by TMS in Figure 21. The remainder of analyses presented use data that has been normalised. I discovered several metabolites that exhibited a weak correlation along a latitudinal gradient. The top 25 metabolites that correlate with a latitudinal gradient are presented in Figure 23. Of these 25 metabolites, only four were significant after correcting using FDR with 2-(methoxyimino)-pentanedioate showing a weak negative correlation ($r = 0.35$, $p < 0.001$), arabinose showing a weak negative correlation ($r = -0.31$, $p = 0.003$), phosphoric acid showing a weak negative correlation ($r = -0.29$, $p = 0.005$), and urea showing a weak negative correlation ($r = -0.24$, $p = 0.04$) (Figure 23).

Using a heatmap, I explored variation in the TMS derivatised metabolite profile of *P. reticularis* between individuals and locations (Figure 22). There was less variation in the metabolite abundance between individuals when compared to the MCF heatmap (Figure 14), and some visually distinct patterns emerged between locations, in particular Mangaroa & Seabush both exhibited lower abundances of metabolites that other locations.

I constructed an unsupervised PCA model for the TMS dataset which showed the general structure of the data (Figure 24). The first two principal components cumulatively explain 62.1 % of the total variation (PC1 explains 46.1 %, PC2 explains 16 %). When visualising in two-dimensions (Figure 24 & Figure 25), I was able to see clearer groupings in the TMS data set in relation to location than compared to MCF. When visualising in three-dimensions (Figure 26), a greater degree of separation between locations was apparent.

The PERMANOVA results (Table 11) confirmed my PCA observations, showing that only location played a significant role in the metabolite composition of *P. reticularis*, explaining 31.1 % of the variation observed in the data set ($p < 0.001$, $R^2 = 0.311$). I performed pairwise

comparisons on the location factor to understand which locations significantly differed from one another (Table 12).

The classification model (using the random forest algorithm) had an overall out-of-bag (OOB) error rate of 0.278, meaning the model correctly identifies the location of a sample 72.2 % of the time. Again, Kaingaroa had the greatest OOB error rate of all sample locations with 0.58 (Table 13, Figure 27). I also used random forest to determine metabolites that have the greatest impact on the accuracy of the model and could be used as biomarkers in the future (Figure 28). These 15 metabolites accounted for a mean decrease in accuracy of 33.4 % when permuted in the model, with the top 5 metabolites accounting for a 15.2 % decrease in accuracy.

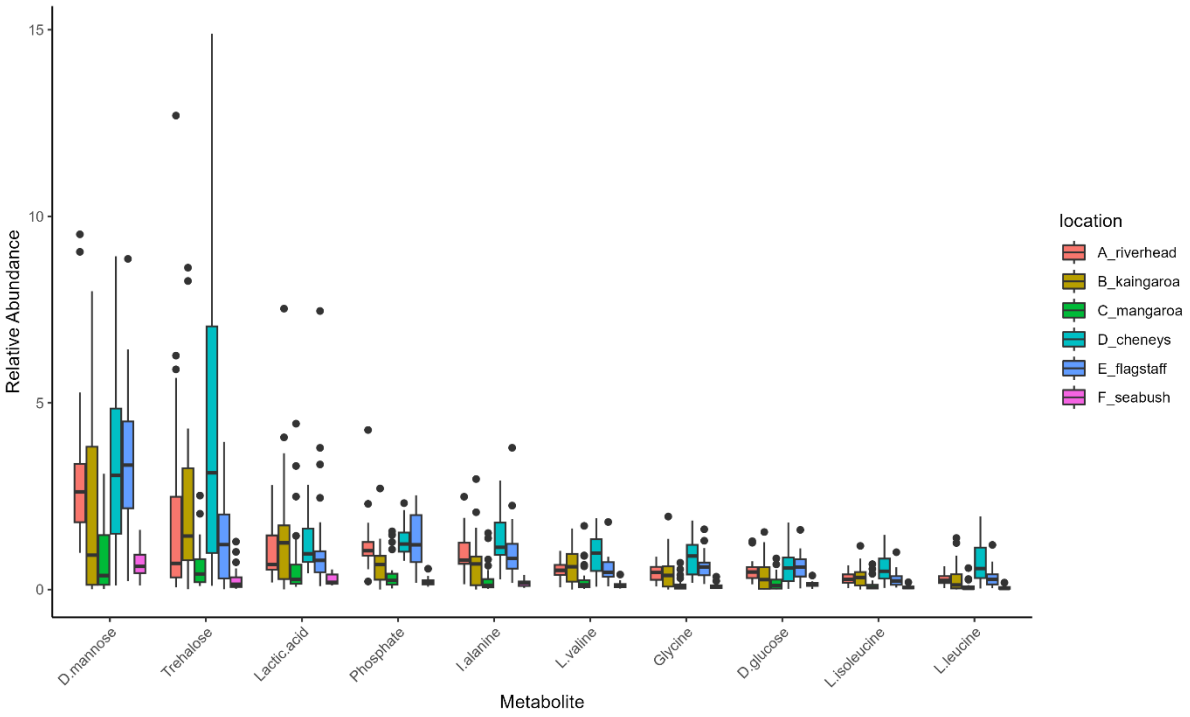

Figure 21. The top ten most abundant compounds derivatised by TMS using untransformed data, grouped by location.

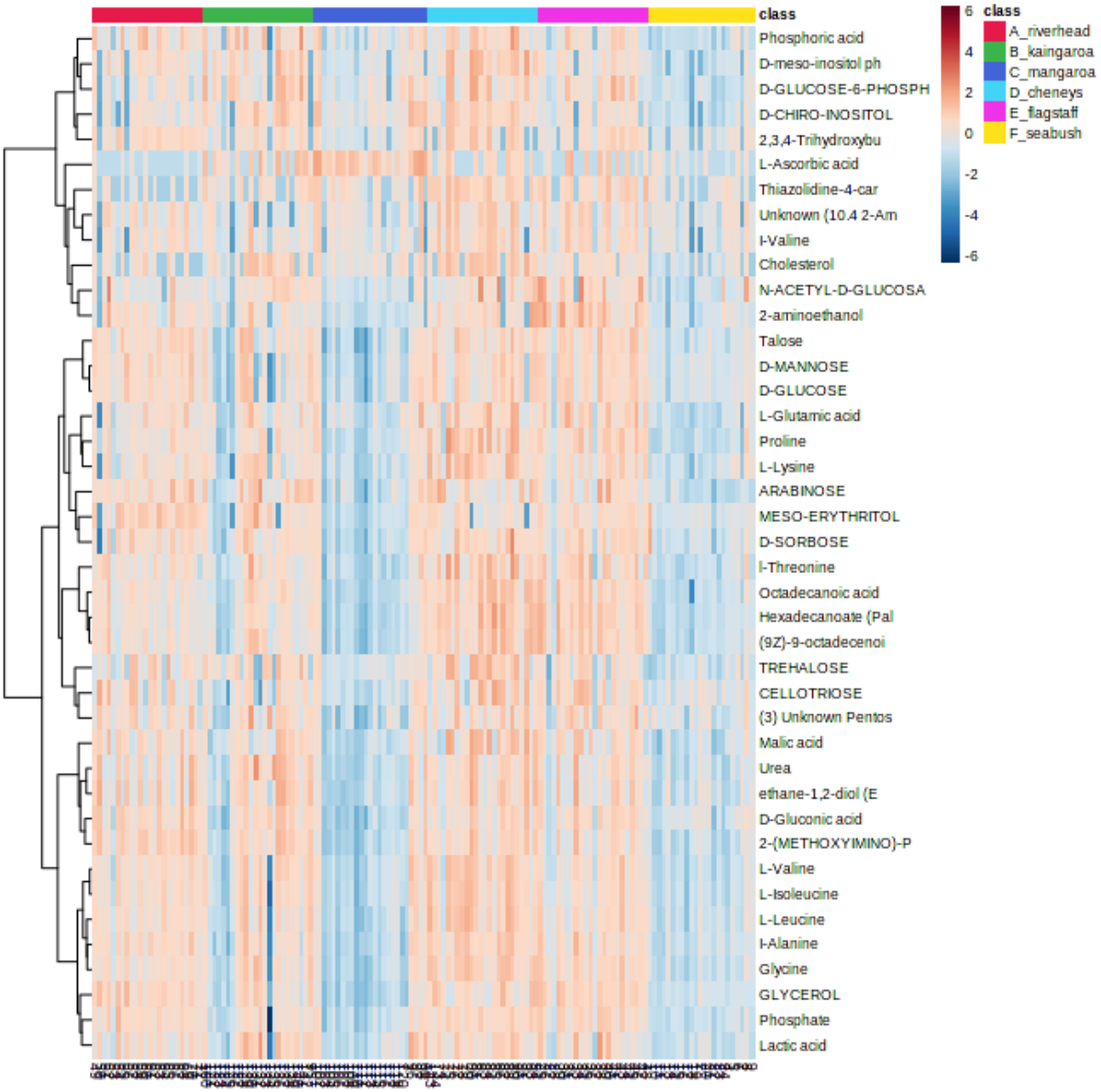

Figure 22. The heat map represents the metabolite profile of larvae collected from six different locations and derivatised using TMS. Clustering of metabolites was performed with a Euclidean distance measure and the Ward algorithm in MetaboAnalyst.

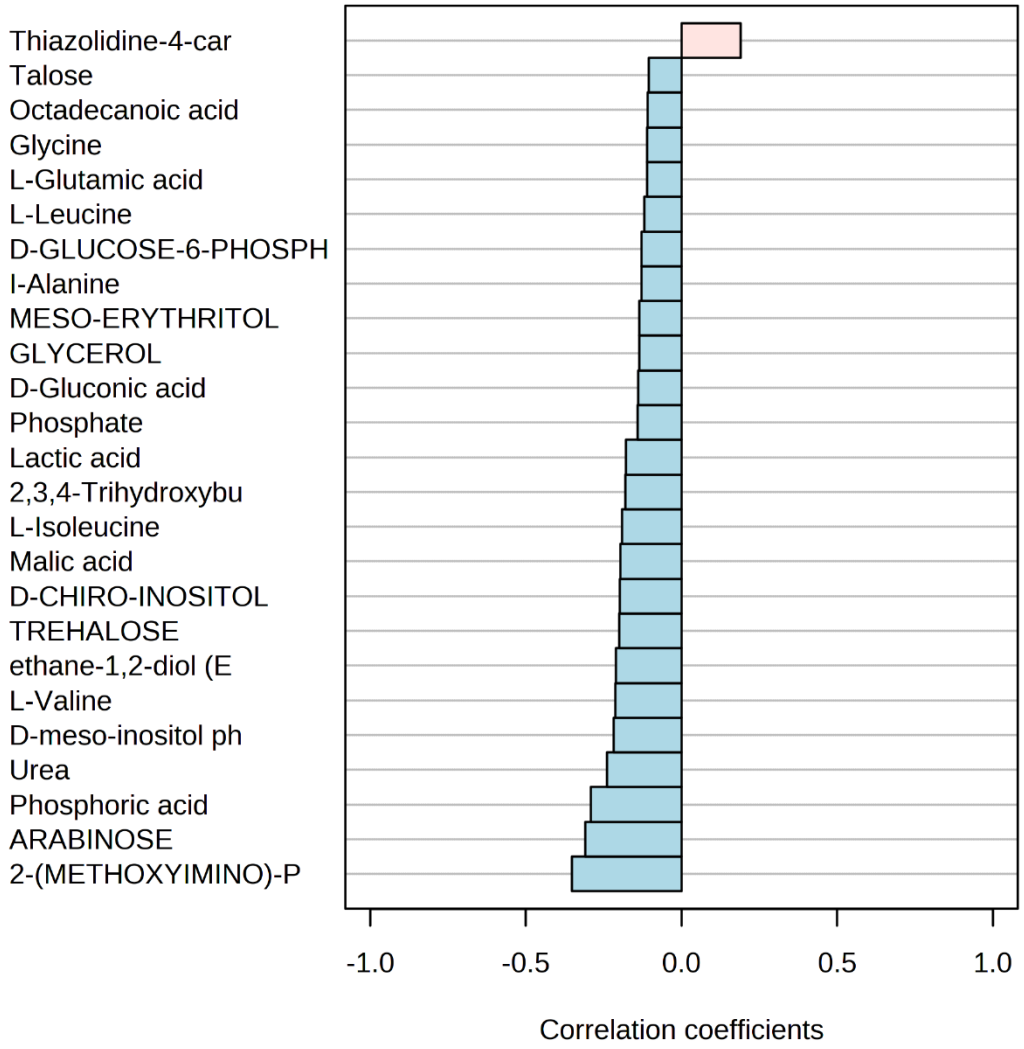

Figure 23. Top 25 compounds identified by TMS that are correlated with a north to south pattern. Pearsons r was used as the correllation coefficient.

Figure 24. Pairwise score plots between the first five TMS principal components. The variance explained by each principal component is presented in the corresponding diagonal cell. The first five principal components accounted for 79% of the explained variance.

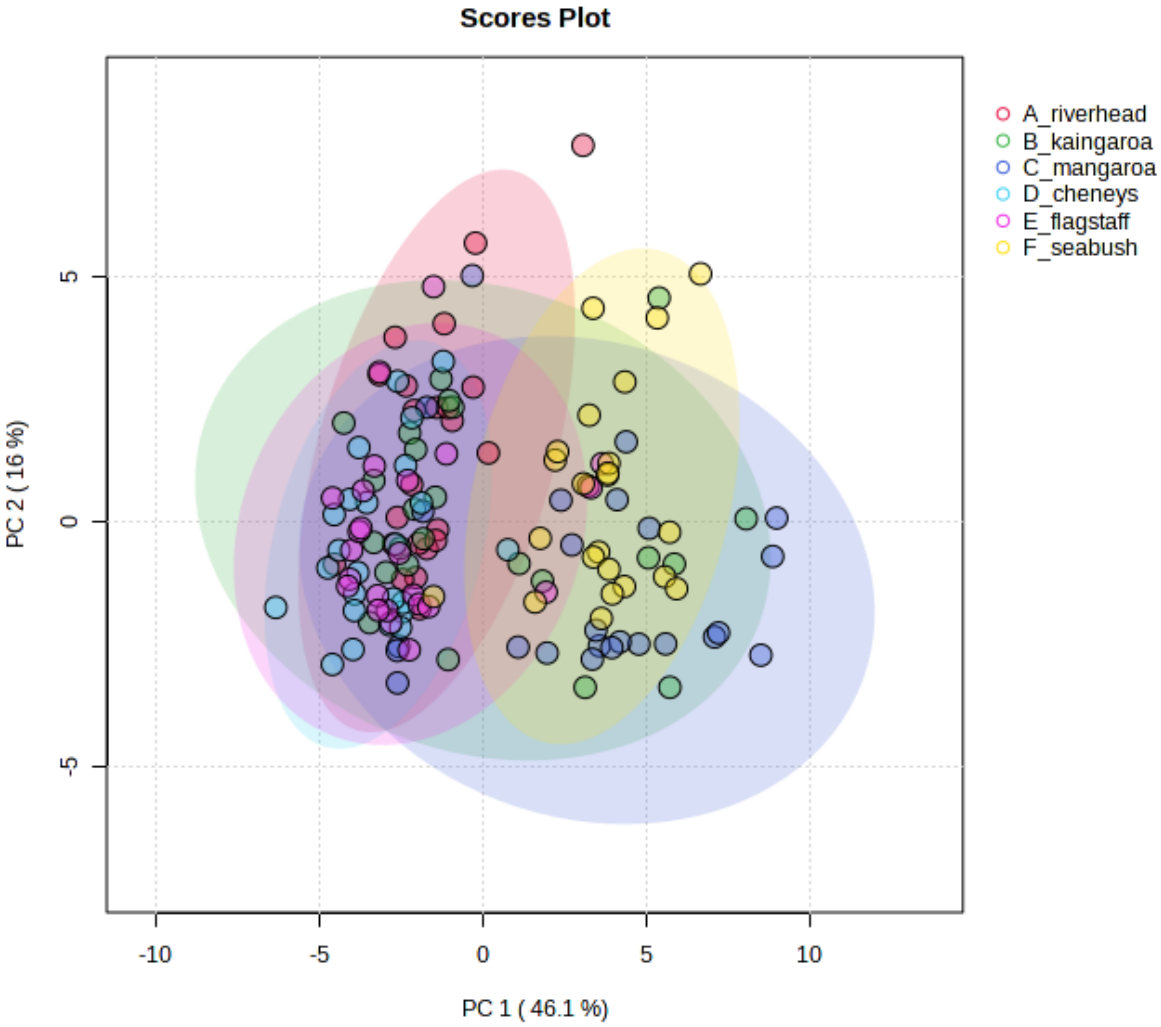

Figure 25. Scores plot between TMS principal component 1 and 2, with explained variances shown in brackets on respective axis.

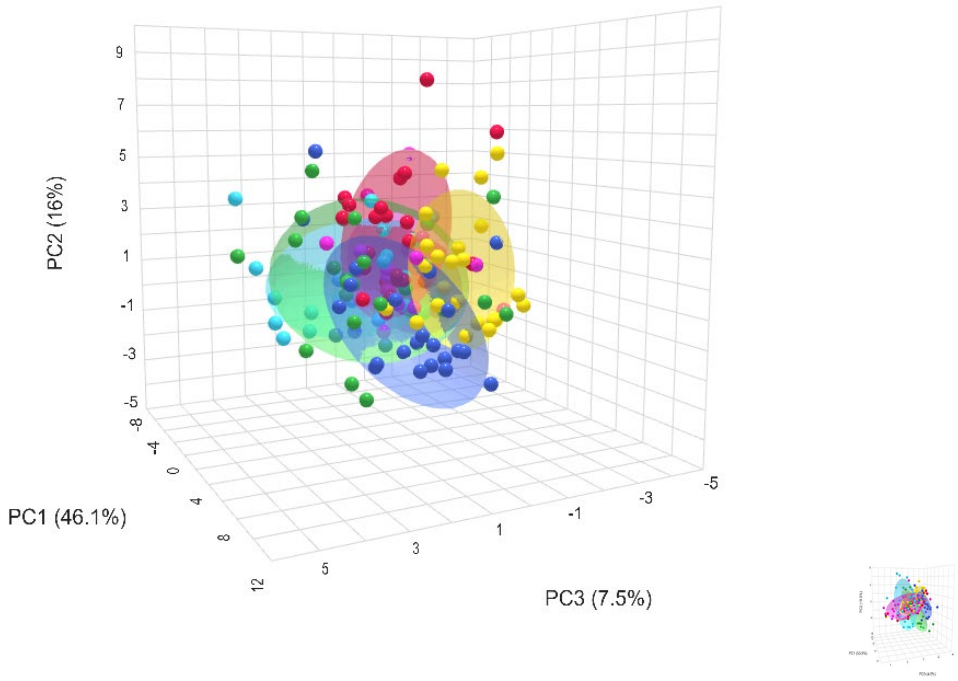

Figure 26. A 3D scores plot between the first three TMS principal components. The explained variances are shown in brackets. 95 % confidence intervals are shown.

Table 11. PERMANOVA results for the normalised TMS metabolites.

	Df	F	R^2	p-value
Location	5	13.29	0.311	< .001
Log temperature	1	1.41	0.007	0.195
Larval weight	1	1.39	0.007	0.203
Log	11	1.27	0.065	0.117
Log temperature:larval weight	1	1.92	0.009	0.093
Larval weight:log	13	1.35	0.082	0.065
Residual	111		0.520	
Total	143		1	

Note: Df = Degrees of freedom, F = F statistic

Table 12. Pairwise comparisons between locations for TMS metabolites. All P values were corrected using the Benjamini Hochberg correction.

	Riverhead	Kaingaroa	Mangaroa	Cheneys	Flagstaff
Kaingaroa	0.002				
Mangaroa	0.000	0.002			
Cheneys	0.000	0.000	0.000		
Flagstaff	0.002	0.002	0.000	0.009	
Seabush	0.000	0.000	0.002	0.000	0.000

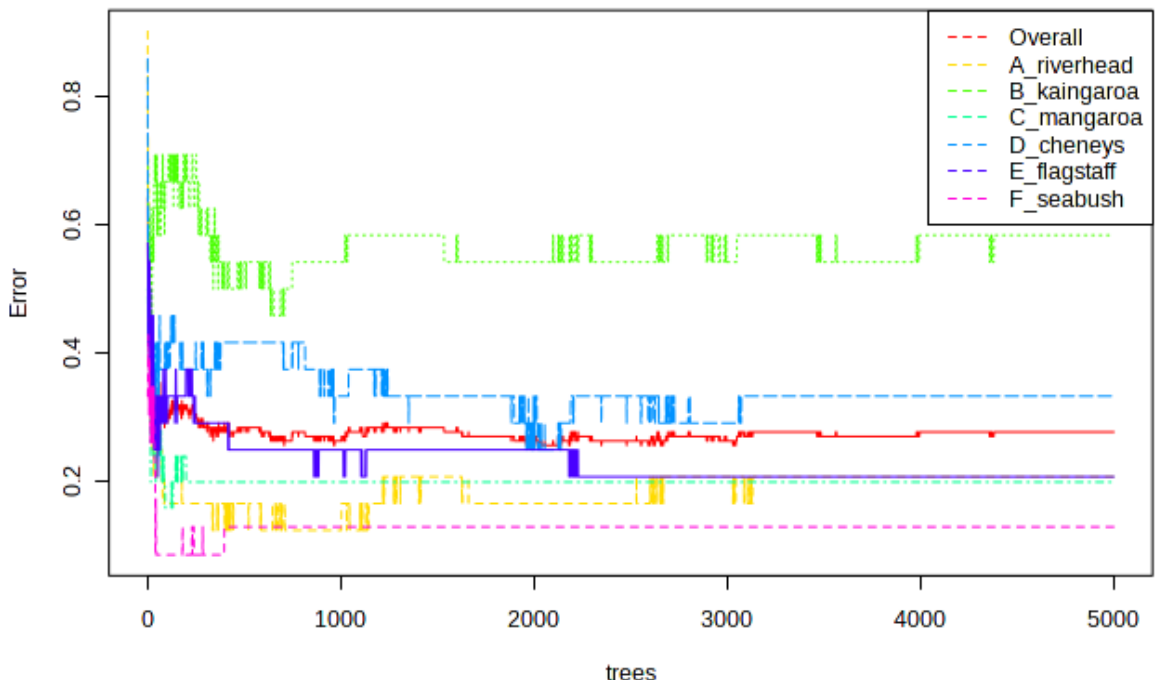

Figure 27. Cumulative error rates of the random forest classification model for metabolites identified with TMS over 5000 permutations.

Table 13. Random Forest Classification Performance

	Riverhead	Kaingaroa	Mangaroa	Cheneys	Flagstaff	Seabush	Class Error
Riverhead	19	0	0	1	3	1	0.21
Kaingaroa	0	10	4	6	0	4	0.58
Mangaroa	0	3	20	0	0	2	0.2
Cheneys	2	2	0	16	4	0	0.33
Flagstaff	2	0	0	1	19	2	0.21
Seabush	0	1	2	0	0	20	0.13

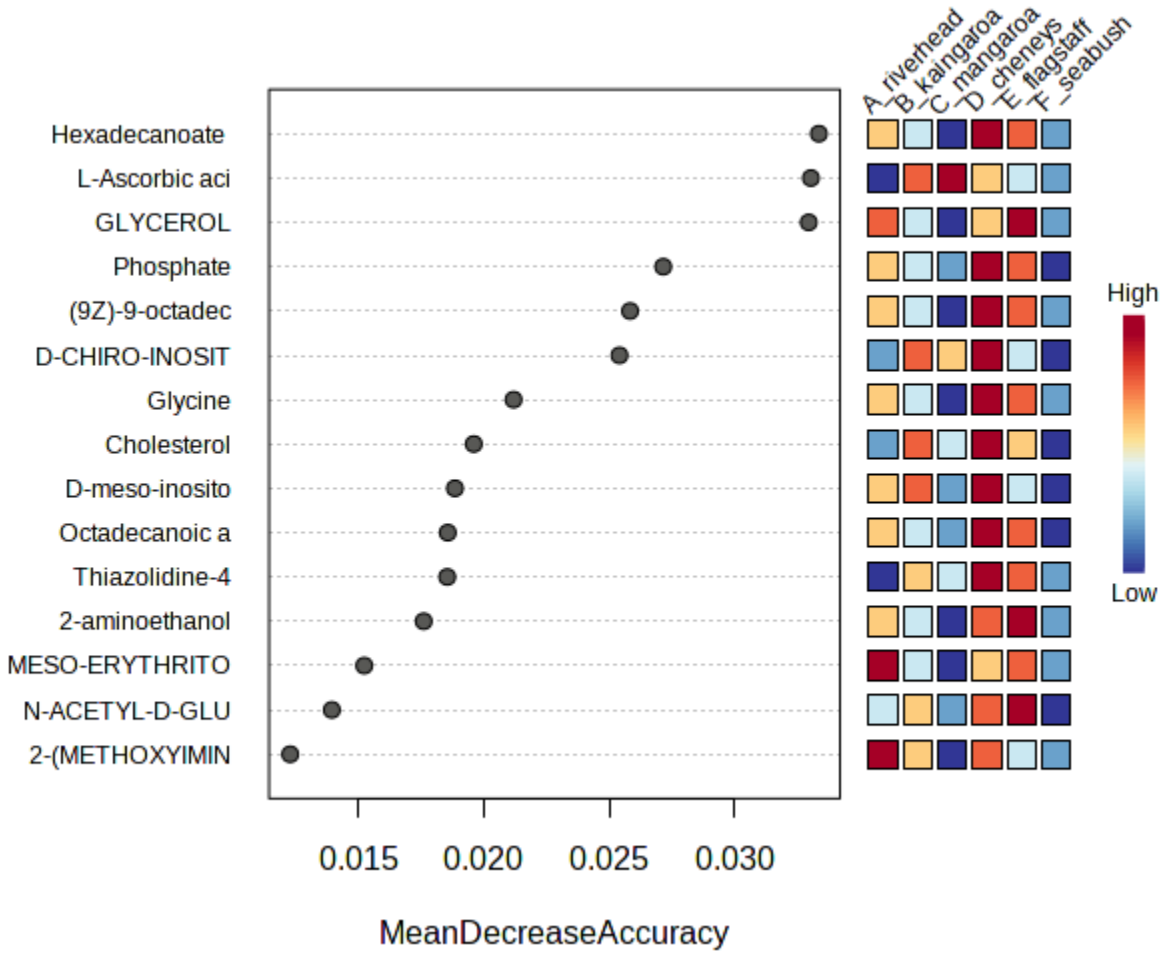

Figure 28. Top 15 significant features (metabolites) identified by the random forest model. Removal of the identified compound results in a mean decrease in the accuracy of the model. The heat map on the right of the model shows the relative concentration of the compound by location. Top 15 account for a 33.3% reduction in model accuracy.

Discussion

Ascertaining the provenance of an edible insect would allow for identification of products that have been sourced from geographical areas where an insect population might be under threat. I assessed the viability of an untargeted, GC-MS based approach to fingerprinting an edible insect, the larvae of *P. reticularis*, using two different derivatisation techniques. I used an untargeted GC-MS approach to create a metabolomic profile of 144 *P. reticularis* samples from six geographic locations along a latitudinal gradient. I identified 43 compounds using TMS derivatisation and

103 compounds using MCF derivatisation for a total of 146 compounds. I found that a RF model trained using the TMS derivatised metabolite profile had the highest accuracy, predicting the correct location of a sample 72.2 % of the time. In comparison, a RF model trained using the MCF derivatised data set had an accuracy of 68.1 %. I found that samples from one location, Kaingaroa, had a much lower accuracy in the model regardless of the derivatisation technique, suggesting that individuals sampled from this population had variable metabolite profiles that overlapped with that of other populations. Despite this, the PERMANOVA analysis found Kaingaroa to be significantly different from all other sites, except for Cheney's in the MCF data set. Whilst the RF model performed reasonably well, there is room for improvement. Future research comparing different classification models would assist with this. For example, models such as soft independent modelling by class analogy (SIMCA), support vector machine (SVM) and linear discriminant analysis based on principal component analysis (PCA-LDA) yielded similar if not better results in the finger printing of hazelnuts (Klockmann et al., 2016)

There was considerable variation observed in the metabolomic profiles between individuals in the MCF data set (predominantly amino acids, fatty acids and organic acids) and there was little visible distinction between locations in the heatmap. I saw less variation between individuals in the TMS derivatised samples (predominantly sugars and sugar alcohols) and, visually, I was able to see some distinction between locations. This distinction was also visible in the PCA and was confirmed with PERMANOVA, with location explaining a greater amount of the variance in the TMS data than in the MCF data (31.1 % vs. 12.3 %). This suggests that TMS may be a better derivatisation technique than MCF for assessing differences between populations of *P. reticularis*.

I found several compounds whose relative abundance correlated with a north-south latitudinal gradient. Of particular interest were arabinose and urea, which are related to gut and metabolic activity. Urea is a nitrogenous waste product of metabolic processes in insects and arabinose is a five-carbon sugar that can be converted by gut bacteria into essential aromatic amino acids

(Scully et al., 2013, 2014). Both compounds had a negative correlation as latitude increased (i.e., became more southerly), and I propose that this relates to the activity levels of the insects along these gradients. The most southerly field sites, Flagstaff (Dunedin) and Seabush (Invercargill), were visited at the beginning of November, when it was still very cool, and the larvae were noticeably sluggish indicating a lack of feeding activity and most likely a lowered metabolic rate. There are few studies investigating metabolomic variation along natural gradients. *Arabidopsis lyrata* ssp. *petraea*, a plant, has been shown to have significant natural variation in metabolism between locations, and exhibit adaptations to cold that are population specific along a latitudinal gradient (M. P. Davey et al., 2008, 2009). A decrease in the concentration of essential amino acids as latitude increased was found in a range-expanding mite, *Tetranychus urticae*, which was suggested to be due to reduced exploitation of resources at the margin of the organisms range, with lower feeding rates resulting in lower concentrations of EAA (Van Petegem et al., 2016).

Mannose and trehalose are two compounds with low molecular weights that are important for freeze tolerance in insects. Both of these compounds were found in high relative abundance in the samples. Larvae were collected over the course of November, a period where larval activity would be increasing following diapause. Cessation of diapause is believed to occur as mean temperatures increase (Edwards, 1961b; Rogers et al., 2002). For Auckland, there is evidence this occurs around August and presumably later in the year at lower latitudes (Edwards, 1961b). Cold hardiness is likely to be important to the survival of diapausing *P. reticularis*, particularly in southern locations where below freezing temperatures can occur and this may explain the high levels of mannose and trehalose. The concentration of low molecular weight sugars and sugar alcohols has been shown to differ in concentration between geographical localities in another cerambycid species, suggesting that this may be linked to environmental temperature (Feng et al., 2014). Curiously, the *P. reticularis* larvae collected from the most southerly field site, Seabush, had

the lowest levels of mannose and trehalose which is the opposite of what would be expected if these compounds are important for freeze tolerance.

Proline, followed by alanine, were the most abundant amino acids found in the larvae of *P. reticularis*. Many insects are capable of oxidising proline for use as a fuel and most beetles use a combination of proline and carbohydrates for flight, whilst some beetle groups exclusively use proline for flight (Auerswald, 2000; Gäde, 1997a, 1997b; Gäde & Auerswald, 2002; Teulier et al., 2016). In laminine cerambycids, both proline and glycogen are used by flight muscles (Gäde & Auerswald, 2000). When proline is used by flight muscles, it undergoes partial oxidation resulting in the production of alanine which is then stored in the fat body and then used to re-synthesise proline (Gäde & Auerswald, 2002). It is likely that the high abundance of these two amino acids is related to their importance in the metabolism of the larvae, and storage of these compounds will be important for the adult life stage which does not feed.

Future research could investigate how the metabolome varies temporally and with diet. The metabolomic fingerprint of a grub is likely to vary over time. It would be logical to next investigate how the metabolome alters at different stages of the season and life cycle, such as selecting larvae before, after and during diapause. This would help to validate the use of fingerprinting in assessing geographic provenance, whilst also providing insights into how the metabolome changes during different aspects of the life history of this species. I controlled for diet by only collecting larvae from pine logs, however, it is commonly held that the taste of *P. reticularis* is "sweeter" when it has come from a matai log (pers. comms.). This suggest that diet may alter the compounds attributable to flavour in the larvae and presents an interesting future direction for research.

In summary, this study used an untargeted GC-MS method to characterise the metabolomic profile of *P. reticularis* along a latitudinal gradient. By utilising two derivatisation techniques, MCF and TMS, I was able to identify metabolites comprised of amino acids, organic acids, fatty acids,

sugars, and sugar alcohols, and from these profiles predict which location a larvae came from with reasonable accuracy, with the TMS data set providing the greatest accuracy. I found compounds that correlated with a latitudinal gradient, offering insights into how environmental cues influence the metabolism of *P. reticularis*. Further investigation is required into the pattern I observed in sugars implicated in freeze tolerance, trehalose and mannose, which did not follow the environmental gradient I expected. Proline and alanine were prominent amino acids identified and this is likely indicative of their importance in the metabolism of both larval and adult life stages. This preliminary study presents opportunities for future research such as investigating a temporal gradient and the role of diet on the metabolomic fingerprint of this species.

Acknowledgements

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Chapter 5 The static allometry of traits associated with fitness in *Prionoplus reticularis* (Coleoptera: Cerambycidae)

Abstract

Scramble competition mating systems are characterised by intense competition among males to locate and mate with females. Despite the prevalence of scramble competitions in nature, little is known about how selection influences male traits important for scramble competition, and their patterns of sexual dimorphism and allometry. The mating system of the cerambycid *Prionoplus reticularis* exhibits the hallmarks of a scramble competition so I set out to determine the degree of sexual dimorphism and patterns of allometry in traits predicted to be important in scramble competition: body size, wing size and antennal size. I also assessed the influence of body size and wing traits on locomotory performance in male huhu using a flight mill. I observed pronounced sexual dimorphism between male and female individuals across all measured traits, with the exception of wing aspect ratio. Female traits were consistently larger on average than males, with the exception of the antennae. Male antennae were significantly larger on average than female antennae which suggests sexual selection has generated longer antennae but smaller body size in males. I did not observe positive allometry for any male or female traits. Females showed negative allometry in antennal length, wing aspect ratio, and wing loading whilst all other traits exhibited isometry. In contrast, all male traits demonstrated negative allometry, except for wing loading which was isometric. This suggests that whilst sexual selection is likely driving sexual dimorphism, larger males do not benefit from disproportionate investment in wings or antennae. Instead, stabilising selection likely generates the negative allometries of traits I observed, possibly due to biomechanical constraints. In the three areas of flight performance I explored, I discovered that wing loading was significant and positive, suggesting that as wing loading

increases, so too does flight distance, duration and speed. This result suggests that relatively heavier males may have the energetic reserves to fly faster and for longer, contrary to studies of other taxa. Overall, I highlight the study of patterns of trait investment in scramble competitors as a potentially fruitful avenue of further research.

Introduction

Scramble competition mating systems are characterized by intense competition among males to locate and mate with females, then continue to move through the environment investing in mate acquisition rather than defence (S. T. Emlen & Oring, 1977; Herberstein et al., 2017; Thornhill & Alcock, 1983). Such mating systems often involve little courtship, but high levels of multiple mating by both males and females, and therefore a high degree of sperm competition. Mating systems dominated by scramble competition are likely to be very common in the animal kingdom (Herberstein et al., 2017). Despite this, the way that selection influences male traits involved in scramble competition is less well-understood, than for more dramatic mating systems such as leks, and those that involve male defence of females or their critical resources (Emberts et al., 2023; M. Fea & Holwell, 2018; Fiske et al., 1998; Palaoro et al., 2022; Walker & Holwell, 2018). Among these more well-studied mating systems, significant research attention has focused on sexual dimorphism, allometry and the selective benefit of male signals, conspicuous ornaments, and exaggerated weaponry (Bonduriansky, 2007; Bonduriansky & Day, 2003; Eberhard et al., 2018; McCullough & O'Brien, 2022). In contrast, less is known about how selection influences male traits important for scramble competition, and their patterns of sexual dimorphism and allometry.

Due to the importance of locating mates, sometimes over long distances, strong selection on sensory traits in males is predicted under scramble competition (Thornhill & Alcock, 1983). Darwin predicted that sexual selection should influence the evolution of the 'organs of sense' in much the same way as for ornaments and weapons (Darwin, 1871; Elgar et al., 2019). He

proposed that males with sense organs better able to detect females should find and mate with more females and these traits would be selected for. This is particularly the case among scrambling species and higher investment in the size and sensitivity of male sense organs such as eyes, ears and antennae has been demonstrated for many species (reviewed in Herberstein et al., 2017). For example, most male moths show longer and more elaborate (pectinate) antennae than females (Symonds et al., 2012), and the number and density of olfactory antennal sensilla have been shown to be higher for males in a wide range of insect species including mantises, beetles, stick insects, flies and moths (Holwell et al., 2007; Jourdan et al., 1995; Mark et al., 2018; S. S. Myers et al., 2015; F. Zhang et al., 2021). Eyes can also be sexually dimorphic, with male Bibionid flies having larger eyes than in females, all the better to detect the females against complex backgrounds (Nordström & O'Carroll, 2009; Zeil, 1983). Evidence for a functional relationship between sensory trait size and mating success or the ability to locate mates is however, rare. Where it has been established, research has shown that male *Pseudomantis albofimbriata* (Mantodea) with more trichoid sensilla (a chemosensory sensilla) were able to find females quicker (Jayaweera & Barry, 2017) and *Uraba lugens* (Lepidoptera) males arriving at a trap containing a single female had on average larger antennae compared to a trap containing two females indicating that larger antennae provided an advantage in finding weaker sensory cues (Johnson et al., 2017).

Just as males who can better detect females will be selected for under scramble competition, those who can move more quickly or cover wider distances in search of females, are predicted to have higher mating success (Able, 1999; Andersson, 1994; Husak & Fox, 2008). Locomotion and investment in locomotory traits are known to differ between males and females, often to extremes such as in cases where females are sedentary (e.g. many spiders, Grossi & Canals, 2015) and lack means of dispersal (e.g. flightless female mantises (Barry et al., 2011), moths (Niitsu et al., 2017) and stick insects (Zeng et al., 2020) when compared to more mobile and winged males.

Giant wētā are one of the best studied groups with males with longer legs relative to their body size being more mobile resulting in higher mating success (Kelly et al., 2008). In dragonflies, sexual selection has been shown to act on flight endurance rather than speed with larger wings and increased fat reserves leading to greater endurance and higher mating success (Gyulavári et al., 2014).

In flying species, the morphology of wings are correlated with performance allowing wing metrics, such as aspect ratio and wing loading, to be used as predictors of locomotive ability (Berwaerts et al., 2002; Gibb et al., 2006). Aspect ratio is a metric that represents the relationship between length and width (or area) of a wing, with a high ratio characterized by a longer, thinner wing suited to fast, agile flight (e.g. dragonflies), and a low aspect ratio indicating a shorter, broader wing suited to slow gliding flight (e.g. butterflies) (Fu et al., 2014; Le Roy et al., 2019; Wootton, 2020). Wing loading describes the relationship of body mass to wing area with the energy efficiency of flight increasing as wing loading decreases (Gibb et al., 2006). In noctuid moths, individuals reared in a nutrient poor environment display developmental plasticity with lower than predicted body weights and lower wing loading ratios suggesting an adaptive response tailored to lowering the energetic costs associated with flight (Angelo & Slansky, 1984).

Body size is more complex and it seems that how scramble competition influences male body size depends on its relationship with locomotion (Andersson, 1994; Ghiselin, 1974). In a scramble competition mating system, an individual's reproductive success may be impacted by traits such as energy efficiency, endurance, mobility, manoeuvrability, agility, and aerial ability. If body size interacts with these traits, then selection pressure will lead to sexual size dimorphism (SSD) where one sex will be smaller or larger than the other. For small male SSD, several hypotheses have been put forward to explain this phenomenon. The Ghiselin – Reiss small-male hypothesis proposes that smaller males have an advantage in mate searching as they require less energy per unit of time of mate searching and therefore require less time having to replenish

energy through foraging and can spend more time searching for mates (Ghiselin, 1974; Herberstein et al., 2017; Reiss, 1989). This has been documented in the water strider, Aquarius remigis, where smaller males had greater mating success in a food limited environment (Blanckenhorn et al., 1995). In species where smaller or lighter males might benefit from increased locomotor speed or endurance (e.g. flying species, spiders that climb to females), it is predicted that selection will reduce male body size relative to that of females, and relative to locomotor traits such as wing area and leg length (Crompton et al., 2003; Gyulavári et al., 2014; Moya-Laraño et al., 2002; Vollrath & Parker, 1992).

In species where larger bodied males can move faster or have more energy reserves to cover more distance or roam for longer periods, then larger bodies are likely to be selected for. For example, in some spider species, larger males are associated with greater mating success (Brandt & Andrade, 2007; Foellmer & Fairbairn, 2005) which is suggested to be due to a locomotor advantage on horizontal substrates (Brandt & Andrade, 2007), whilst in the cerambycid *Phoracantha semipunctata*, larger males are able to walk faster and have a greater antennae span, leading to higher mating success through covering a greater area during mate searching (Hanks et al., 1996).

Allometry is the study of the scaling relationship between a trait of interest and body size. It has been broadly classified into ontogenetic, evolutionary and static allometry. Ontogenetic allometry encompasses changes in trait and body size over different developmental stages, evolutionary allometry refers to the relationship between body and trait size among species, and static allometry (henceforth referred to as allometry), refers to how a trait correlates with size among individuals of the same developmental stage and species (Klingenberg & Zimmermann, 1992). Allometry is represented as the mathematical expression $y = bx^a$ which relates a trait of interest, y, to a measure of body size of an organism, x, with the y-axis intercept represented as b and the slope, of particular interest to evolutionary biologists, estimated as a (Huxley, 1924).

The slope of the line can be classified as being positively allometric ($a > 1$), negatively allometric ($a < 1$), or isometric ($a = 1$) (Huxley & Teissier, 1936). This is of interest when considering how the size of a trait of interest, for example antennae, scales between with body size amongst individuals of a species. If, after measuring a sample of individuals, I find that the relationship between antennal length and body size is greater than one (i.e. $a > 1$), this is described as positive allometry and I can infer that larger individuals are investing disproportionately in that trait in comparison to smaller individuals.

Many traits under sexual selection have been shown to exhibit positive allometry. Both ornaments and weapons frequently scale positively with body size in males, but not females (Dale et al., 2007; D. J. Emlen, 2008; Rico-Guevara & Hurme, 2019; but see Bonduriansky, 2007). In contrast, genitalia and sexually selected contact structures involved in courtship show negative allometry, due to the functional constraints of successful male-female interaction (Eberhard, 2009; Eberhard et al., 1998). It is unclear how sensory and locomotory traits under sexual selection will scale with body size. The pattern of allometry for any traits represents the variation in the marginal fitness gains that individuals of different sizes receive by investing more or less in those traits (Bonduriansky & Day, 2003). Therefore, for sensory or locomotory traits, if larger individuals stand to gain fitness benefits from investing in the development of disproportionately larger antennae or wings, then I would predict positive allometry to evolve. However, there are likely to be strong functional and biomechanical constraints that curtail exaggeration. For example, larger wing length or area is likely to only provide additional locomotor performance up to a point, where mobility would then be reduced with further exaggeration (Frankino et al., 2005; Rayner, 1988). Such constraints might drive the evolution of negative allometry, or lead to no deviation from isometry.

The huhu beetle, *Prionoplus reticularis* (Cerambycidae: Prioninae), is an iconic New Zealand long horned beetle whose larvae feed on the dead and decaying wood of certain native and exotic tree

species (Edwards, 1959). The sex ratio of adult beetles is roughly 1:1, however light trapping is biased towards male capture (Edwards, 1956, 1961c). The adult beetle is large, short lived and non-feeding, living for around two weeks upon the energy reserves accrued from its two to three year xylophagous larval stage (Edwards, 1961b, 1961c). Upon emergence, the female produces a male attracting pheromone setting up the conditions for a scramble competition mating system where males try to find spatially dispersed females to mate with within a finite time period determined by their energy reserves. There is also a report of *P. reticularis* males engaging in combat displays with other males, where the display escalates through several stages to grappling with the fore legs until one individual is thrown onto its back (Edwards, 1961c).

I set out to (1) determine the degree of sexual dimorphism in measures of body size, wing size and antennal size for *P. reticularis.*, (2) determine the patterns of allometry for sensory (antennae length, ninth flagellomere length), locomotory (wing length, wing area, wing aspect ratio and wing loading) and combat (tibia and femur) traits for *P. reticularis* and, (3) assess the influence of body size and wing traits on locomotory performance in huhu using a flight mill. Specifically, for (2) I predict that positive allometries indicative of sexual selection will exist in male traits optimized for detecting females quickly (chemosensory structures i.e., antennae), traits that improve male mobility (locomotory traits i.e., wing size including wing aspect ratio and loading) and positive selection on traits that improve combat ability in males (grappling ability, i.e., femur and tibia length of fore legs).

Methods

Field site and Collection

I collected adult huhu beetles (n = 112) from Riverhead Forest, Auckland, New Zealand using a mercury vapour light at night between November 2021 and February 2022. Individuals were stored in ventilated plastic specimen jars with a piece of cardboard for beetles to gain purchase and rest. Upon return, each individual had half a staple super glued to the pronotum which

allowed for easy attachment to flight mill via a magnet. To identify each individual, a number was written on the left elytron using acrylic paint (Molotow Extra Fine 1mm Pen). Individuals were acclimatised in the dark at room temperature for 1 hour before being attached to flight mills. The weight of each individual was taken before attachment to a flight mill using a milligram scale (8028 Series, Guangzhou Juanjuan Electronic Technology Co., Ltd.). After flight mill tests (see below), individuals were then placed into a freezer and traits of interest were dissected, attached to graph paper and digitised using a flatbed scanner (HP Envy 5000 series).

Measurements of Morphological Traits

Traits of interest were measured using ImageJ (NIH, 1.50i). The locations of linear measurements taken is visualised in Figure 29. I measured total body length (from mandibles to tip of left elytron, mm), pronotum width (between two pairs of pronotal spines, mm), femur length (along ventral edge, between proximal bend and distal femoral spur), tibia length (along ventral edge, between proximal bend and distal tibial spur), antennal segment length (pedicel, scape, flagellomere 1 to 9), total antenna length (sum of antennal segments), elytron length (from base of integument to distal end of medial suture) and hind wing length (henceforth called wing length, humeral plate to distal end of radial anterior 4), wing area (calculated by tracing the wing edge in ImageJ and measuring area). For wing aspect ratio ($2(wing\ length)^2/\ wing\ area$) and wing loading ($wet\ body\ mass/2(wing\ area)$), I doubled the wing area and wing length to account for only measuring a single wing. All measurements were taken from the right-hand side of the insect where possible (if damaged, the left-hand side was used).

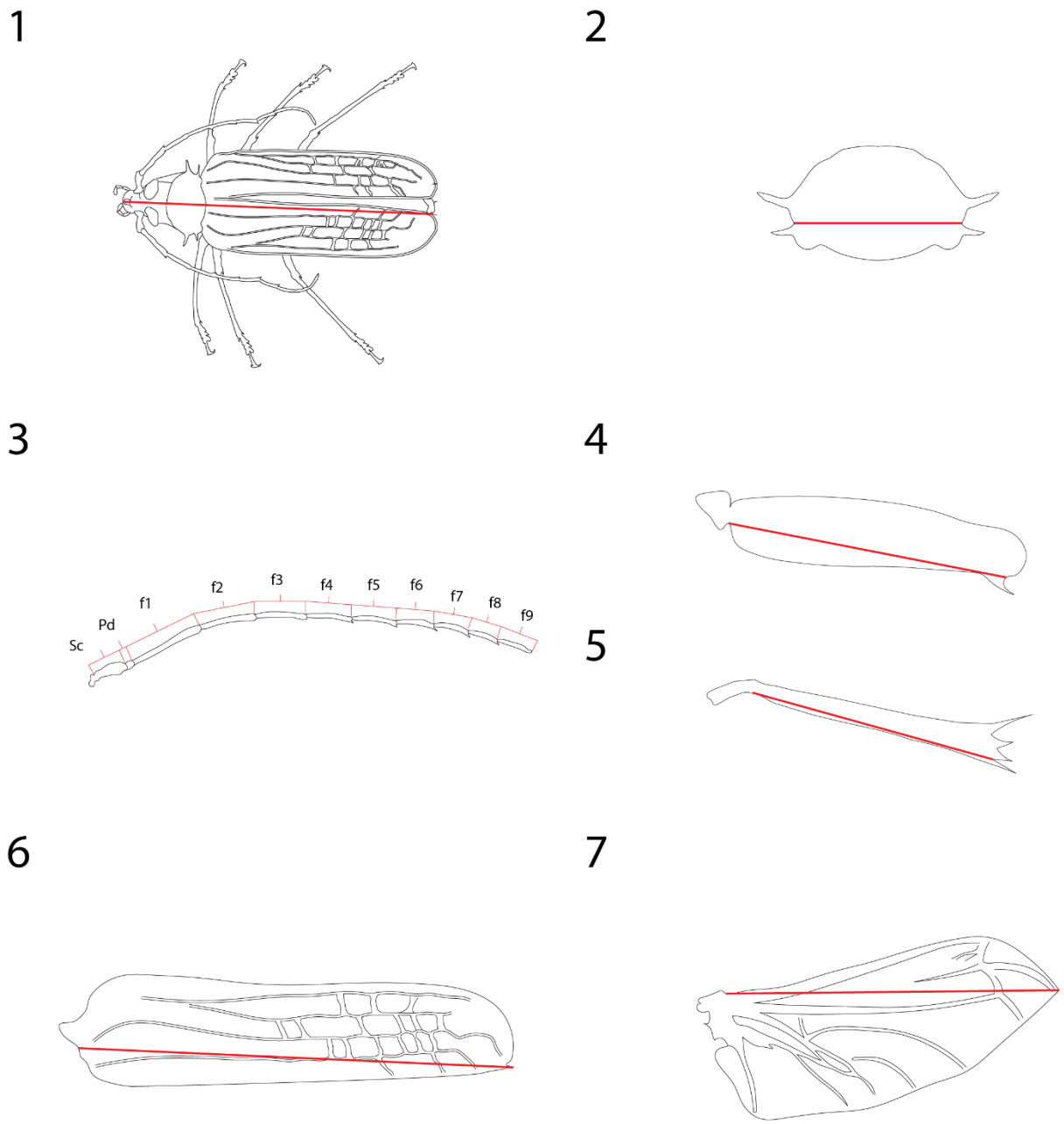

Figure 29. Morphometric measurements taken. (1) Body length; (2) pronotum width; (3) Antennal measurements: Sc = scape, Pd = pedicel, f1:9 = flagellomere 1 to 9. Total antennal length was the sum of these measurements; (4) femur length; (5) tibia length; (6) elytron length; (7) wing length. Wing area was calculated by tracing the outer margins of the wing. Body length was measured using callipers. All other measurements taken in imageJ.

Flight Mill Design

Six flight mills were constructed and attached to a microprocessor (Arduino Uno Revision 3) to record flight information (Figure 30). A 3D printer was used to produce the shaft and arms of each flight mill from polylactic acid (PLA). The axis upon which the flight mill arm pivots was comprised of a polyethylene pipette tip inserted into a 0.5 ml polyethylene Eppendorf tube, this material has very low friction, allowing for the arm to rotate freely (Figure 30 B). The flight mill arm was placed over the Eppendorf tube. To extend the length of the arms, stiff paper clip wire was attached to either end. To allow for ease of attachment specimens, a neodymium magnet was attached to the end of the extended flight mill arm which would then be attracted to the half-staple attached to the pronotum of the beetles (Figure 30 D). At the end of the opposite flight mill arm, a plasticine counterweight of the specimen was attached (Figure 30 A).

Each flight mill had a hall effect sensor (Honeywell 2SS52M Omnipolar Hall Effect Sensor) coupled with a 10K ohms pull-up resistor and 5V power supply attached to the shaft (Figure 30 E). On the flight mill arm, close to the axis, a neodymium magnet was secured with epoxy resin, close to the centre of the axis (Figure 30 C). Each flight mill was connected to the same microprocessor using single core, flexible flat cable. A high precision real time clock (RTC) module was connected to the microprocessor along with a micro-SD card reader module. The microprocessor was programmed using Arduino programming language, a C++ variant. The schematics for the electronic components are presented in Supplementary Figure 33.

Upon each rotation of the flight mill arm, the magnetic field of the neodymium magnet causes the hall effect sensor to complete an electrical circuit, sending a signal to the microprocessor to record the flight mill identification number (1 - 6) and time (both UNIX and POSIX format) to a text file on the micro-SD card. For example, if a rotation was completed on flight mill three at 0013h on the 26th of January 2022, it would be recorded as follows:

3, 1643156007, 2022-01-26T00:13:27

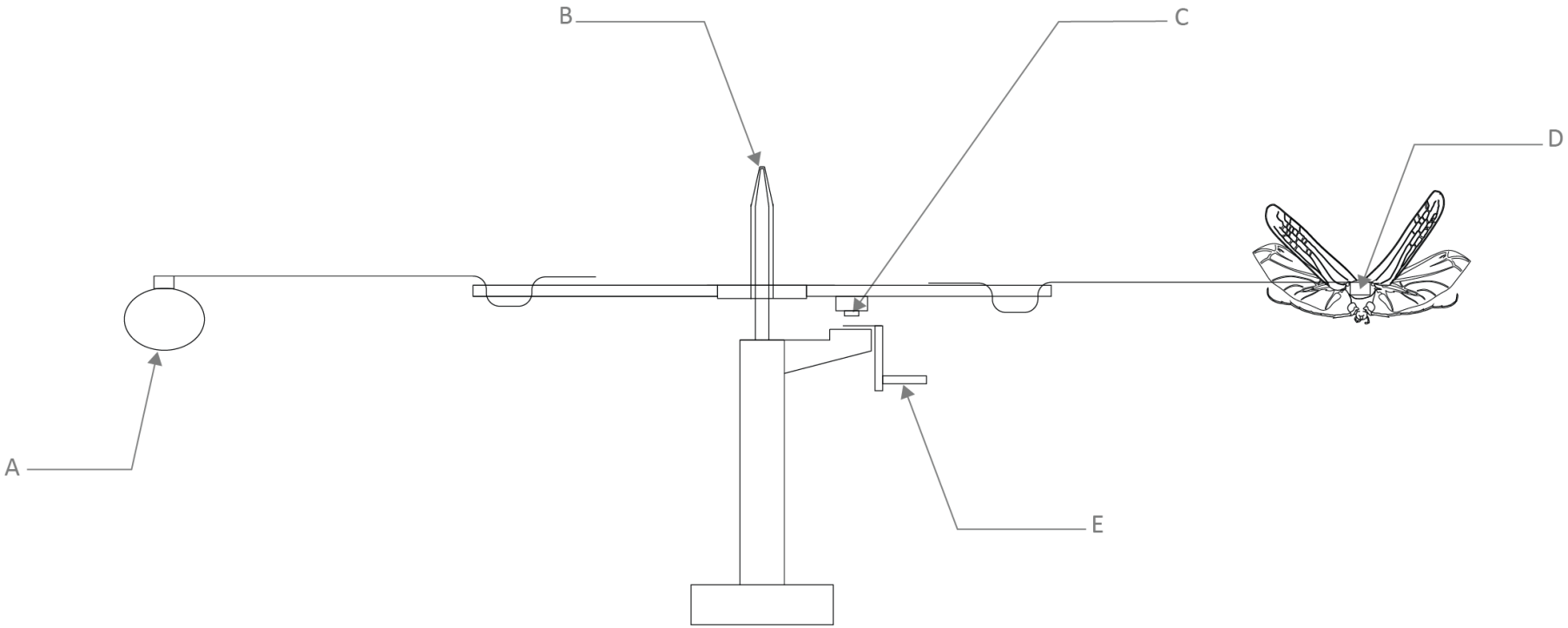

Figure 30. Flight mill design. A. Plasticine counterweight, matching insect weight. B. Low friction, polypropylene axis. C. Neodymium magnet trigger. D. Neodymium magnet attached to pronotal staple on specimen. E. Hall effect sensor (wired to Arduino, not pictured).

Data Processing

Flight mill data were cleaned using R 4.2.3 (R Core Team, 2022) and the tidyverse ecosystem (H. Wickham et al., 2019). I attached n = 103 *P. reticularis* to flight mills, producing 4,018,162 data points from n = 68 individuals. Firstly, I removed any data points that had been recorded with an incorrect date stamp i.e., occurring outside of the experimentation period (44 data points). I then grouped all responses by date and time and summarised to provide the number of revolutions per second (rps), providing 176,121 data points. In some instances, an individual would cease flying over the top of the hall-effect sensor, causing the sensor to stay in an on position, leading to abnormally high revolutions per second. To account for this, I filmed five individuals (n = 5) and recorded maximum revolutions per second, taking the highest value (1.6 rps), I then doubled this value and filtered out any results greater than 3 rps resulting in a final amount of 23,876 data points for analysis from n = 55 individuals.

Statistical Analysis and Software

Allometry

I used the power function $y = \alpha X^{\beta}$ where y is the trait of interest, X is pronotum width, α is the intercept and β is the slope (Huxley, 1924). I linearised this by log transformation to $\log_{10} y = \log_{10} \beta + \alpha \log_{10} X$ which allowed the use of an ordinary least squares (OLS) linear regression to fit a line to the data, estimate the slope and intercept and perform a test to assess for deviation from isometry ($\alpha \neq 1$) using the smatr package (Warton et al., 2012; version 3.4-8). There has been debate regarding the appropriateness of different regression methods used in evolutionary biology, with OLS at risk of underestimating allometric slopes if there is measurement error in body size measurements. However, this bias is negligible when measurement error is low and OLS is still viewed as preferable in comparison with other methods (Kilmer & Rodríguez, 2017). To account for this, all measurements were taken by a single researcher (NWB), and I thrice repeated measurements of traits on 20 individuals to ensure that measurement error was low.

Flight mill

I calculated distance flown (flight mill circumference x number of revolutions (mm)), duration (time spent flying in seconds) and speed (m/s) for each individual.

To assess for sexual dimorphism in flight behaviour, I compared the average distance, duration and speed between males and females. Due to an imbalance in sample size between males and females, I performed a Levene's test which revealed the groups being compared did not have approximately equal variance for distance and duration ($p = 0.006$, $p = 0.01$ respectively) but was approximately equal for speed ($p = 0.639$). Due to this, I performed a non-parametric Kruskal-Wallis test.

To assess how wing dimensions affect flight performance in males, I used three generalised linear models (GLM) with a gamma distribution with a log-link function to predict (1) average flight duration in seconds, (2) average distance in millimetres, (3) average flight speed in metres per second, using body size (pronotum width in millimetres), wing aspect ratio and wing loading as predictors. For each model, I used the R package "Performance" (Lüdecke et al., 2021) to visualise the data and confirm the assumptions of normality of residuals, normality of random effects, linear relationship, homogeneity of variance, and multicollinearity were met. All statistical analyses were conducted in R (R Core Team, 2022; version 4.2.3).

I fitted three general linear models (GLM), estimated using maximum likelihood, to predict the following measures of flight performance (1) average flight distance, (2) average flight duration and, (3) average flight speed with the independent variables body size (pronotum width), wing aspect ratio and wing loading (formula: $flight\ performance\ measure \sim body\ size + wing\ aspect\ ratio + wing\ loading$). Standardized parameters were obtained by fitting the model on a standardized version of the dataset. 95% Confidence Intervals (CIs) and p-values were computed using a Wald t-distribution approximation.

Results

Sexual Dimorphism

P. reticularis shows strong evidence of sexual dimorphism in selected traits with males having larger antennae than females, whilst females were larger in body length, body mass, pronotum width, elytron length, femur length, tibia length, wing length and wing area and wing loading (Full statistical details provided in Table 14). There was no significant difference in wing aspect ratio between males and females. The Kruskal-Wallis tests revealed no significant differences between male and female flight metrics (distance: $\chi2$ (1) = 1.663, p = 0.197; duration: $\chi2$ (1) = 1.419, p = 0.234; speed: $\chi2$ (1) = 0.739, p = 0.390).

Table 14. Descriptive statistics showing sexual dimorphism between male and female adult P. reticularis.

Morphological & Behavioural Traits	Units	Males						Females						t-test results	
		n	Mean (+/- SD)		Max Size	Min Size	CV (%)	n	Mean (+/- SD)		Max Size	Min Size	CV (%)	t	p
Antenna Length	mm	86	41.00	4.35	53.77	27.64	10.62	23*	28.42	3.03	34.43	23.12	10.68	-15.96	0.00
Body Length	mm	88	31.97	2.95	37.85	23.70	9.24	24	34.48	4.01	42.05	26.06	11.63	2.87	0.01
Pronotum width	mm	88	6.79	0.73	8.34	4.91	10.76	24	7.23	0.87	8.52	5.44	11.99	2.28	0.03
Elytron Length	mm	88	23.55	2.41	29.09	16.27	10.24	24	25.92	3.16	31.78	19.62	12.20	3.42	0.00
Femur Length	mm	88	6.51	0.66	8.02	4.79	10.19	24	7.02	0.77	8.16	5.29	10.92	2.96	0.01
Tibia Length	mm	88	6.33	0.66	7.88	4.39	10.37	24	7.32	0.85	8.80	5.41	11.59	5.29	0.00
Wing Length	mm	88	26.61	2.69	34.03	19.14	10.11	24	29.86	4.21	37.92	21.93	14.09	3.59	0.00
Wing Area	mm2	88	226.19	47.28	345.96	110.59	20.90	24	288.94	74.43	431.98	154.88	25.76	3.92	0.00
Wing Aspect Ratio	-	88	3.18	0.24	3.87	2.57	7.59	24	3.14	0.21	3.47	2.71	6.82	-0.81	0.42
Wing Loading	-	88	1.73	0.30	2.41	1.20	17.26	13	2.38	0.84	4.58	1.39	35.40	2.78	0.03
Body Mass	mg	88	792.89	260.20	1505.00	324.00	32.82	13	1268.00	397.16	1843.00	636.00	31.32	4.18	0.00

Note: CV = coefficient of variation. Significant differences in mean trait sizes between sexes were assessed using Welch t tests. P-values were corrected for multiple comparisons using Benjamini–Hochberg method. *Both antennae were damaged in one female so was excluded from the antenna length analysis.

Allometry

Full allometric statistical outputs are provided in Table 2 and Figure 4. In summary, the wing length and area and tibia length of *P. reticularis* exhibited negative allometry for males and isometry for females. Antennal length of *P. reticularis* exhibited negative allometry for both males and females. Ninth flagellomere length exhibited isometry in both males and females. Wing aspect ratio exhibited very low correlation with pronotum width (wing aspect ratio male, r2 = 0.063, female, r2 = 0.071). Wing loading exhibited isometry for males and negative allometry for females.

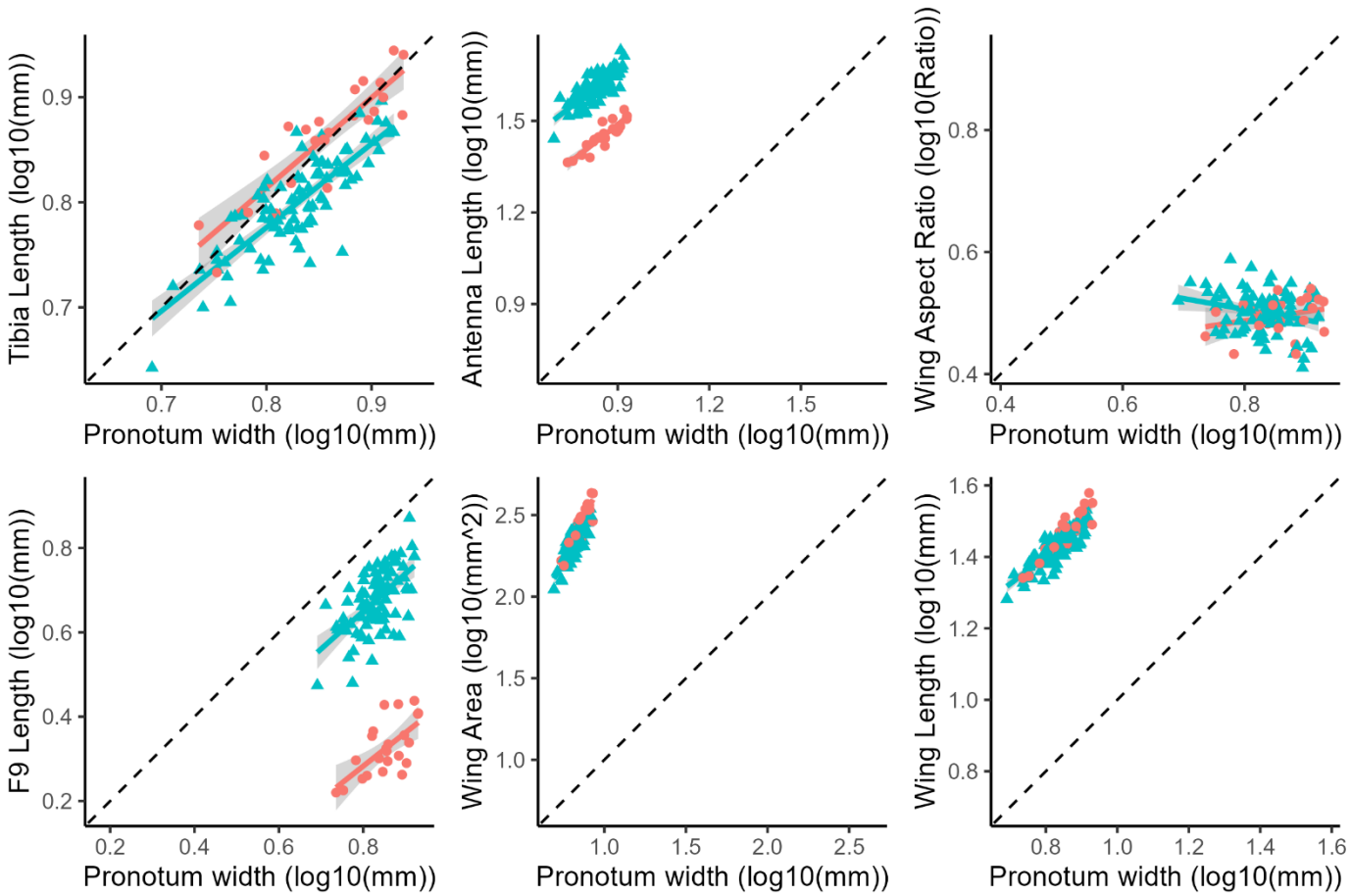

Figure 31. Allometric slopes (± SE) male (blue triangle) and female (red circle) P. reticularis. Both x and y axis have been log10 transformed. The dashed line shows the line of isometry.

Table 15. Estimated allometric slopes and slope test results for log10 transformed male traits of interest against pronotum width (proxy for body size). The slope test assesses evidence that the slope is significantly different from a hypothesised value of 1. The test statistic r is the correlation between residual and fitted values.

	Males							Females						
Trait	n	Intercept (α)	Slope (β)	H0: Variables uncorrelated		H0: Slope not different from 1		n	Intercept (α)	Slope (β)	H0: Variables uncorrelated		H0: Slope not different from 1	
				r2	p	r	p				R^2	p	r	p
Antennal Length	86	0.972	0.769	0.602	0.00	-0.347	0.00	23	0.779	0.787	0.812	0.00	-0.489	0.02
Length F9	88	-0.064	0.892	0.344	0.00	-0.087	0.42	24	-0.351	0.792	0.429	0.00	-0.221	0.31
Femur Length	88	0.160	0.785	0.690	0.00	-0.378	0.00	24	0.128	0.836	0.817	0.00	-0.384	0.06
Tibia Length	88	0.139	0.796	0.680	0.00	-0.350	0.00	24	0.128	0.857	0.779	0.00	-0.300	0.16
Wing Length	88	0.791	0.762	0.657	0.00	-0.396	0.00	24	0.549	1.077	0.846	0.00	0.166	0.44
Wing Area	88	0.468	0.850	0.720	0.00	-0.273	0.01	24	0.365	1.002	0.836	0.00	0.004	0.98
Wing Aspect Ratio	88	2.947	-0.175	0.063	0.02	-0.867	0.00	24	2.668	0.150	0.071	0.21	-0.843	0.00
Wing Loading	88	-0.549	0.939	0.354	0.00	-0.0473	0.66	24	0.992	-0.745	0.089	0.32	-0.591	0.03

Note: F9 = ninth flagellomere. Wing area has been reduced to one dimension by square root prior to log transformation.

Wing traits and their impact on flight performance

The exponentiated predicted values from each model along with observed data are plotted in Figure 32. Exponentiated coefficients and confidence intervals are presented in Supplementary Table 16.

Average flight distance

The model's explanatory power is substantial (Nagelkerke's $R^2 = 0.30$). The model's intercept, corresponding to body size = 0, wing aspect ratio = 0 and wing loading = 0, is at 6.85 (95% CI [3.68, 10.05], $t(55) = 4.25$, $p < .001$). Within this model, the effect of body size is statistically non-significant and negative ($\beta = -0.06$, 95% CI [-0.34, 0.22], $t(55) = -0.40$, $p = 0.692$; Standardised $\beta = -0.04$, 95% CI [-0.23, 0.15]). The effect of wing aspect ratio is statistically non-significant and negative ($\beta = -1.19e-03$, 95% CI [-5.26e-03, 2.85e-03], $t(55) = -0.64$, $p = 0.521$; Std. $\beta = -0.05$, 95% CI [-0.23, 0.13]). The effect of wing loading is statistically significant and positive ($\beta = 1.07$, 95% CI [0.45, 1.70], $t(55) = 3.38$, $p < .001$; Std. $\beta = 0.31$, 95% CI [0.13, 0.49]).

Average flight duration

The model's explanatory power is substantial (Nagelkerke's $R^2 = 0.32$). The model's intercept, corresponding to corresponding to body size = 0, wing aspect ratio = 0 and wing loading = 0, is at -1.19 (95% CI [-3.78, 1.41], $t(55) = -0.91$, $p = 0.365$). Within this model, the effect of body size is statistically non-significant and positive ($\beta = 5.40e-03$, 95% CI [-0.23, 0.24], $t(55) = 0.05$, $p = 0.963$; Std. $\beta = 3.65e-03$, 95% CI [-0.15, 0.16]). The effect of wing aspect ratio is statistically non-significant and positive ($\beta = 2.36e-04$, 95% CI [-2.95e-03, 3.42e-03], $t(55) = 0.16$, $p = 0.876$; Std. $\beta = 0.01$, 95% CI [-0.13, 0.15]). The effect of wing loading is statistically significant and positive ($\beta = 0.77$, 95% CI [0.27, 1.26], $t(55) = 2.96$, $p = 0.003$; Std. $\beta = 0.22$, 95% CI [0.08, 0.37])

Average flight speed

The model's explanatory power is weak (Nagelkerke's $R^2 = 0.09$). The model's intercept, corresponding to corresponding to body size = 0, wing aspect ratio = 0 and wing loading = 0, is at 0.73 (95% CI [-0.99, 2.46], t(55) = 0.82, p = 0.413). Within this model, the effect of body size is statistically non-significant and negative (β = -0.06, 95% CI [-0.21, 0.09], t(55) = -0.78, p = 0.433; Std. β = -0.04, 95% CI [-0.14, 0.06]). The effect of wing aspect ratio is statistically non-significant and negative (β = -1.00e-03, 95% CI [-3.07e-03, 1.07e-03], t(55) = -0.97, p = 0.330; Std. β = -0.04, 95% CI [-0.14, 0.05]). The effect of wing loading is statistically significant and positive (β = 0.38, 95% CI [0.03, 0.73], t(55) = 2.14, p = 0.032; Std. β = 0.11, 95% CI [9.52e-03, 0.21])

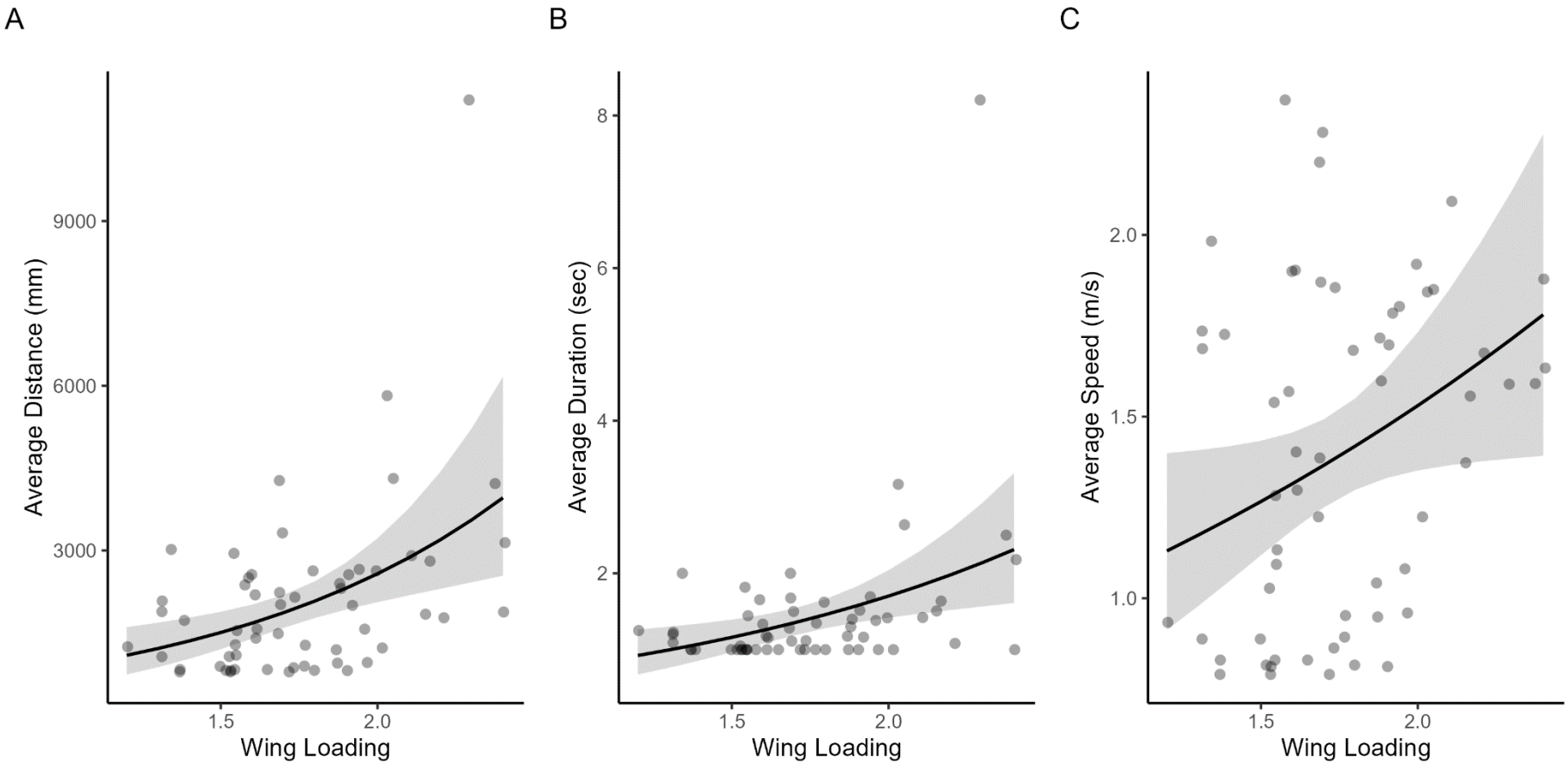

Figure 32. Predicted values of flight performance plotted against wing loading with aspect ratio and pronotum width held constant. Plotted over observed data with confidence intervals at the 95% level. A = Average Distance, B = Average Duration, C = Average Speed.

Table 16. Predictive models of flight performance parameters and wing traits in P. reticularis.

Dependent Variables	Model Fit					Coefficients					
	AIC	AICc	BIC	Nagelkerke's R^2	Sigma	Independent Variables	Exp. Coefficient	Exp. CI low	Exp. CI high	t	p
Average flight distance	983.74	984.87	994.12	0.30	0.60	(Intercept)	946.47	39.76	23091.13	4.25	0.000
						Pronotum Width	0.95	0.71	1.25	-0.40	0.692
						Wing Aspect Ratio	1.00	0.99	1.00	-0.64	0.521
						Wing Loading	2.93	1.57	5.45	3.38	0.001
Average flight duration	92.75	93.88	103.14	0.32	0.49	(Intercept)	0.31	0.02	4.11	-0.91	0.365
						Pronotum Width	1.01	0.80	1.27	0.05	0.963
						Wing Aspect Ratio	1.00	1.00	1.00	0.16	0.876
						Wing Loading	2.15	1.30	3.53	2.96	0.003
Average flight speed	76.93	78.07	87.32	0.09	0.33	(Intercept)	2.08	0.37	11.69	0.82	0.413
						Pronotum Width	0.94	0.81	1.09	-0.78	0.433
						Wing Aspect Ratio	1.00	1.00	1.00	-0.97	0.330
						Wing Loading	1.46	1.03	2.07	2.14	0.032

Note: CI = Confidence Interval at 95%. t-statistic & p-values were computed using a Wald t-distribution approximation.

Discussion

In my investigation of sexual dimorphism in *P. reticularis*, I observed pronounced differences between male and female individuals across all measured traits, with the exception of wing aspect ratio. Female traits were consistently larger on average than males, with the exception of the antennae. Male antennae were significantly larger on average than female antennae which suggests sexual selection is acting on antennal length. Contrary to my initial hypothesis, an analysis of trait allometries did not reveal the positive allometry that is frequently used to infer the influence of sexual selection (Kodric-Brown et al., 2006; but see Bonduriansky, 2007). Females showed negative allometry in antennal length, wing aspect ratio, and wing loading whilst all other traits exhibited isometry. In contrast, all male traits demonstrated negative allometry, except for wing loading which adhered to isometry. In the three areas of flight performance I explored, I discovered that wing loading was significant and positive, suggesting that as wing loading increases, so too does distance, duration and speed. As *P. reticularis* does not feed as an adult, the single objective of males is to mate with females which presents a fascinating study system to explore importance of different traits to success in a scramble competition mating system.

Sensory traits

The male-biased antennal sexual dimorphism apparent in *P. reticularis* is consistent with prionine antennae in general (Linsley, 1959), indicating the importance of detecting female chemical cues to the overall reproductive fitness of the male in this group (Barbour et al., 2006; Hanks & Millar, 2016; Paschen et al., 2012; Rodstein et al., 2009; J. D. Wickham et al., 2016). Increased antennal size/surface area has been associated with male fitness, improving the ability to detect weaker signals from females in Lepidoptera (Johnson et al., 2017). The observed elongation of male antennae in *P. reticularis* therefore may represent the optimising of mate-searching efficiency in this species.

Antennae are important sensory receptors, that are likely to be under different selective pressures. Directional selection is likely to have driven the increase in male antennal length compared to females. However, antennal length in both sexes of *P. reticularis* exhibited negative allometry suggesting that stabilising selection is occurring in both sexes, driving negative allometry. It appears that there is an interplay between directional and stabilising selection and I suggest that directional selection on the males is occurring within the constraints of stabilising selection as the allometric slope is very similar in both sexes (Male $\beta = 0.769$ & female $\beta = 0.787$).

The interplay of different selective pressures is likely very important in determining the maximum length or area of the antennae. As antennae provide information vital to male fitness they are likely to be under strong directional selection e.g., longer, pectinate antennae in males to detect chemosensory information on chemical cues involved in mate searching (Cardé, 2021; Johnson et al., 2017). This selection frequently leads to spectacular antennal morphologies which increase the fitness of the individual. However, there must be a trade-off; otherwise, I might expect the antennae to become even longer or with even greater surface area. This trade-off is likely to be one of aerodynamics, with drag increasing with antennal surface area resulting in greater energetic costs (Jaffar-Bandjee et al., 2020) and imparting a stabilising selective pressure leading to negative allometry. I conducted several laboratory experiments to determine the strength of olfactory response in males to female cues, however I was unsuccessful in eliciting mating behaviour in artificial conditions, indicating that unknown environmental factors may be important in this mating system. Future research would benefit from conducting field trials to explore and quantify behavioural responses.

Locomotory Traits

Morphological traits associated with dispersal (wing length and wing area) displayed negative allometry in males and isometry in females. Negative allometry in male wing traits is a pattern

seen in several other beetle groups (Kawano, 1995, 1997; Kelly, 2022). Kelly (2020) found that small body size and larger wing size in males, led to greater mating success in *Popillia japonica*. However, this did not translate to positive allometry for wing area in this species, instead negative allometry was present with stabilising selection proposed to be at play on this trait (Kelly, 2022).

I found that flight performance (distance, duration and speed) increased with wing loading, but body size and wing aspect ratio did not influence flight performance. Increased wing loading is associated with higher wingbeat frequency, an increase in airspeed but a decrease in energetic efficiency suggesting the sustainability of flight is lower (Byrne et al., 1988; Dudley, 1999). Different wing loading has been shown to be intimately related with defensive strategies in Neotropical butterflies, with species with high wing loadings relying on speed to escape predators, whilst species with low wing loadings fly in a slow and jaunty fashion relying on aposematism to avoid predation (Chai & Srygley, 1990). Therefore, the benefits to flight performance received from high wing loading values are likely to result in increased male fitness by maximising the number of females a male can get to. Whilst endurance (sustainability of flight) has been posited to be important and thus favour lower wing loading values, this likely depends on the spatial distribution of the female population (Husak & Fox, 2008). If females are located within a short distance of one another, then speed is likely to be a more important factor (Kelly, 2020).

Aspect ratio is important for agility and manoeuvrability. However, I did not see evidence of sexual dimorphism for this trait and it was not significant in the flight performance models. Aspect ratio had the lowest coefficient of variation (CV) of all of the traits I measured (Table 14) suggesting that this trait may be very constrained across body sizes. On the other hand, wing loading and body mass had the highest CV of traits measured, this suggests a great deal of variation within the sample population which is likely due to the differing ages of individuals. As

the fat reserves of an individual are finite, the mass of the organism will decrease over time as these reserves are depleted whilst the wing area remains the same size. Wing loading will therefore decrease with age for each individual, resulting in more efficient flight over time but with a decrease in flight performance.

Body size

I found evidence for female-biased sexual size dimorphism (SSD) in *P. reticularis*, with males exhibiting significantly smaller and lighter body size compared to females. Small body size in males is thought to be beneficial in scramble competition, increasing their mobility and/or endurance (Andersson, 1994; Ghiselin, 1974; Herberstein et al., 2017; Kelly et al., 2008). One theoretical framework that may explain this is the Ghiselin-Reiss small-male hypothesis which suggests that in species exhibiting a scramble competition mating system, smaller males have a selective advantage in acquiring mates (Ghiselin, 1974; Reiss, 1989). The advantage is derived from a trade-off between allocating time and energy from feeding to mate searching. Smaller males, which require less energy per unit of time, can dedicate more time to energetically costly mate searching instead of seeking food, thus increasing the chance of encountering and mating with females.

In the context of female-biased SSD observed in *P. reticularis*, the Ghiselin-Reiss hypothesis provides a possible explanation. As the adults do not feed, instead surviving on larval energy reserves, a lower body size should increase the energy efficiency of flight. Flight efficiency is related to wing loading, a biomechanical variable relating wing surface area to body mass, with a lower wing loading value corresponding to increased flight efficiency, and a higher wing loading values associated with faster flight and higher body temperatures (indicating a greater loss of energy through heat) (Chai & Srygley, 1990; Dudley, 1999). Smaller, lighter males (in comparison to females) have a lower wing loading value decreasing the energy required for flight allowing for

more efficient use of the finite energy supply thereby extending the length of time available to find spatially dispersed females.

Females are significantly heavier than males, and this was reflected in their larger wing loading value indicating a lower efficiency when flying. However, as mate searching is not required for the female, instead relying on a chemical cue to attract males, flying efficiency is less likely to be an issue other than when finding an oviposition site. This indicates that selection pressure is occurring on males but does not appear to be acting upon females and this drives the sexual dimorphism I saw.

Scramble competition and the selective pressures at play

Male success in scramble competition is governed by the rate at which they find and mate with females. Therefore, I predicted that as for male weapons and ornaments, positive allometry would be evident in sensory and locomotory traits. I did not find evidence of this, instead finding that all male traits of interest exhibited negative allometry. This suggests that the traits do not play a purely sexual function; traits with both sexual and non-sexual functions typically demonstrate isometry or negative allometry as observed here (Bonduriansky, 2007; Fromhage & Kokko, 2014).

In scramble competition, male fitness is linked to finding and mating with as many females as possible (Andersson, 1994; Ghiselin, 1974; Herberstein et al., 2017; Thornhill & Alcock, 1983) and has led to spectacular examples of sexual dimorphism such as the wingless, sedentary females in several species of bag moth relying on pheromones to attract males (Niitsu et al., 2017; Rhainds et al., 2008). In the case of *P. reticularis*, the female is fairly sedentary, attracting mates with a chemical cue and flying only when finding an oviposition site. This, combined with finite energy reserves, places selective pressure on male locomotory and mate searching traits.

One reason I might see negative allometry rather than positive allometry is that small males may be investing disproportionately in wing length and area as it provides an increase in marginal fitness gain for small males whilst larger males do not see a marginal fitness gain by investing further in wings, perhaps being constrained by a biomechanical or developmental constraint. A developmental constraint is a limitation or restriction that is imposed by the processes of development on the range of possible phenotypes that can evolve in a population (Frankino et al., 2005; Smith et al., 1985). However, determining the relative role of selection and developmental constraints is challenging without experimentally manipulating trait and assessing changes in fitness. Frankino et al. (2005) artificially altered the wing loading in a population of butterflies by selecting for differences in fore wing area. Over several generations this led to different allometries between the experimental group and the wild-type control. The wild-type were observed to have greater fitness, mating more frequently with females. This showed that the short-term evolution of new allometries was not limited by developmental constraints, instead natural selection appears to be exhibiting strong stabilising selection on male wing loading in this species.

In summary, my investigation provides evidence for selective pressures on traits associated with fitness in a scramble competition mating system. Most traits examined demonstrated female-biased sexual dimorphism apart from antennal length which showed strong male-bias indicating the importance of detecting female chemical cues to male fitness. I did not find positive allometry indicative of sexual selection. Instead, negative allometry was prevalent in several traits, suggesting the influence of stabilizing selection. Wing loading was positively correlated with flight speed, distance, and duration. My findings provide insights into the trade-offs and constraints influencing the evolution of sexual dimorphism in *P. reticularis*, highlighting the need for future investigation, such as using artificial selection to tease apart and fully understand the dynamics at play.

Supplementary Material

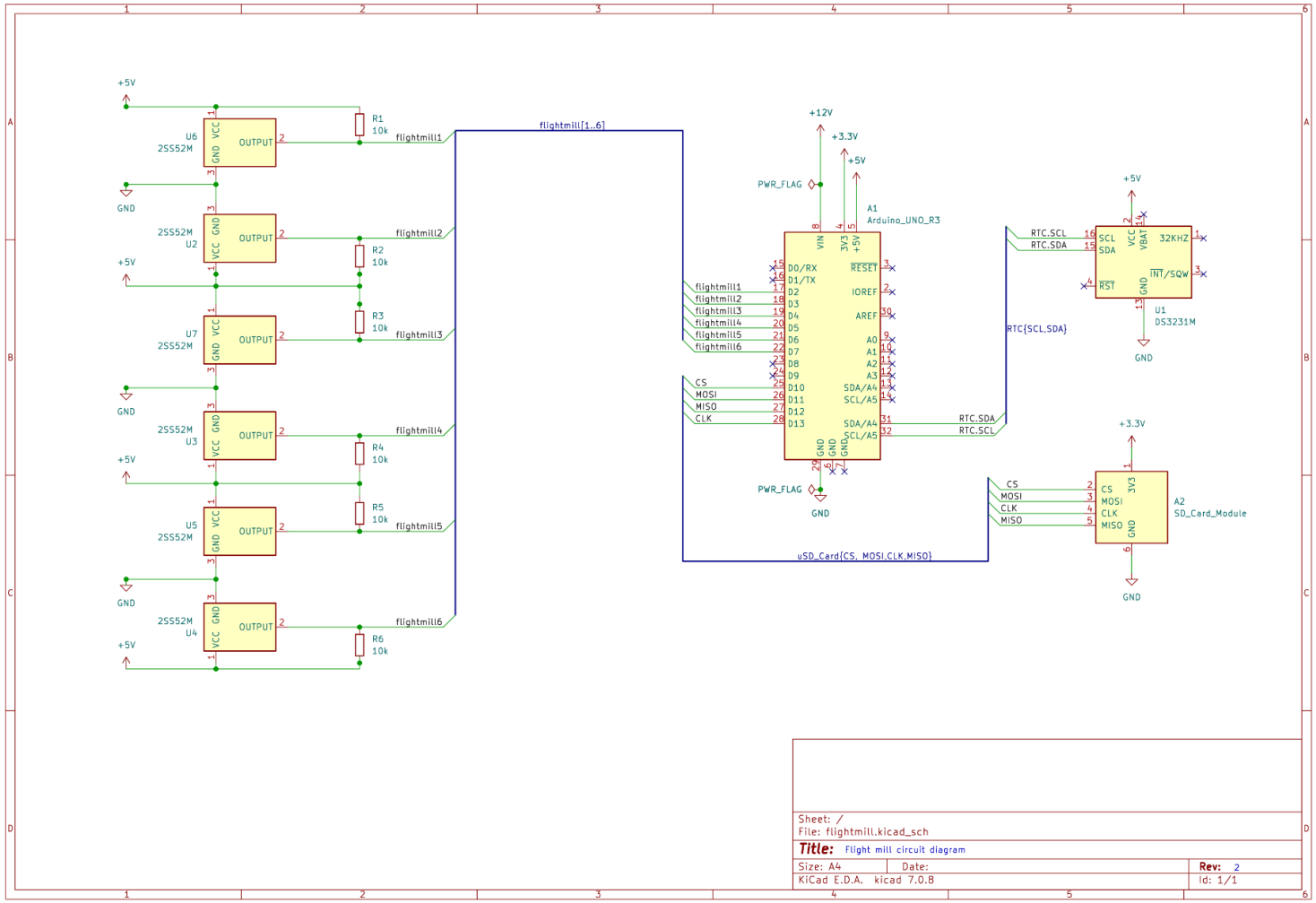

Figure 33. Flight mill electronic schematics. Microprocessor is an Arduino Uno Revision 3 connected to a real time clock (DS3231M), Micro SD Card module, and six Honeywell Hall Effect Sensors (2SS52M)

Chapter 6 The antennal ultrastructure of a prionine longhorn beetle, *Prionoplus reticularis* (White)

Abstract

Antennal sensilla are fundamental to an insects sensory perception of its environment. In species where one sex produces pheromones for attracting mates, I can predict antennal and sensilla morphology to be sexually dimorphic. *Prionoplus reticularis* (Prioninae; Cerambycidae; Coleoptera) is large longhorn beetles exhibiting a scramble competition polygyny mating system with sexual dimorphism present in antennal length, suggesting chemical cues are of importance in male mate searching. I investigated antennal ultrastructure morphology and sexual dimorphism in this species using scanning electron microscopy. I identified several sensilla that were only present in males: sensilla filiformia, sensilla auricillica, wall pore hairs and distal sensilla chaetica. These sensilla are known to have chemosensory and mechanosensory functions in other insect groups, suggesting these senses play an important role in male mating strategies.

Introduction

An animal's perception of its surroundings is vital for its survival and reproduction, and for insects the antennae fulfil a key role in reception of sensory cues to inform its behavioural decisions (Elgar et al., 2018). Antennae can be host to a variety of ultrastructures involved in mechanoreception, hygroreception, and thermoreception but are particularly associated with chemoreception (D. Schneider, 1964). Chemoreception relates to the detection of chemical cues in the environment (Zacharuk, 1980) and many insects are particularly influenced by such cues (Szyszka & Galizia, 2015), using them to locate host plants or prey (Geier et al., 1999; Paschen et al., 2012), make decisions about food quality (Haverkamp et al., 2016), choose appropriate

habitats and oviposition sites (Paschen et al., 2012), avoid predators (Kannan et al., 2022) and locate and select mates (Johansson & Jones, 2007). Chemoreception can be further categorised into olfaction, analogous to smell, the perception of volatile molecules in the air, while gustation, analogous to taste, is the detection of non-volatile chemicals from surfaces (B. H. King & Gunathunga, 2023). Whilst the antennae are comprised of just three units; the basal scape and pedicel, and a flagellum, these can be readily modified as reflected in the incredible diversity in antennal morphologies across insect taxa (Elgar et al., 2018).

The basic sensory unit of the antennae is a sensillum, which is an innervated ultrastructure comprised of sensory nerve cells, trichogen and tormogen cells, and sometimes auxiliary cells (D. Schneider, 1964). Sensilla have been classified according to their structural morphology (e.g., peg and socket, hair, dome), wall thickness (e.g., thin walled, thick walled, single walled, double walled), presence and distribution of pores (e.g., terminal pore, wall pore, aporous, uniporous, multiporous, pitted, grooved) and innervation (e.g., number of sensory nerve cells, dendritic branching) (Altner & Prillinger, 1980). Whilst it is problematic to label the function of a sensilla based on its morphology without supporting electrophysiological evidence, there are sometimes clues which can help us to provide a tentative role to a sensillum type; for instance many chemosensory sensilla will usually have pores visible in the cuticle, through which odour molecules pass (Altner & Prillinger, 1980).

Darwin suggested that sexual selection, through female mate choice, could explain the seemingly bizarre array of traits that appear in some animal species, e.g., the "gorgeous plumage and strange antics" of male birds in attracting a mate (Darwin, 1859) that would seem conspicuous to predators and at odds with natural selection. Sensory traits that improve the ability of males to discover females were also postulated by Darwin to be under the influence of sexual selection (Darwin, 1871), but these have largely remained unstudied in the context of recent sexual selection research ((Elgar et al., 2018). The reproductive benefit of finding a mate first can

explain selection for longer antennae. For example, male *Uraba lugens* moths arriving at a trap containing a single female had, on average, longer antennae than males arriving at a trap containing two females, suggesting that longer antennae length improves the receptive ability of males to find a female pheromone plume at lower concentration (Johnson et al., 2017).

In species of insects that rely on female sex pheromones to attract mates, I frequently observe sexual dimorphism in the antennae. This may be obviously visible through an increase in antennal surface area through length of antennae and branching of the flagella, or, less visible to the human eye, through differences in the sensilla on the antenna (Elgar et al., 2018). Morphology, density and distribution of sensilla have been shown to differ across a variety of insect orders. In the Mantodea, female sex pheromones are used by *Pseudomantis albofimbriata* as sensory cues in mate finding, whilst *Ciulfina biseriata* uses visual cues (Holwell et al., 2007). Sensilla basiconica, associated with olfaction, are only present on the antennae of males of P. albofimbriata. In contrast, *C. biseriata*, which used vision in mate searching, did not exhibit sensilla basiconica or dimorphism in the antennae (Holwell et al., 2007). Pheromones are well known in the Lepidoptera, and sexual dimorphism in antennal morphology is, unsurprisingly, common. Differences in the density and distribution of sensilla between sexes have been attributed to differences in importance of different sensory cues, e.g. female sex pheromones for males and host plant volatiles in females (Castrejón-Gómez et al., 1999; Hansson et al., 1995; Mark et al., 2018). Female produced sex pheromones appear to explain sexual dimorphism exhibited in *Clitarchus hookeri* (Phamatodea) with increased density of sensilla basiconica and sensilla trichodea, sensilla associated with olfaction, present in males (S. S. Myers et al., 2015). In some cases, the driver of sexual dimorphism of antennal ultrastructures can be less clear. This is the case with two species of closely related cave wētā (Orthoptera), *Pachyrhamma waitomoensis* and *P. acanthocera*. Several sensilla subtypes associated with chemoreception are absent in male *P.*

waitomoensis that are not absent in male *P. acanthocera*, which may be due to differences in the structural complexity of the respective species' habitats (M. P. Fea et al., 2019).

Distinctive long antennae are a characteristic feature of cerambycid beetles, giving rise to their common name - long horn beetles (Allison et al., 2004). Typically, the antennae are filiform, however in some instances, these antennae may be strikingly modified, with comb-like flabellate flagellomeres, densely setose segments and, in one instance, a modified terminal flagellomere to form a venomous stinger (Berkov et al., 2008; Q. Wang et al., 2017). Sexual dimorphism is common in the cerambycids, and this is often most pronounced in the antennae with males having longer antennae than females. This suggests that there may be sexual differences in the sensilla present on the antennae which allow for the detection of chemical cues of differing importance to each sex.

Within the cerambycids, there are seven major types of sensilla present on the antenna (recently standardised in Haddad et al., 2023) which are: Böhm bristles, sensilla chaetica, chemosensory hairs (this term is used for any large, porous, hair-like sensilla i.e., sensilla trichodea and porous sensilla chaetica which have been used interchangeably in the literature), sensilla basiconica, dome shaped organs, sensilla coeloconica, and sensilla auricillica. Whilst only a few studies of sensilla in cerambycids have included electrophysiological data (Álvarez et al., 2015; Lopes et al., 2002; S. Wang et al., 2005; Wei et al., 2018), likely function of a sensilla can be supported by evidence from studies in other groups.

Böhm bristles are found on the antennal socket and between scape and pedicel in most insect orders and are mechanosensory in function (D. Schneider, 1964). These bristles are important in proprioreception, indicating the position of the antennae in relation to the body which has been shown to be important in positioning the antennae for flight (Krishnan et al., 2012). Sensilla chaetica are primarily mechanoreceptive in function (D. Schneider, 1964) with neurons of long and distal subtypes eliciting an action potential in response to movement whilst the stout subtype

did not (Álvarez et al., 2015). Stout sensilla chaetica also do not show evidence of chemosensory functionality (MacKay et al., 2014; Sun et al., 2010).

Chemosensory hairs are divided into wall-pore hairs and terminal pore hairs which are proposed to have an olfactory and gustatory function respectively (Altner & Prillinger, 1980). Wall pore hairs, featuring multiporous walls and inflexible sockets, have been shown to have an olfactory function in Diptera (Clyne et al., 1997). Terminal pore hairs, featuring flexible sockets and a single apical pore, have been experimentally investigated in Lepidoptera and Blattodea with these structures showing both chemosensory and mechanosensory responses indicative of a contact chemoreceptive sensilla (Chun & Schoonhoven, 1973; Lambin, 1973).

Sensilla basiconica are small and cone shaped, with thin, multiporous walls and sit on raised bases (Haddad et al., 2023) with three sub-types: stout, thin, and grooved peg sensilla. Sensilla basiconica have been shown to play a role in olfaction with electrophysiology studies showing responses to host plant volatile compounds, sex pheromones and volatiles associated with burnt timber (Álvarez et al., 2015; Dyer & Seabrook, 1975; Wei et al., 2018).

The euonymous dome shaped organs are raised areas of the integument and are likely to have a thermosensory or hygrosensory function (Merivee et al., 2003, 2010). Sensilla coeloconica, or "pit pegs", are small pegs that emerge from shallow depressions and are have a thermosensory or hygrosensory function if aporous (Waldow, 1970), and chemosensory if porous (Pophof, 1997).

Sensilla auricillica are cone like sensilla with a flattened depression, described as shoe-horn shaped. Electrophysiological studies have shown these sensilla to respond to host plant volatiles and sex pheromones in Lepidoptera (P. Anderson et al., 2000; Ansebo et al., 2005).

Prioninae are particularly noted for sexually exaggerated antennae and the flagellomeres of the antennae can be serrate, pectinate, imbricate or flabellate (Linsley, 1959). There is evidence to suggest that male and female prionines are both attracted to semiochemicals released by their

host plant (Paschen et al., 2012), however only males respond to volatile female sex pheromones (Barbour et al., 2006; Edwards, 1961c; Paschen et al., 2012; Rodstein et al., 2009; J. D. Wickham et al., 2016). Mating systems with female produced sex pheromones, as found in the prionine beetles, tend to fit the category of scramble competition polygyny, whereby males try to mate with as many females as quickly as possible (Dickinson, 1992; Goldsmith et al., 1996; Goldsmith & Alcock, 1993).

The huhu beetle, *Prionoplus reticularis* (Cerambycidae: Prioninae), is the only member of the prionine family recorded in New Zealand (Sopow & Bain, 2017). The larvae feed on dead timber of native and exotic host plants (Edwards, 1959; Sopow & Bain, 2017) and are present across New Zealand. Adults are short-lived, non-feeding, large, and sexually dimorphic with males having longer antennae than females (Edwards, 1961c). A male-attracting olfactory signal is produced by female *P. reticularis*, and it has been noted that females attract males whether they are visible or covered (Edwards, 1961c). *P. reticularis* are also attracted to alpha- and beta- pinene lures suggesting that olfaction is important in the discovery of oviposition sites (Pawson et al., 2020). Stimulation of the antennae of either male or females elicits a defensive response, where the head is raised and lowered, the antennae flail and the jaws are opened and will seize on anything it comes into contact with (Edwards, 1961c). If this display occurs between two males, it can also lead to combat with the pair grappling one another with fore legs to flip one another whilst the mandibles seize any object that they come into contact with (Edwards, 1961c).

There are a paucity of studies in the literature of antennal ultrastructures in prionines with only males from two species, *Titanus giganteus* and *Prionus imbricornis*, being imaged using scanning electron microscopy (SEM) (Dvořáček et al., 2020; Haddad et al., 2023). Both studies found the sensilla in prionines to be markedly different to other cerambycid sub-families with an absence of sensilla chaetica and the presence of an unidentified sensilla resembling sensilla basiconica but

unlike those found in other cerambycid groups. Dvořáček (2020) has suggested that sensilla chaetica are absent in Prioninae, however this requires further evidence.

In this study I use SEM to categorise the ultrastructures of both male and female *P. reticularis* to assess whether there is evidence of sexual dimorphism present in antennal sensilla and to expand knowledge of prionine antennal sensory architecture.

Methods

Sample Preparation

Specimens of *P. reticularis* were collected using a light trap at Riverhead Forest, Auckland, New Zealand in January 2021. The right-hand antennae were excised from four male and four female specimens. Using methods adapted from Haddad et al., (2023), the antennae were soaked in ethanol overnight, then rinsed in deionised water, and left to soak in dilute detergent overnight, before sonicating for 2 – 3 seconds at 60 Hz. This was repeated a second time before a final rinse in 70% ethanol and then soaking overnight in 95% ethanol.

Antennae were mounted on stainless steel stubs with carbon adhesive tape then sputter coated with gold using a Leica EMACE200 (Leica Microsystems, Germany) for 60 seconds.

Scanning Electron Microscopy

Ultrastructural imaging was performed on a TESCAN CLARA field-emission gun scanning electron microscope (Tescan-Clara, Czech Republic) with the gun operated at 5 kV, a spot size of around 4 nm and a working distance between 11- and 12.5-mm. Images were taken of each segment of the antennae, from the scape to the ninth flagellomere.

Measurements

SEM Images were analysed in ImageJ v. 1.50i (C. A. Schneider et al., 2012). Following the methodology of Fea et al. (2019), I measured length of sensilla from the base to the apex, the

width of base, width of tip and socket width for sensilla in both sexes (2 female individuals and 4 male individuals). The mean and standard error are reported.

Terminology

Ultrastructures were classified based on their morphology, insertion, surface features, porosity (porous or aporous) and location of pores (wall or terminal pore). For ease of comparison, I use the sensilla naming conventions outlined by previous authors, in particular the recent review of cerambycid antennal ultrastructures by Haddad et al. (2023) who standardised the sensilla of cerambycids into the following broad categories: Böhm bristles, sensilla chaetica, chemosensory hairs, sensilla basiconica, dome shaped organs, sensilla coeloconica, and sensilla auricillica. These categories were further subdivided into subcategories. Haddad et al. (2023) prioritised the terminology of Dyer and Seabrook (1975) who were first to publish on cerambycid antennal ultrastructure morphology, whilst also using the terminology of Altner and Prillinger (1980) to replace the inconsistent use of suspected chemosensory sensilla chaetica and sensilla trichodea found in the literature with the terminology "wall-pore hairs" and "terminal-pore hairs". I use the term sensilla filiformia from Faucheux (2011) to describe the long, ciliated sensilla which are prevalent in the male specimens examined.

Results

The whole antennae of the adult male and female *P. reticularis* are shown in Figure 34 A & B. The male and female antennae are both serrate and comprised of the scape, pedicel and nine flagellomeres. The surface of the antennae is covered in cuticular scales that are roughly hexagonal in shape with the exception of the base of the scape and the pedicel where it inserts into the head and scape. The scape is cylindrical, with a slight kink on the lateral surface. The pedicel is pocillovous in shape, constricted at the mid-point before widening at the distal end. The flagellomeres are largely the same shape, with the distal end of flagellomeres one to eight protruding sharply on the distal, ventral edge, giving the serrated appearance of the antennae.

Above this protrusion is a small, sharp spur, mirrored on both the lateral and medial faces. The ventral surface of the antennae is densely pitted, with each pit containing several sensilla. The dorsal, medial surface has fewer pits, usually containing a sensillum. The terminus of the ninth flagellomere is rounded and laterally compressed with well-defined pits. The male flagellomeres are densely covered in sensilla filiformia with flagellomeres 6 – 9 at least as long if not longer than flagellomeres 1 – 5. The female flagellomeres do not have the sensilla filiformia found in the males, however they are densely pitted and flagellomeres 6 – 9 are reduced, being smaller than flagellomeres 1 – 5. Average (arithmetic mean) measurements for individual sensilla length are presented in Table 17.

Table 17. Mean length of individual sensilla for both sexes (where present). n.b. one grooved peg sensilla was found on a female antennae, however the orientation was not conducive to measuring.

Sensilla Type	Sex	Mean Length (μm)	±SE
Bohm bristles	f	72.19	9.21
	m	67.12	7.36
Sensilla chaetica distal	f	-	-
	m	81.81	2.57
Sensilla chaetica long	f	429.5	48.5
	m	385.77	-
Sensilla chaetica stout	f	83.55	20.26
	m	78.58	4.39
Sensilla filiformia	f	-	-
	m	327.23	5.073
Sensilla basiconica broad	f	7.36	0.28
	m	6.69	0.36
Sensilla basiconica thin	f	14.60	1.81
	m	15.20	0.54
Grooved peg sensilla	f	-	-
	m	4.38	0.77
Sensilla auricillica	f	-	-
	m	7.98	0.98
Terminal pore hair	f	111.91	-
	m	80.23	7.70
Wall pore hair	f	-	-
	m	200.00	-

Böhm Bristles

Böhm bristles are present on the condyle, scape, and pedicel, particularly in the junction between these antennal sections (Figure 34 C & D). The conical Böhm bristles taper to a point and are set into a concave depression in the surface of the antennae. They are variable in length (Table 17) and have no visible pores on the surface.

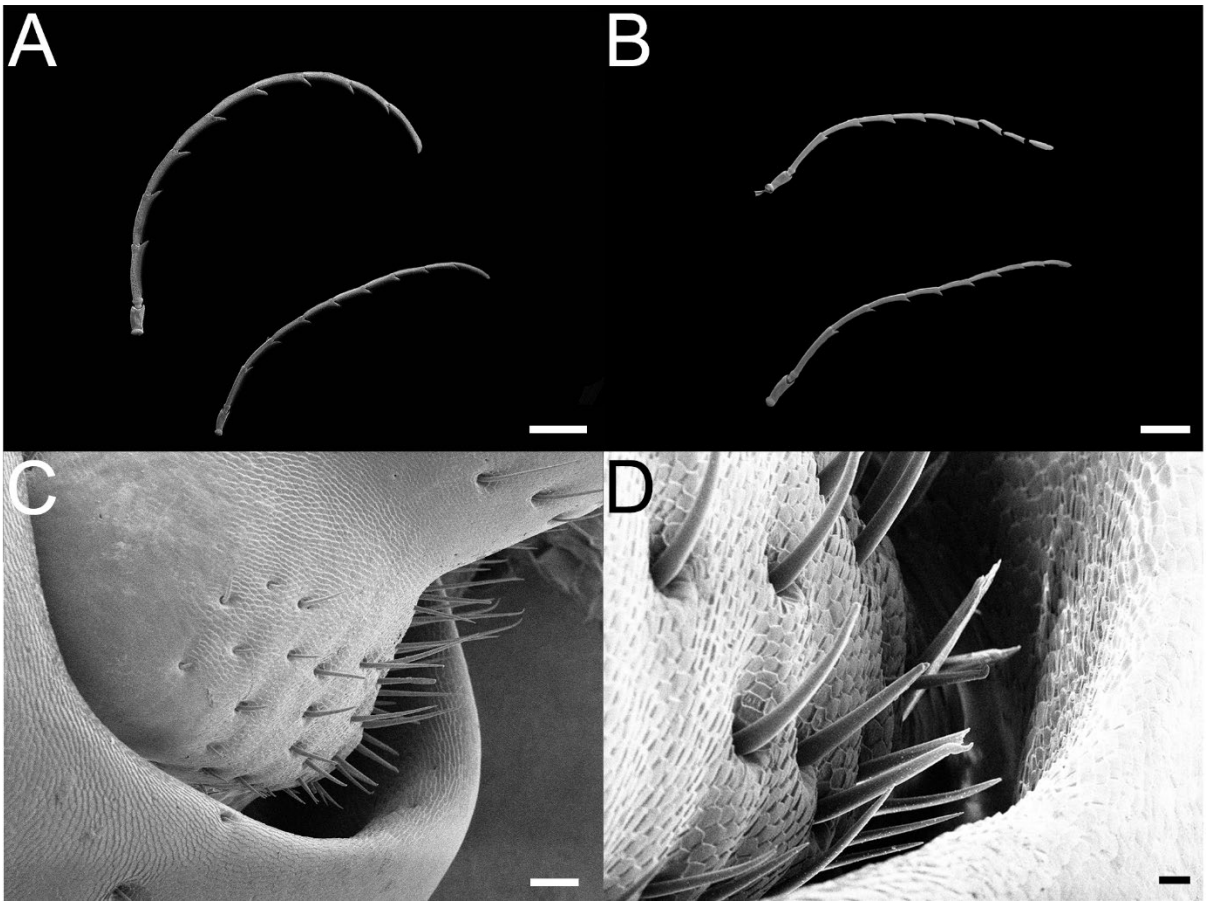

Figure 34. The ex-situ antennae of male (A) and female (B) Prionoplus reticularis. Two individuals of each sex are presented to show variation between antennal size within the same sex. Böhm bristles on the base of the pedicel where it connects to the scape in a female (C) and a magnified view of the Böhm bristles in the same location on a male (D). Scales: 5 mm (A), 5 mm (B), 50 µm (C), 20 µm (D).

Sensilla Chaetica (SC)

Distal, Long and Stout

Distal SC (Figure 35 B) were observed on the serrate protrusions of the antennae, set within a deep socket. There were between one and two present on flagellomeres 1 – 8 and none observed on flagellomere 9. They are slightly striated, with blunt, pointed tips and aporous.

Long sensilla chaetica (Figure 35 C) were rare and, if present, were found on the distal end of the scape and pedicel of both sexes. The surface of the long sensilla chaetica are aporous and striated with longitudinal grooves with the apex terminating in a sharp point.

Of all the SC present, stout SC (Figure 35 A & D) were the most numerous on both male and females. They were present in greatest numbers on the scape and pedicel and the ninth flagellomere in females. They are shorter than other SC and variable in length. In most cases they emerge from a narrow depression in the cuticle and lie flat along the surface of the antennae, their sharp tips pointing distally. The surface of the stout SC is aporous and deeply striated with three corrugated openings present at the base (Figure 35 D). The stout SC in females appear to be more deeply striated than the SC in males.

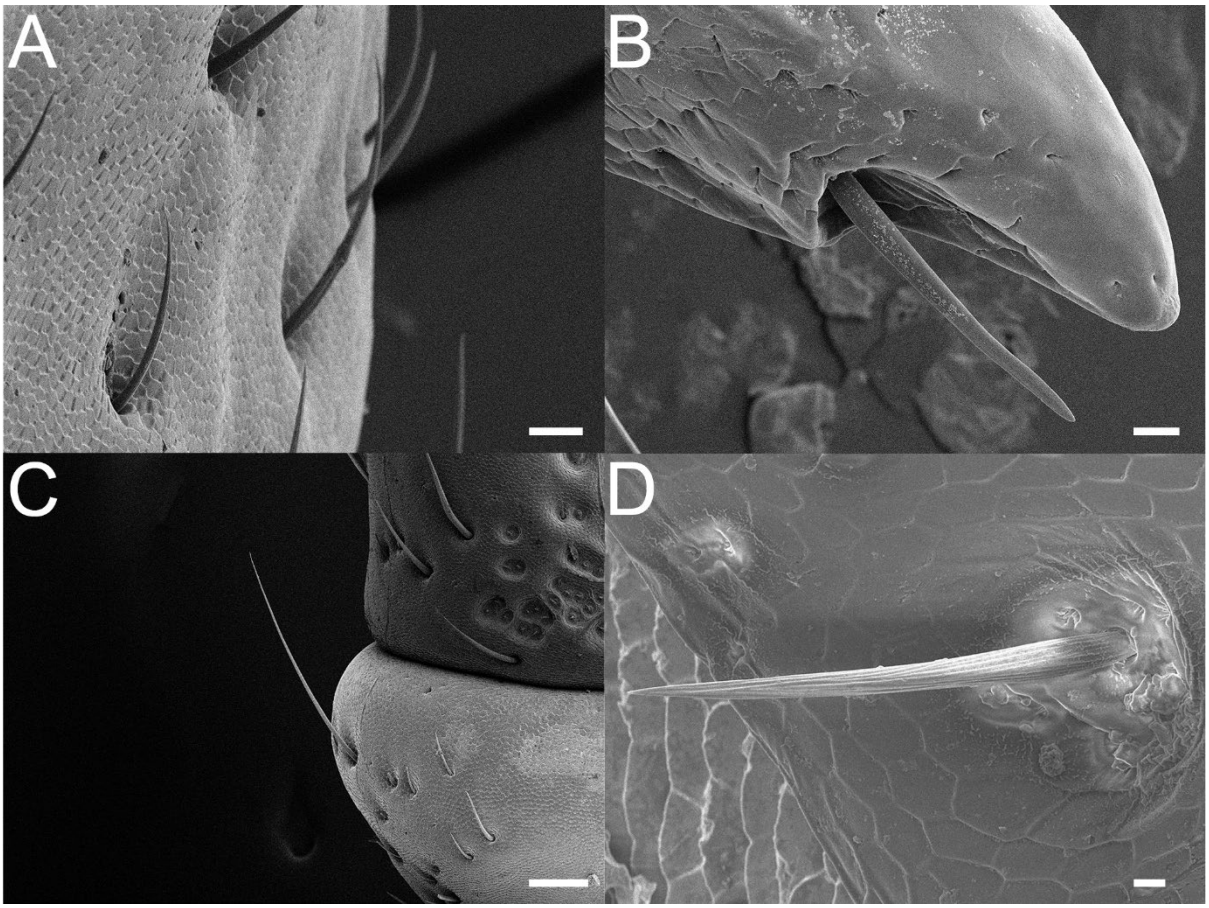

Figure 35. Aporous, stout sensilla chaetica present on the scape of a male (A). A single distal sensilla chaetica with deep insertion on the distal protrusion of flagellomere 5 (B). A long sensilla chaetica, present on the pedicel of a male (C). A stout sensilla chaetica on the flagellomere 5 of a female, showing characteristic glandular openings at the base (D). Scales: 20 µm (A), 10 µm (B), 100 µm (C), 5 µm (D).

Sensilla filiformia

The flagellomeres on the male antennae are densely covered in long, aporous, ciliated sensilla filiformia that are not present on the female antennae (Figure 36 A). I was only able to distinguish one type of sensilla filiformia present, characterised by their wavy, ciliated and flexible appearance, frequently entwining around one another (Figure 36 D). These structures give a slight pubescent appearance to the male antennae that is visible to the naked eye. Longitudinal striations run from the base of these sensilla up to around one third of the length of the structure. Several sensilla filiformia were broken at the base or apex which revealed a hollow structure through its length (Figure 36 C). The sensilla are situated in shallow insertions and surrounded by two to three corrugated openings (Figure 36 B).

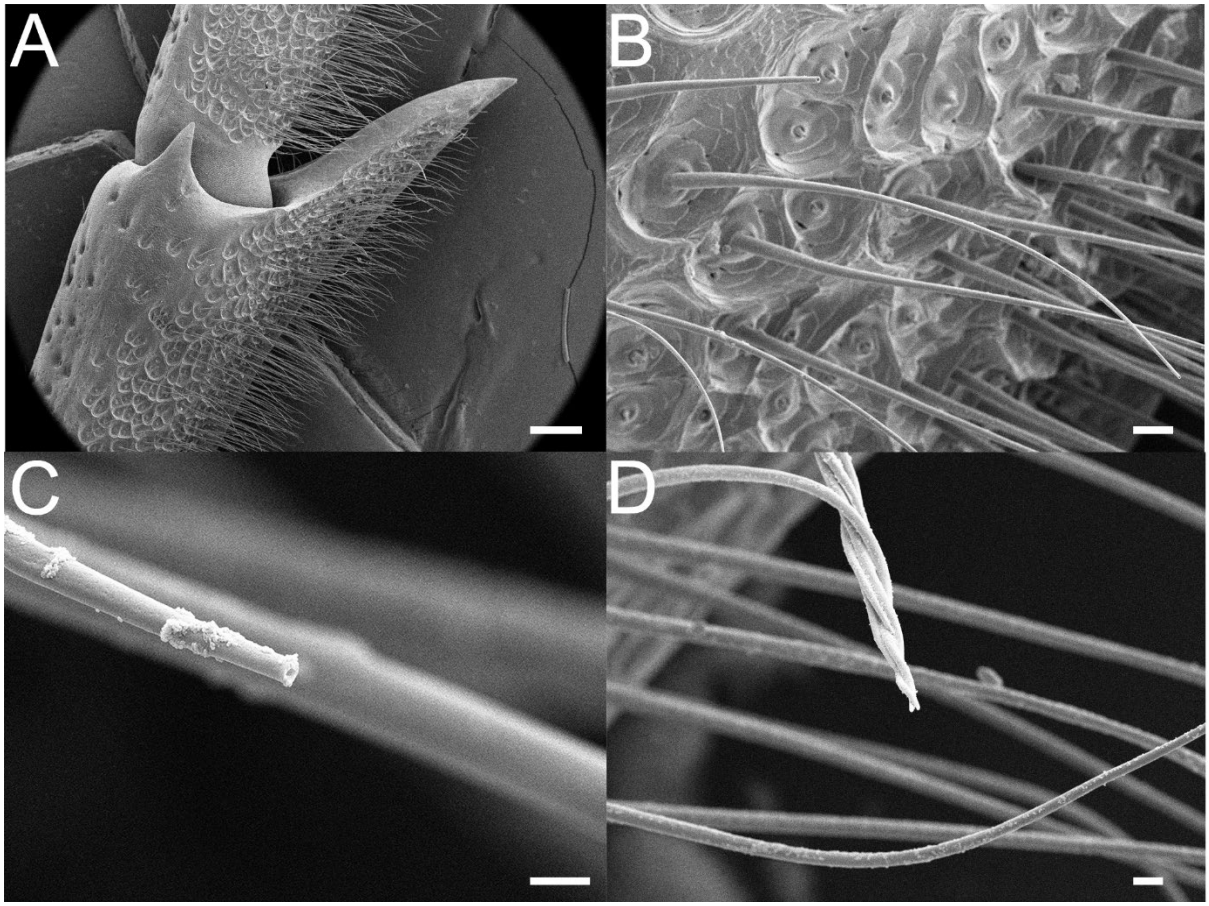

Figure 36. The ciliated appearance of the male antennae is derived from dense covering of sensilla filiformia shown here on flagellomere 2 (A). Sensilla filiformia emerge from a field of sensilla on the second flagellomere of a male. Glandular openings are visible surrounding the pits of various sensilla (B). The broken tip of a sensilla filiformia, exhibiting the hollow interior (C). A group of sensilla filiformia entwined around one another, demonstrating their flexible nature (D). Scales: 200 µm (A), 20 µm (B), 2 µm (C), 5 µm (D).

Sensilla Basiconica

Stout & Thin Sensilla Basiconica

Stout sensilla basiconica (Figure 37 A & C) were present on all flagellomeres in both sexes however were more prevalent on females than males. They are shorter than thin sensilla basiconica with a broader base and a blunt tip. The stout SB sits within a shallow socket on flagellomeres 1 – 8 and a much deeper socket on flagellomere 9. I found possible evidence of pores on the surface of the sensilla. I also found one bifurcated example of a short sensilla basiconica which is suspected to be a developmental issue.

Thin SB (A, B & D) were rarer than stout sensilla basiconica but present on all flagellomeres in both sexes. They are longer and narrower than stout sensilla basiconica and have a sharper tip and variable in size. They were usually found amongst groups of stout sensilla basiconica, and more frequently on the distal flagellomeres particularly flagellomere 9.

Grooved Peg Sensilla

Grooved peg sensilla (Figure 38) are a sub-type of sensilla basiconica that were infrequently found on flagellomeres 1 – 9 in both sexes but appeared to occur more in males. The sensilla are in broad, shallow, concave craters on the surface of the flagellomere. The wall and floor of the craters are wrinkled in texture. The base of the sensilla is broad, smooth, and circular in circumference, gradually tapering before narrowing distally to form deep, vertical grooves resembling digits.

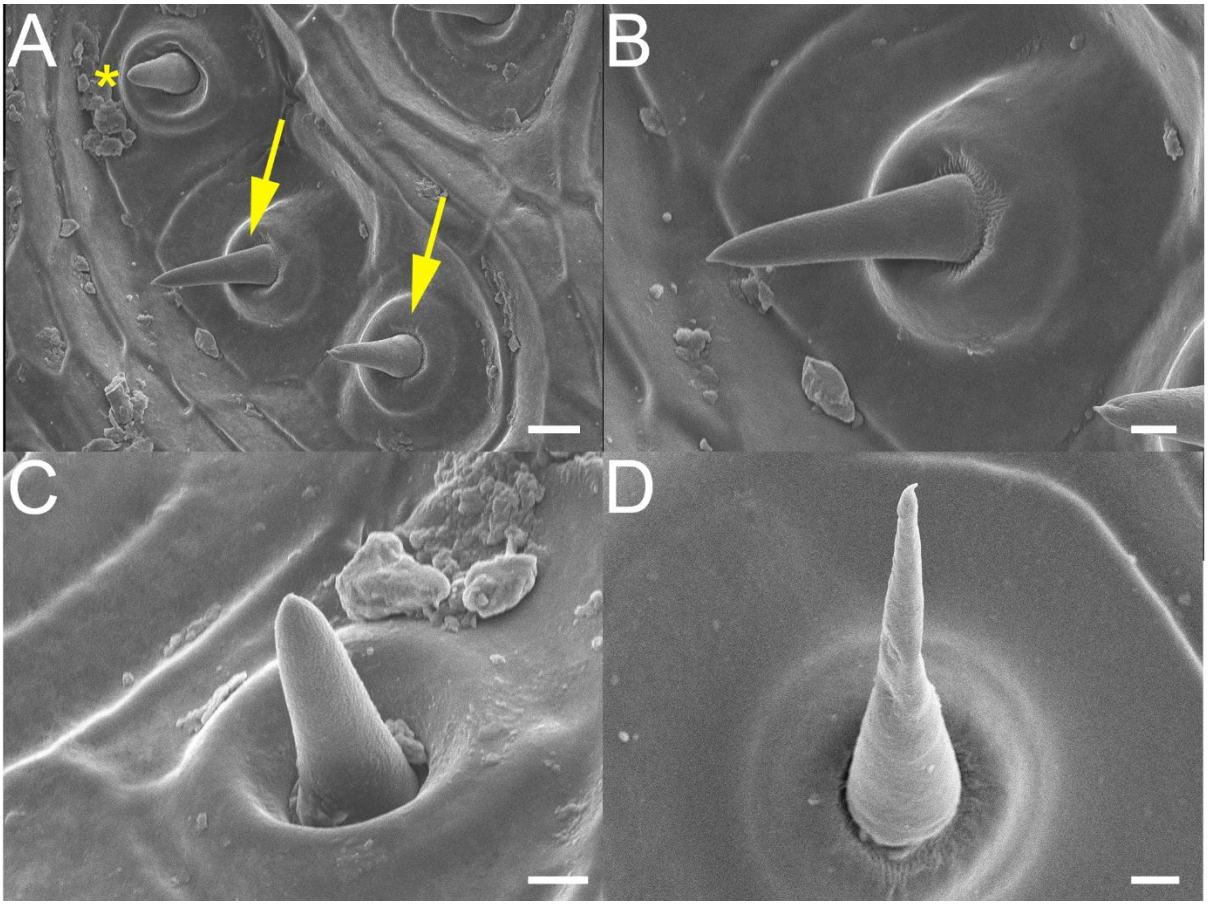

Figure 37. A single stout (asterisk) and two thin sensilla basiconica (arrows) within a depression on the pedicel of a female specimen (A). A thin sensilla basiconica on the female pedicel (B). A stout sensilla basiconica on the female pedicel (C). A thin sensilla basiconica on the male fourth flagellomere (D). Scales: 5 µm (A), 2 µm (B), 2 µm (C), 2 µm (D).

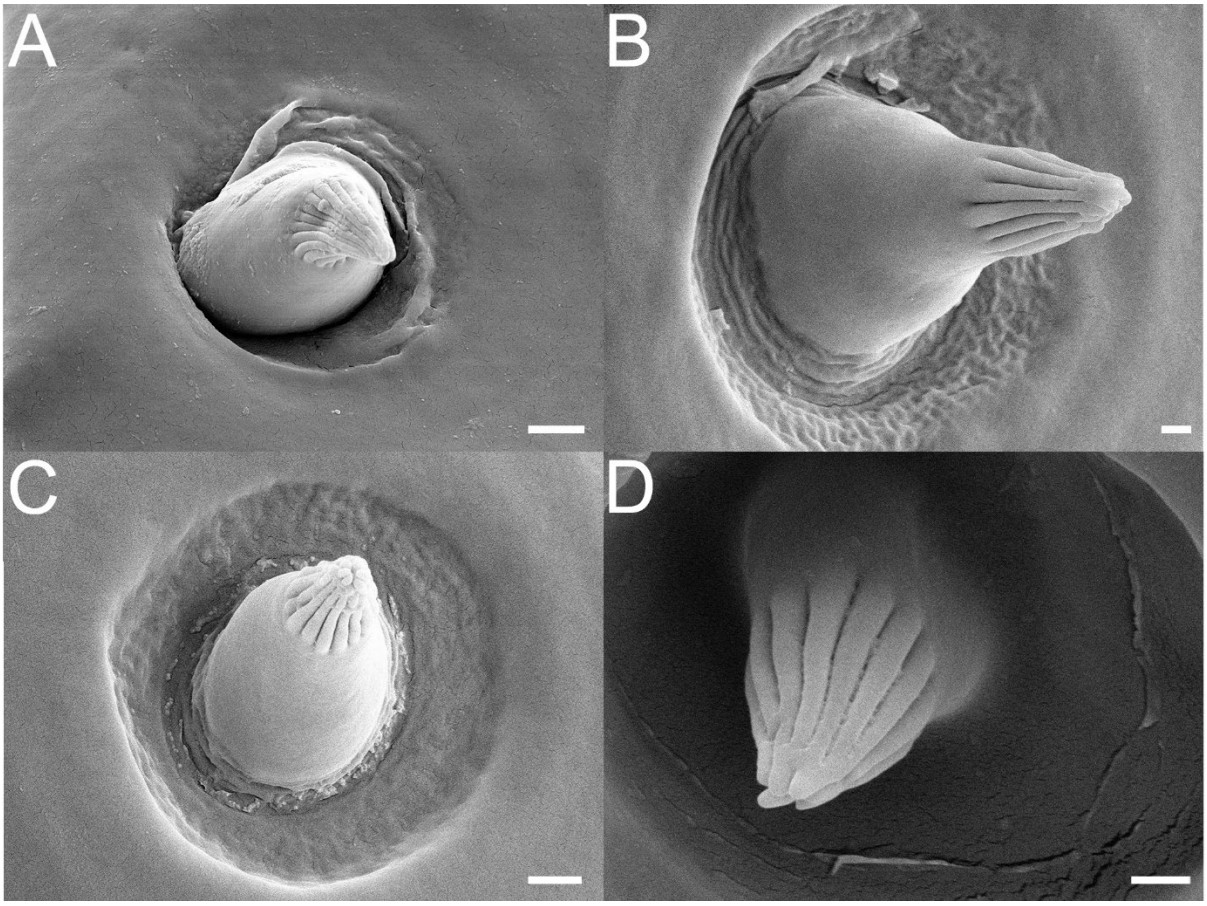

Figure 38. A grooved peg sensilla found on the pedicel of the female (A). A grooved peg sensilla found in a shallow depression on the second flagellomere of a male (B). A grooved peg sensilla found in a shallow depression on the second flagellomere of a male illustrating the variation in the base size of this sensilla (C). A grooved peg sensilla found in a moderate depression on the ninth flagellomere of a male illustrating the increase in depression depth on the terminal flagellum (D). Scales: 1 µm (A), 500 nm (B), 1 µm (C), 500 µm (D).

Chemosensory Hairs

Terminal Pore Hair & Wall Pore Hair

I found one candidate for a terminal pore hair (Figure 39 C & D) typically found on the edges of flagellomeres. They emerge from depressions in the cuticle and run parallel to the cuticle. The sensilla is robust in appearance with a broad base rising to a blunt tip. There appears to be a furrow at the tip of the sensilla which may imply a terminal pore.

I found one example of a possible wall pore hair sensilla (Figure 39 A & B) on the ninth flagellomere of a male. The sensilla is long, straight and hair like (but shorter than the surrounding sensilla filiformia) with smooth walls. The surface appears to have rows of pores suggesting an olfactory role.

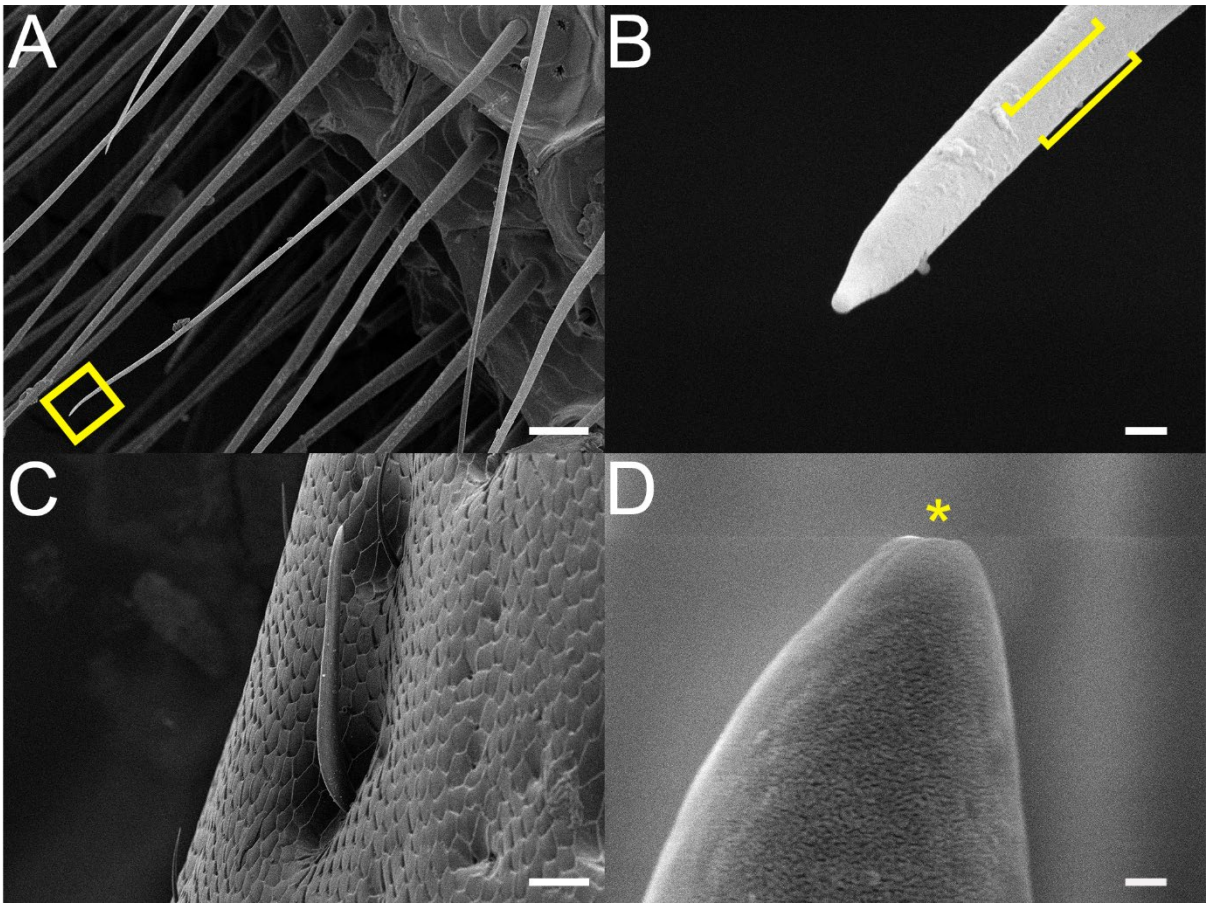

Figure 39. Proposed candidate for wall pore hair on the ninth flagellomere (box) (A), enlarged tip of wall pore hair, arrows showing lines of possible pores (B). A proposed candidate for a terminal pore hair(C) flagellomeres. A magnified view of the blunt tip of the sensillum in (C) showing pore at the apex (asterisk). Scales: 20 µm (A), 500 nm (B), 20 µm (C), 200 nm (D).

Sensilla Auricillica

Sensilla auricillica (Figure 40) were irregularly present on flagellomeres 1 – 9 for both male and female antennae. They emerge from a broad socket with the antennal cuticle forming a collar around the base. The general appearance is similar to those found by Zhang et al. (2015), a conical sensilla that has been longitudinally compressed with a concave depression from the base to the apex giving the appearance of an ear with the apex terminating in a papilla. There was some variation in shape and torsion, with some having broader, rounder bases than others. The surface is densely covered in pores, suggesting a chemosensory function.

Dome-shaped Organs and Sensilla Coeloconica

I did not find any evidence of dome-shaped organs or sensilla coeloconica present on the antennae of *P. reticularis*.

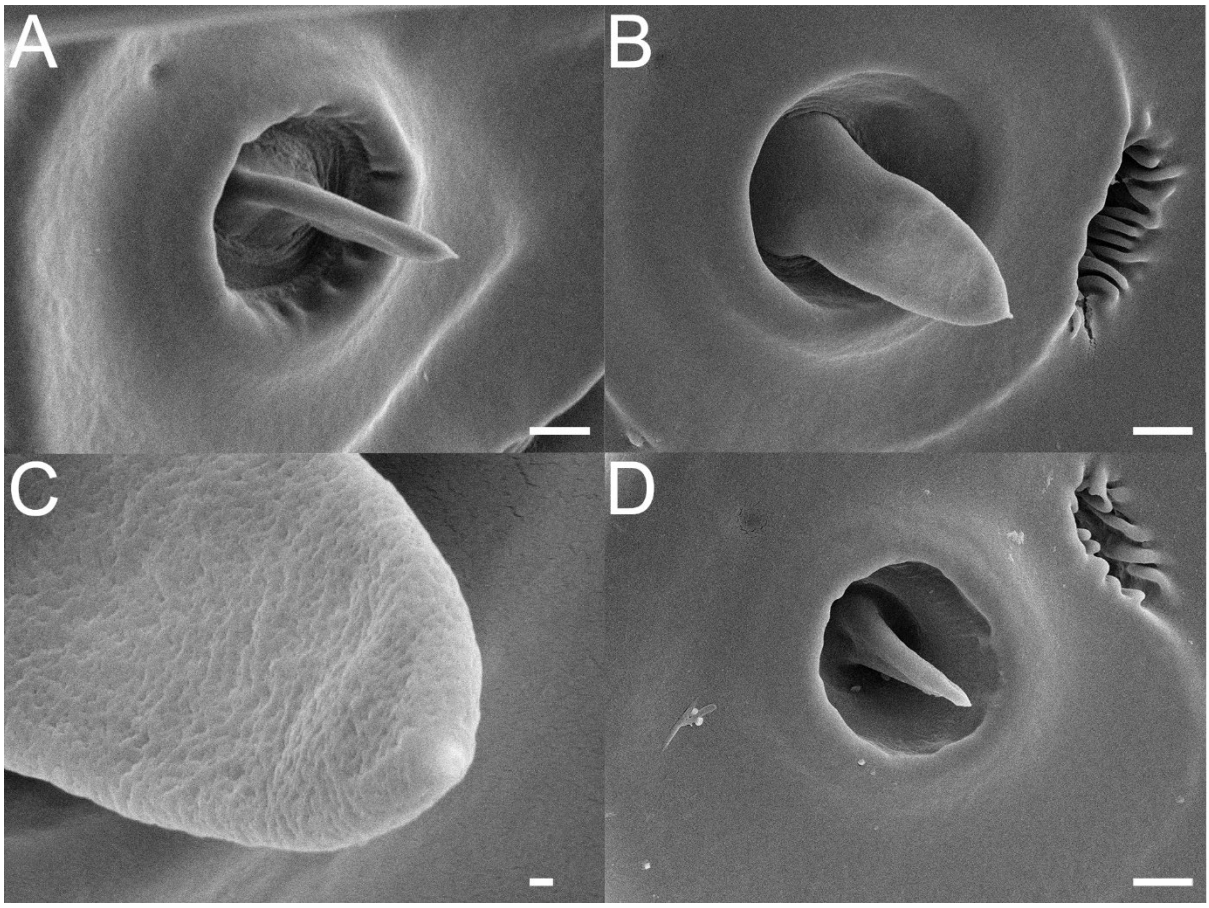

Figure 40. sensilla auricillica found on the first flagellomere (A) a sensilla auricillica on the sixth flagellomere demonstrating a broader, flatter shape (B), an enlarged view of B showing the numerous pores on the surface (C), sensilla auricillica on the sixth flagellomere with a shorter, narrower profile and torsion (D). Scales: 2 µm (A), 2 µm (B), 200 nm (C), 2 µm (D).

Sensilla on the Ninth Flagellomere

On the ninth flagellomere of males, I found several different sensilla types that emerge from inside deep pits surrounded by cuticular collars (Figure 41). Two of the sensilla appear to be similar to sensilla basiconica but inset into the cuticle. I tentatively label these as sensilla basiconica broad (Figure 41 B) and thin (Figure 41 C & D) however further examination using transmission electron microscopy is required.

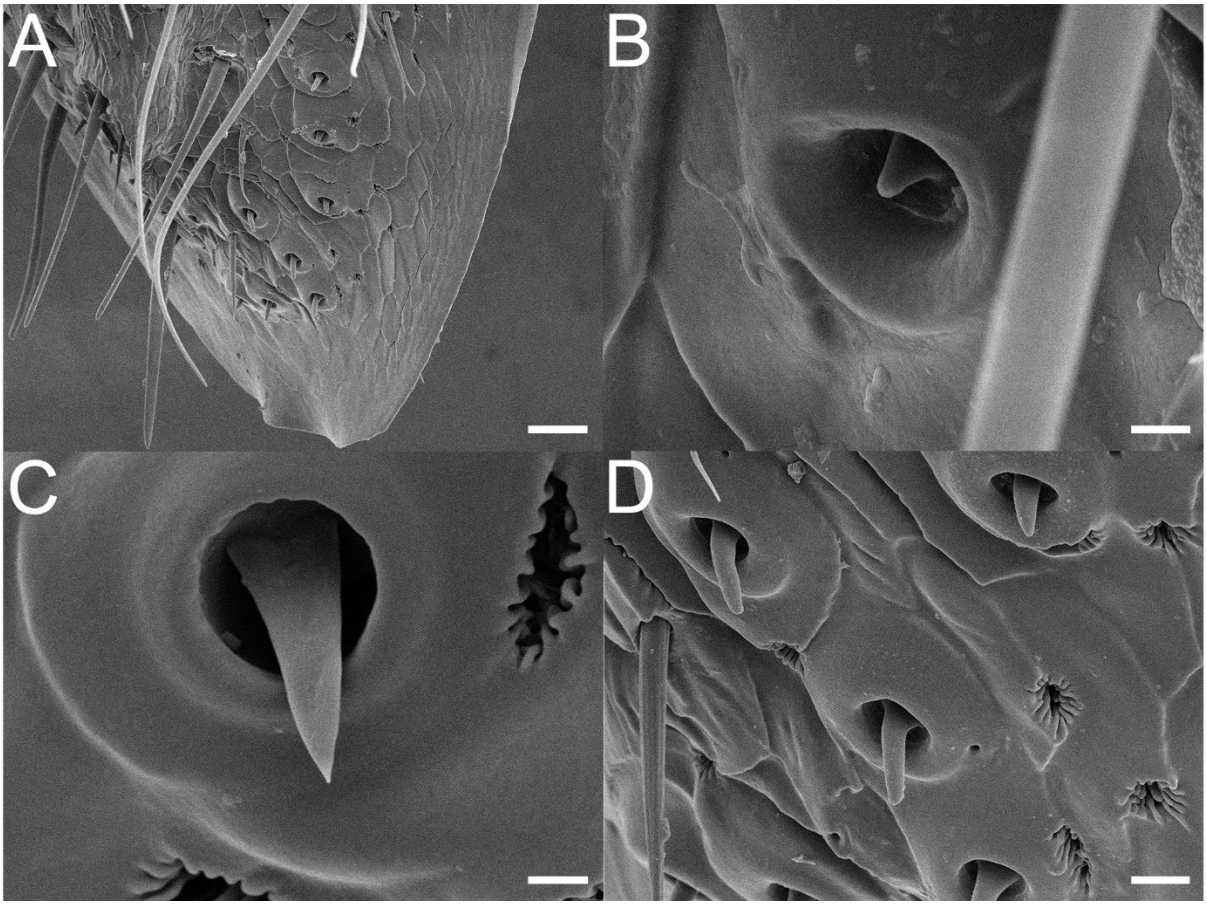

Figure 41. The tip of the ninth flagellomere of the male, showing the cuticular collars that enclose the sensilla (A), tentative broad sensilla basiconica sensilla (B), tentative thin sensilla basiconica (C),tentative thin sensilla basiconica (D). Scales: 20 µm (A), 2 µm (B), 2 µm (C), 5 µm (D).

Discussion

I described the sensilla ultrastructures present on the antennae of male and female *P. reticularis* providing the first ultrastructure investigation of an endemic prionine and first investigation of a prionine in the Meroscelisini tribe. The most abundant antennal sensilla present on the antennae of both sexes, sensilla basiconica, are associated with chemoreception further supporting the importance olfaction plays in this species perception of its environment (Edwards 1961; Pawson et al., 2020).

I found evidence of sexual dimorphism in the antennal sensilla, with several sensilla types only found on the male antennae. The most abundant ultrastructure, present only in males, were sensilla filiformia suggesting that these sensilla are important in male behaviour. Also present only in males, but in low numbers, were sensilla auricillica, wall pore hairs and distal sensilla chaetica. Grooved peg sensilla were common on male antennae but rarely seem on females. Sensilla auricillica showed a multiporous surface and are proposed to be involved in the reception of female produced sex pheromones. The sensilla chaetica of the female antennae featured much deeper, more pronounced striae than male sensilla chaetica. Whilst the functional significance is unknown, the sensilla chaetica may be related to the female assessing physical characteristics of suitable oviposition sites, as I have observed female *P. reticularis* using their antennae (and ovipositors) to interact with substrates prior to laying eggs.

There are only two studies investigating the antennal ultrastructures of the Prioninae, both showing highly unusual antennal ultrastructures when compared to other cerambycid subfamilies. The antennae of both sexes of *P. reticularis* were covered in the sensory pits reported in the other two prionines studied (Dvořáček et al., 2020; Haddad et al., 2023) and contained a sensillum similar in structure to the sensilla described variously as coeloconica (Dvořáček et al., 2020) and unidentified prionine sensilla (Haddad et al., 2023). I agree that there are differences to the sensilla basiconica found in other cerambycid subfamilies (Haddad et al., 2023) but suggest

that these are likely still sensilla basiconica with two subtypes identified - thin and broad. The sensilla in the sensory pits were typically surrounded by two to three corrugated, semi-circular glandular openings that are seen in other prionines, although the function of these is unknown (Dvořáček et al., 2020; Haddad et al., 2023). It has been suggested that sensilla chaetica may be absent in the Prioninae (Dvořáček et al., 2020) however I found examples of several sensilla chaetica subtypes on both male and female antennae.

Perhaps the most striking difference to other prionines is the dense pubescence of sensilla filiformia present on only the male antennae. This sexual dimorphism indicates that there is a role in reproductive behaviour, perhaps in mate searching or combat. Sensilla filiformia have been reported present in both sexes of the cerambycinine, *Phoracantha recurva* and are hypothesised to be mechanosensory receptors possibly related to wind detection (Faucheux, 2011). In this species, both sexes scramble to reach an ephemeral resource, stressed Eucalyptus spp., which may indicate that whatever the cue these sensilla receive is equally important to both sexes.

Electrophysiological data in Orthoptera supports a mechanoreceptive role for Sensilla filiformia, with detection of slight vibrations being of importance for predator avoidance (Gnatzy & Tautz, 1980; Steinmann & Casas, 2017). I can only speculate as to why sensilla filiformia are present in males. Whilst both males and females of *P. reticularis* are mobile, males likely spend more of their time in flight searching for females and could be perceived to need to process information relating to air movement (such as orientating to face the prevalent air current) or have an increased risk of encountering predators (such as *Ninox novaeseelandiae*, Edwards, 1961c). Other possible roles for mechanoreception in *P. reticularis* is in male-male combat and mate guarding. These sensilla may provide information relevant to fights, such as opponent size. Males engaged in mate guarding post-copulation outstretched their antennae and attempted to orientate themselves towards individuals that make contact with the antennae (pers. obs.). These sensilla

would be excellent candidates for electroantennography and transmission electron microscopy work in the future combined with behavioural experiments.

Sensilla are fundamental to how insects perceive their surroundings and understanding the sensilla present on antennae can allow us to make inferences into the life history of a species and its sensory ecology. Sexual dimorphism in the types, distribution, and morphology of antennal sensilla suggest that sexual selection is acting on the antennae, and in the case of *P. reticularis*, provides evidence for the importance of chemical cues for males in mate-searching. I also suggest that future research should explore the role of sensilla filiformia in males which may provide further insight into the mating system of *P. reticularis*.

Chapter 7 General Discussion

The study of insects as food is a multidisciplinary field, requiring research and researchers that can bring together different areas of knowledge to tackle the challenges that are found in this area. The aims of this thesis were twofold, to explore the social licence and cultural history of insects as food in Aotearoa New Zealand (Chapters 2 & 3), and to explore the biology of a well-known edible insect, the huhu beetle *Prionoplus reticularis* (Chapters 4, 5 & 6).

The success of an industry is contingent on its acceptability to the society in which it operates, and, in this thesis, I found Aotearoa to have a rich history of past insect use (Chapter 2) and a social licence for the nascent industry developing around edible insects. Survey participants' responses were nuanced showing that people were generally very open to eating insects and had a reasonable degree of knowledge and experience of edible insects in Aotearoa. However, this contrasted with insect ingredients being described more negatively than other more "conventional" ingredients (Chapter 3).

In Chapter 2, I collated a comprehensive catalogue of insects that have been used as food in traditional and more recent settings, which found considerably more edible insect species in Aotearoa than has previously been reported in the literature. Whilst there are several well-known native edible insect species documented, it was the off-hand references in the grey literature to lesser-known native insects used by Māori that hints at the knowledge that may have been lost or hidden following European settlement. Future efforts should be made to ensure mātauranga is not lost and this would benefit from kaupapa Māori led research. Recent work by O'Connor et al. (2023) is an excellent starting point for this, and demonstrated that there is (1) mātauranga regarding insect species and their uses that is not recorded in the literature and, (2) interest in greater access to traditional food types and the customs surrounding them. WAI262 will play an

important role in shaping the edible insect sector and provides the opportunity to look at different ways of making insects accessible, for instance through the lens of food sovereignty.

We may benefit from the learnings of other countries' efforts to preserve traditional knowledge of edible insects. In Japan, a successful initiative connected older generations with school children to pass on their knowledge of insect use (Nonaka & Yanagihara, 2020). Chapter 2 also included a surprisingly large number of non-native insects used in contemporary ways, indicative of the diverse cultural make-up of Aotearoa and its connections to the rest of the world. One particularly fascinating and somewhat surreal highlight was discovering the export of wasp nests from Aotearoa to Japan in the 1990's. This no longer appears to occur, although at a recent conference I was approached by researchers from a Japanese university who indicated there is still a demand for this export. This raises the prospect of harvesting invasive species, which are plentiful in Aotearoa, for export overseas. This would dovetail nicely into government initiatives such as Predator Free 2050, although whether it is wise to encourage a sunset industry is debatable and may lead to an unwanted outcome to protect wasps to ensure livelihoods remain intact.

Chapter 2 also addresses a complicated legislative framework which makes it difficult for start-ups to produce food-grade products. I found a concerning trend indicating insect businesses struggle to be viable in Aotearoa. Over a ten-year period, out of the eight companies I found trading, only two appear to still be active, suggesting challenges to achieving commercial success in this market. Currently, the easiest route to market would be through wild harvesting of insects which carries risks of exploitation if not managed carefully. The viability of the industry is likely to be related to a variety of reasons, but one obvious area for improvement are regulations governing the production of insects for human consumption. Streamlining legislation, whilst maintaining food safety standards, would allow greater innovation in this space whilst providing consumer confidence in the safety of products. Whilst not explored in this thesis, by comparison

Australia has a greater number of insects as food and feed companies and an industry body lobbying for the support of the industry. It would be prudent to learn from the successes of our neighbour, with which we already share some common legislature, as we develop our own industry. In the interim, start-ups could diversify their cashflow by producing insects for pet & livestock whilst they build capacity to sell to humans, allowing them access to a less regulated market reducing compliance costs whilst also honing mass production techniques necessary to make a product competitive.

Chapter 2 also found that companies selling edible insect products have relied heavily on sustainability and nutritional claims in their marketing, however there is little validation of these claims such as a life cycle analysis of their product, instead relying on research produced in other countries. It is well established (Halloran et al., 2016) that the sustainability of insects is very context dependent on the specific production techniques and species used and caution should be used when presenting generalised statements on the environmental benefits of insects. This is an area where researchers can play a role in supporting businesses, by investigating sustainability claims made in the context of Aotearoa. This will help increase consumer trust in the sector and potentially provide a competitive edge to products from this country compared to products imported from overseas.

The sustainability claims we saw in Chapter 2 appear to have permeated into the public discourse for insects as food. In Chapter 3, I found that words associated with cricket flour clearly matched the marketing techniques employed by companies e.g., as being environmentally friendly ("green", "environmental", and "alternative") and nutritious ("high protein", "gluten free"). Cricket flour was the insect-based ingredient with the highest positive sentiment score, ranking higher than conventional meat products. This presents tangential evidence that marketing of cricket flour has been quite successful and is a strategy that could be replicated with other insect products, such as "sky prawns". Huhu grubs and grasshoppers had a neutral

sentiment rating similar to lamb, shrimp, beef and tofu and words associated with them related to their perceived flavours and textures, or to cultural references associated with these items. As the overall sentiment is neutral and they are largely associated with food, these species may have a low barrier to entry as a commercial food product.

There was a high degree of knowledge amongst participants, with 498 of 620 participants able to name one or more species of insect used as food, and 367 participants having eaten an insect in the past. I also found several attributes that predict the likelihood of eating insects in the future, with likelihood increasing with prior experience, existing knowledge, and a low food neophobia score. This suggests strategies to increase the acceptance of insects as food should focus on education, tasting experiences and incorporating into familiar dishes and by reducing the "insect-ness" of the product (e.g., a powder or flour).

In Chapter 2 I suggested that the easiest native insects to rear in NZ, these are *Locusta migratoria* and *Teleogryllus commodus* both of which are being farmed. *P. reticularis* could be another candidate albeit a challenging one due to it long lifecycle, but there have been strides in reducing to under one year in laboratory conditions, this, combined with the well-known status as a food in NZ society (Chapter 3), would make it the next most promising candidate.

A key finding in Chapter 3 was the tension between people's stated openness to eating insects and the predominantly negative sentiment of words used to describe most insect products. This may indicate an unconscious bias against some insect ingredients and could be indicative of a value-action gap amongst participants, i.e., expressing a willingness to eat insects, whilst this doesn't translate into the action of eating insects. This may also explain the limited success of businesses discovered in Chapter 2 and is an area that warrants further research attention.

In Chapter 2, I found several references to the flavour of *P. reticularis* varying based on the timber they are found in. This suggested that (1) biotic and abiotic factors influence the metabolic

compounds within larvae, analogous to the influence of *terroir* on the metabolomic profile of wine and, (2) this quality might allow us to assess the provenance of an edible insect, i.e., fingerprint it to a particular location. Using GC-MS, I found that the metabolomic profile of *P. reticularis* varied between locations and that a random forest classification model trained on this data was reasonably successful at determining the location of collection for a huhu grub. It would be worthwhile, however, to compare different models (e.g., SVM, SIMCA) to attempt to improve classification accuracy.

The ability to distinguish the provenance of an edible insect product provides several potential benefits. With the increase of trade agreements containing regional protections, it allows the detection of food fraud (e.g., a product being labelled as from a high value location but is actually from somewhere with a lower value). It also allows legislators or stakeholders to place restrictions, and detect infringement of those restrictions, on the sale of insects from certain locations, for instance due to concerns over sustainability.

However, it must be noted that I only explored spatial variation, and to validate this method I would need to further explore the effects of temporal variation and dietary variation on metabolites. There is already indirect evidence of diet influencing the metabolite profile of *P. reticularis* (Chapter 2) and future research could compare the effects of different timber on the profile of the larvae. Given the mātauranga associated with the impact of timber on flavour, future research would benefit from collaboration with Māori to elucidate the physiological processes behind this. This research may also benefit insect farmers, given that the flavour of farmed insects is held to be inferior to wild-harvested insects (Evans et al., 2017, p. 179), a phenomenon likely driven by differences in diet.

The time of year will influence the metabolite profile as physiological processes such as cold tolerance and diapause entail changes to metabolism. I saw differences in two sugar compounds, mannose and trehalose, which are known to be important for freeze tolerance. They were the

most abundant sugars identified in the larvae and varied along a latitudinal gradient, although in the opposite direction to what was expected. I have not been able to identify why this might be. I would expect these compounds to be more abundant in the cooler South and less abundant in the warmer North. It is likely that a controlled laboratory experiment would be required to tease apart what is happening here.

One element, I was unable to control for was the gut microbiome which is likely to interact with the metabolome and would be a fruitful area to investigate in the future. The gut microbiome is known to vary across the geographical locations of *Costelytra zealandica* and this is likely the case in *P. reticularis* (H. Zhang & Jackson, 2008). The gut microbiome of *P. reticularis* has been investigated in the past, but just from one location (Reid et al., 2011; Viswam et al., 2019). I found the five-carbon sugar, arabinose, to be weakly correlated with latitude, and in insects this sugar is utilised by gut bacteria which raises the possibility that the metabolomic profile is partially influenced by gut contents.

In Chapter 4, I also found proline and alanine to be the most abundant amino acids present, and these compounds are known to be important metabolites for powering flight in adult beetles. In Chapter 5, I investigated traits associated with flight and found that heavier males flew faster for longer. It would be interesting to investigate if proline and alanine abundance correlates disproportionately with increasing larval mass. Larger larvae, which in turn become larger adults, may have comparatively more of these amino acids than their smaller conspecifics providing better flight performance as an adult. If this is the case, then investigating the abiotic and biotic factors that govern larval size, could also assist in manipulating desired nutritional quality and weight. From previous work I have done on *Hermetia illucens* (Diptera: Stratiomyidae), black soldier fly, larval density and diet can greatly influence larval size (Birrell, 2018) and this is likely to also be the case in *P. reticularis*.

The majority of research on *P. reticularis* has been through the lens of its role as a forestry pest (Chapter 2). Other than the seminal work of Edwards (1956, 1959, 1961b, 1961c, 1961d, 1961a, 1965), little is known of many aspects of the life history of this species. However, understanding the behaviour of an insect species is crucial to successful rearing in a captive system. I sought to address this knowledge gap by investigating the mating system of *P. reticularis* (Chapter 5).

Chapter 5 highlighted how allometric patterns are a powerful tool for understanding the interplay of different selective pressures on an animal. The mating system of *P. reticularis* is characterised by scramble competition where males attempt to find and mate with as many females as possible. This puts selective pressure on male traits that improve their ability to acquire mates. Locomotion and olfaction are critical for acquiring mates and this likely drives the sexual dimorphism I found, with males having longer antennae than females, and females being larger in all other respects. I hypothesised there would be positive allometry in male traits (e.g., antennae and wing size) with larger males investing disproportionately in these traits. This was not the case however, with most male traits exhibiting negative allometry. I attributed this to stabilising selection, with biomechanical constraints likely restricting excessive investment in traits.

In Chapter 5, I also designed and built six flight mills using a 3-D printer and low cost, everyday laboratory consumables and electronic components. This presents a novel way to collect data on flying species on a budget, or with limited access to laboratory space. I also developed a novel way of attaching beetles to the flight mill. Typically, low temperature hot glue is used, however this still appeared to cause discomfort to the beetle, potentially impacting their behaviour, and requires you to do this multiple times if the beetle needs to be reattached. To avoid this, I used a quick drying super glue to attach half of a staple to the pronotum of the beetle. This allows for the easy attachment of the beetle to a neodymium magnet which can then be removed and reattached as required.

In Chapter six, I further investigated the sexual dimorphism in antennae size observed in Chapter 5. Using scanning electron microscopy, I observed several sensilla that are only present on male antennae which have roles in chemoreception and mechanoreception in other taxa. This again emphasises the importance of detecting females using sensory cues for males of this species. The sexual dimorphism was particularly striking in the dense sensilla filiformia present only on the male antennae. These have not been reported on any other prionine beetle (Haddad et al., 2023) and in other insect orders have been attributed to mechanoreception (Faucheux, 2011). In hawk moths, the speed of flight and pectinate antennal morphology are optimised to slow the passage of air and ensure the capture of odour molecules (Jaffar-Bandjee et al., 2020). I hypothesis that the dense sensilla filiformia on the male antennae of *P. reticularis* perform a similar role, acting as a mechanoreceptor to provide dynamic information on flight whilst also slowing air flow over the antennal surface to ensure pheromone molecules are captured by chemosensory sensilla.

I attempted a variety of different experiments to quantify mating success, strength of olfactory responses and captive breeding in this species, however these were unsuccessful due to a variety of reasons such as the beetles not exhibiting normal behaviour in a confined environment, the relatively short breeding window in which adults are active, and a pandemic and lockdowns corresponding with field seasons. Whilst disappointing at the time, these challenges did drive innovation (particularly in the construction of the flight mills) and highlighted some of the challenges of trying to rear *P. reticularis* in a closed system for the production of insect protein. With the benefit of more field seasons, I suspect it would be possible to overcome these issues and eventually devise a rearing protocol. However, if commercialisation was the goal, a simpler solution might be to pursue semi-domestication similar to the production of palm weevils in the tropics. In this scenario, a solution might be to prepare an attractive ovipositioning medium, perhaps with kairomones attractive to female *P. reticularis*, such as α- & β- pinene lures developed

for monitoring forestry pests (Brockerhoff et al., 2006). Following this, it might be possible to allow females in the wild to inoculate this media. If so, this would be a straightforward way to proceed and could be done in tandem with further research into captive breeding.

Concluding remarks

In summary, there are challenges faced by the edible insect sector but, with proper support from academia and government, there is great potential for growing the industry further in Aotearoa. Culturally, there is an acceptance and existing awareness that insects can be food and this may reduce the barrier to uptake that is seen in other Western nations. The rich cultural history, and diverse array of edible insect species available, offers an opportunity to showcase edible insect species whilst preserving and celebrating traditional food practices. A sustainable source of domestically produced protein could provide increased food security and sovereignty to communities in Aotearoa, whilst diversifying our agricultural sector. Reaching high densities in our pine plantations, the huhu grub is perfectly placed to champion this movement. I hope this thesis can act as a foundation for future work to build upon whilst also adding to our understanding of a charismatic native insect, *Prionoplus reticularis*.

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