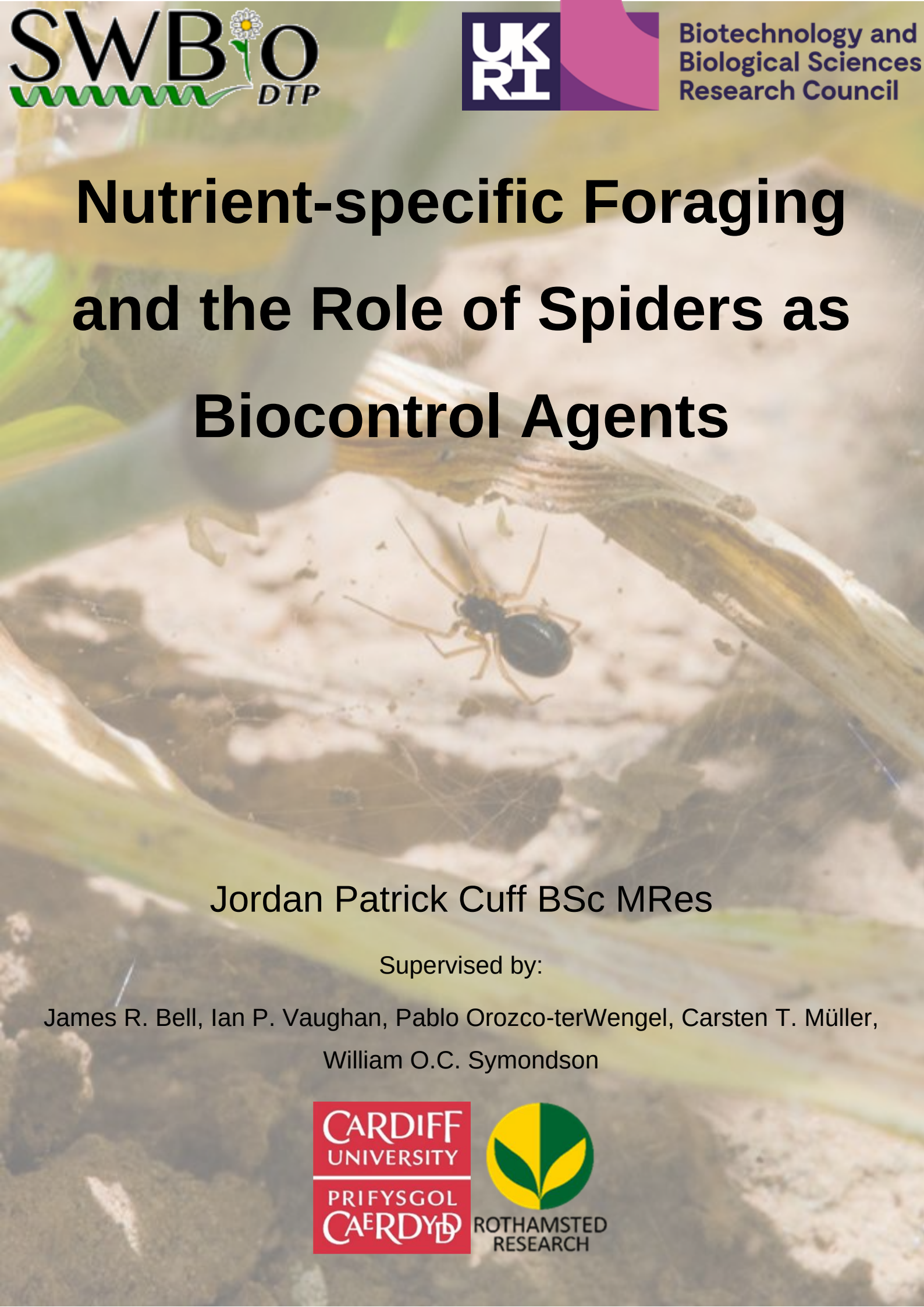
Nutrient-specific Foraging and the Role of Spiders as Biocontrol Agents



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Acknowledgements

I hold a great sense of pride in that which I have achieved over the last 1508 days. I do, however, owe these accomplishments in their entirety to the incredible family, friends, colleagues and wider support network who have carried me through these tumultuous times. Just as Samwise Gamgee said to Frodo on the ascent of Mount Doom in J.R.R. Tolkien's *Lord of the Rings*, this unfathomably encouraging community has often said, sometimes without saying anything at all, "I can't carry it for you, but I can carry you". Without lightening that load, I honestly do not believe that I would have completed this thesis in any form, let alone in the form that I proudly present herein.

I have been warned that eight pages of acknowledgements is unorthodox, unwieldy and frankly ridiculous (paradoxically exacerbated by this paragraph justifying the length), but despite my best efforts, I could not comfortably present this thesis whilst acknowledging those without which it would not have been possible any more concisely. The work herein, whilst the product of field, lab, computational and desk work conducted largely in solitude, is a product not of one individual's achievements but of an expansive community. This thesis is also the embodiment of my own personal and scientific growth over four-and-a-bit years, for which I must acknowledge a great many people. For those uninspired to read the whole thing, that you are reading this at all (whenever in the future that may be) makes you a part of this journey, so thank you (you can skip ahead now).

Foremost, I will never sufficiently express my gratitude to my incredible, inspirational and enduringly encouraging wife, Bhavana. Whilst this thesis has consumed the majority of my time for the last four-and-a-bit years, my greatest and proudest achievement of this time was my marriage to the love of my life on 19th October 2020. Through every trial and tribulation, Bhavana's support has granted me the perseverance and tenacity to succeed even when the temptation to walk away altogether was otherwise overwhelming. From cheering me on through every triumph and disaster, to almost constantly offering me fruit (usually plums) whilst working from home, every action, however great or small, was my greatest source of motivation, knowing that I have our whole life together to look forward to.

Similarly, the consistent and profound support of my family, particularly my parents and brother, provided me the serenity and security to complete the PhD in the knowledge that, regardless of the outcome, all would ultimately be well. Tenacity has been my greatest strength throughout these years and is inherited from the two most perseverant and diligent people I know: my parents, from whom I also learned never to compromise on compassion and community, regardless of circumstance. My brother, Matthew, through counsel, nostalgia and humour, has lifted my spirits high on some of their lowest days. Despite the obscurity of

leaving a comfortable occupation to pursue several years of tireless and poorly-paid work ultimately to answer the question “what do spiders eat?”, my family have ceaselessly supported my strange pursuits, encouraged my every success and consoled my repeated failures. Failure is the inevitable company of the scientist and must be befriended to find success, but without the support of my family I doubt entirely that it would have been company I could have kept for long.

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शादी से मेरा परिवार और बड़ा हो गाया है। अब मैं गुप्ता भी हूँ और मैं ऐसे ज़्यादा ख़ुश नहीं हो सकता हूँ। मैं अन्कल आन्टी स्वाति शैहन और अर्पित को बहुत बहुत बहुत धन्यवाद बोलना चाता हूँ। आपका सहारा मेरा लिये बहुत महत्व रखता है और मैं इंतिज़ार नहीं कर सकता हूँ उस दिन के लिये जब हम अगली बार मिलैनगे।

Unfathomable gratitude goes to my supervisors, who probably had no idea what they were signing up for when they decided to take a chance on me. I am incredibly appreciative of their guidance, humour, wisdom and friendship, and for entertaining my many tangential and oftentimes tenuously linked ideas. I will not falsely claim for even a moment that I was, at the very least on paper, the ideal candidate for a PhD when I applied. I would argue that I learned to be a researcher, an entomologist and a scientist after the point at which I was selected for this PhD, so I am forever grateful for the “risk” taken on me and I hope that my supervisors would all consider that it payed off!

My supervisors greatly enhanced my passion for arachnology, agricultural entomology, molecular ecology, ecological chemistry and wider science, and guided me through many of the most turbulent parts of my PhD with calm and collected insight. To my primary supervisor, Bill Symondson, I am forever indebted for his astute scientific guidance, mentorship and, above all, friendship. Bill would consistently warn me of straying too far from my project, but ultimately gave me the freedom to explore ideas away from my main work, thus allowing me to grow through my own successes and failures. It is through these explorations and the many calculated conversations that I shared with Bill that I believe I truly began to grasp what it means to be a scientist.

I owe a great deal of my progress to my external Rothamsted Research supervisor, James Bell, who offered me consistent guidance, encouragement, mentorship and advice, particularly enhancing my arachnological passion and my broader career development (he also lent me his incredible spider collection which I must remember to return as soon as COVID allows it). Ian Vaughan's cool and collected approach to science, not to mention his statistical wizardry, was invaluable in assuring my confidence in an area of my project that I surely would otherwise have felt entirely overwhelmed by. Carsten Müller's ability to make anything that would otherwise be dry and humourless a fun rollercoaster through ecological chemistry is greatly appreciated, especially during all of the pitfalls associated with developing a novel protocol. Pablo Orozco-terWengel's consistent reassurance, particularly in the first year when the road ahead seemed particularly daunting, and his continuous motivation for me to push harder is to thank for some of the great directions the project (and various tangents) ultimately went.

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The research contained within this thesis would have been impossible without Mr Robert Reader and his family, who kindly allowed us to use his incredible land for our work. Mr Reader has been working with Bill and his research group for many years before my arrival, and his flexibility to best accommodate the plethora of strange research projects and wacky ideas that come to his land via me and those who came before me is truly touching.

I must mention before going further my three-month internship at National Museum Wales with Ben Rowson and the rest of the Mollusca gang. This experience, particularly under the mentorship of Ben, substantially expanded my understanding of scientific collections, the importance of keeping them and my enjoyment of natural history more broadly. I would like to sincerely thank the Mollusca gang for this excellent experience, and I hope that Ben and I can one day reunite to take down the Sith.

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I am incredibly thankful for the various other researchers of the Organisms and Environment division, who consistently supported me and my hare-brained schemes. Lynne Boddy and the fungal ecology alumni for adopting me as an MRes student but continuing to be a source of guidance, wisdom and humour ever since. Rhys Jones for inexplicably believing in me, pushing me to do incredible things and providing me with fantastic opportunities. Rob Thomas for whisking me away to the magical wonderland that is Nant Bran, being the only person whom I've watched cook sausages in the snow and generally being a fantastic human being, whose compassion inspires so many. Jo Cable for mentorship, advice and encouragement, particularly in the quiet hours of the day. Pete Kille for supporting my strange ideas, filling me with confidence for the future through continuous mentorship and for encouraging my curiosity through super cool research.

A great deal of gratitude goes also to my PhD assessor, Wynand Van Der Goes Van Naters, who would regularly challenge my lateral thinking and broader knowledge, ultimately helping me to see the broader context of my work. I would like to thank Esh Mahenthiralingam for regular use of his plate reader and for entertaining the idea of beetle hunting with me (which I still hope we can do one day, pandemics and other major global events permitting). Thanks must also go to Lyndon Tuck for looking after my precious little aphids in all seasons.

The Genomics Hub was a regular haunt of mine at the apex of my molecular work, but I will openly admit that I went there possibly more than was warranted to enjoy the infectiously optimistic and endlessly fun atmosphere created by the likes of Angela, Gina and Trudy. What I predicted to be the most stressful aspect of the work was nothing but enjoyable, for which I am eternally grateful. Similarly, the finance office (or 'money wizards'), particularly Sam, provided me with endless cheer, even when I caused hours of work due to a poor understanding of how to appropriately order things (oops). I'm still mortified that I was spotted dressed as a sequinny bee in Green Man Festival's science engagement zone. Again, the team at stores would always lift my spirits (and my various heavy orders of various acids) on a dull day. In fact, all of the professional services staff in Cardiff University contributed to my unique PhD experience (as well as the running of literally everything necessary for it), for which I am incredibly thankful. This also extends to my second institution, Rothamsted Research. Despite spending less than two working weeks there in total, Donna Fellowes has consistently made my Rothamsted PhD experience even more enjoyable and left me looking forward to every visit to Harpenden.

Finally among the staff, mentors and collaborators that I have had the pleasure of interacting with throughout my time in Cardiff, I would like to expound my interminable gratitude to Hefin Jones who has continually supported me as a scientist, an entomologist and a friend. Despite being one of the hardest working and busiest people I have ever witnessed, Hefin would always make time for not only my often bizarre concerns and questions, but also for every member of the student scientific community who needed him, which never ceases to inspire me. Hefin is truly an exemplar scientist, teacher and human being, and will forever inspire me to be the greatest advocate for science and community that I can be.

I have, through papers, projects and events, collaborated with a variety of incredible researchers across the world. Through each of these partnerships I have learned more about science, research and the wider world. These diversions and “scenic routes” through my PhD were inarguably among the most pleasurable and rewarding milestones of this journey. Whether for papers, grants, outreach or advice, I would like to thank this community, not limited to Shawn Wilder, Mark Telfer, Jörg Spelda, Vasco Elbrecht, Denis Lafage, Mary Gardiner, Rajbir Kaur, Raghavendra Gadagkar, Helen Smith, Geoff Oxford, Bill Parker, Danni Sherwood, Bruce Deagle, Anthony Chariton, Eric Coissac, Fred Boyer, Pierre Taberlet, Simon Jarman, Bastian Egeter, Seirian Sumner, Beth Clare, Sara Goodacre, John Pickett, David Labonte and so many more.

I am excited to continue this ethos of collaborative research, outreach and community in the coming months, for which I am extremely grateful to Efrat Gavish-Regev. To continue researching spiders is an absolute privilege, and to do so in such an exciting environment (by which I mean both Jerusalem and such a welcoming and scientifically astute research group) fills me with anticipation. I am unfathomably grateful for the opportunity to continue research in this area and I can’t wait to see what we will achieve together.

A special thanks must be reserved for the community of Cardiff‘s finest vendors of delicacies (please also view the following as a list of recommendations if you’re after some top-tier sustenance). Brodies Coffee Co energised much of the last stretch of my thesis not only through caffeine-induced ecstasy but also their unwavering humour, optimism and delightful baked goods. Troy Meze Bar, a long-standing love of mine (host to both mine and my wife’s first date and, years later, our pandemic-adapted wedding meal) provided the homely comfort necessary after weeks of frenetic lab work, not to mention they even helped me change my flat car tyre a fortnight from hand-in. Pooja Sweets & Savouries not only provided the mithai for so many celebrations along the way, but also put up with my occasional poor attempts at ordering and conversing in Hindi. Nata & Co. gave me the sunshine-evoking flavour of Portugal even on the dullest days. Vegetarian Food Studio took me on delightful culinary

journeys to Gujarat when the lab got tiresome. Calabrisella gave me the greatest pizza in Cardiff.

Whilst science is the lifeblood often coursing through my veins, music is undoubtedly the oxygen which nourishes it. Countless musicians have fuelled the frenzied lab work and frenetic twitching of my keyboard-worn fingers, ultimately spurring me to the finish line. Given the importance of music to my life, I want to express eternal gratitude particularly to Michael Giacchino, 65daysofstatic, Ravi Shankar, Explosions in the Sky, The Mills Brothers, Comeback Kid, Childish Gambino, Counterparts, Hans Zimmer, The Ink Spots and Ludwig Goransson for the final two months of solid replay value, and the likes of Tallest Man On Earth, Radiohead, The Shins, The Three Keys, Buena Vista Social Club, Surfer Blood, The Beatles, Ben Howard, Nirvana, Haley Heynderickx, Natalia Lafourcade, Novo Amor, Bombay Bicycle Club, Hamilton, Daughter, Foals and The Polar Dream for four years of rhythmic inspiration (and of course Thrips, the greatest Cardiff-based entomological post-folk band I am aware of).

I perplexed many friends in my embarkation on a career path involving the study of undigested spider faeces, but they have bizarrely maintained contact with me regardless. I would firstly like to apologise to those friends, with whom I have missed concerts, surfing trips and generally just seeing them on so many occasions as a result of the tumultuous nature of PhD life (I think it's time to start making up for that). Thanks to Alex Auld for musical musing and the most obscure internet content a person could hope to see, Luke Cartwright for his curious mind and spirit of adventure, Ben Robinson for reverting me back to a ten year old every time I see him, Connor Williams for facilitating my regular return to mosh pits, Davey Hamlen for a beautiful stream of spider photos, George Moore for scenic walks in secluded locations, Mathew Cavaciuti for literally never ceasing to surprise me in every possible way, Niall De'ath for introducing me to the most obscure electronic entertainment out there, and Alvin Szeto for pizza, popcorn and ring shopping.

I had the incredible fortune of starting my PhD alongside an incredible community of scientists from my MRes. JJ, Numair, Rhi and Richard – thank you for keeping the dream alive. No MRest for the wicked. That extends also to Emma Gilmartin who supervised my MRes, but also gave me forest intuition, Radiohead and a refreshed memory of the Spice Girls. A particularly special thanks must, however, be reserved for Rafa, who has consistently pushed me to achieve more than I thought possible, both professionally and personally. Rafa's support was unrelenting, especially during months of lock-down which, against all odds, contain some of my fondest memories, in no small part also thanks also Sabrina. Tootootooroooooo

I have, of course, made many more friends along my wily way too. First, my SWBio egg-brothers, Joel, Siôn and Ed, who made the first year of coursework and endless travelling not

only bearable, but fun. My day-to-day interactions throughout the PhD, however, were mostly blessed by the MolEcol “family” who brightened the dullest days with cheery community spirit and crazy hijinks. For introducing me to this merry band, I would like to thank Jen StockGale, who also provided a great deal of mentorship and advice, and Alex “slug girl” McCubbin, whose humour, knowledge, wit and, of course, fiancé, have filled my life with joy, laughter and Star Wars. Thanks must also go to Iain, mellon, for whom the beacons will always be lit, Dan for being the Jar Jar of my PhD, JUAN MANUEL AGUILAR LEON for drinking my sometimes awful coffee and never failing to make me laugh, Isa Gameiro Aleixo Pais for being a ray of sunshine on the gloomy mornings, Ali for sharing Cardiff’s Egyptian weather with me, Maf for consistently punctual meetings that absolutely never (not once) ran over in the best possible way, Nia for continuing my Peruvian guitar legacy, Josie for so graciously complimenting my 17.5” laptop, Sophie for being such a fantastic desk neighbour (despite me moving desk every few weeks), Ashoka for kindness, conversation and sharing elephant pictures with me, Szabolcs for his hearty chuckles at my terrible jokes, Sen for some fine whisky, Christina for sharing some glorious morning coffees, Jody for making me read fiction that I will never forget, Jez for infinite advice and for not returning the horrid gesture of torturing you in the lead up to your thesis submission, Manon for her musical majesty and for stepping up on that fateful open mic night, Amna for putting up with my broken Urdu and for involving me in some really cool science, Mel for keeping the music alive during long days in the lab, Sam for your WEEN dancing, cockroaches and laughter, Kat for delicious food and friendly chats, and Nina for permanently embedding the mountain chicken song in my brain.

Within the MolEcol group, however, exists a subgroup critically important to all that I have achieved within this thesis: Team Bill (/The Symondson Syndicate).

Lorna, thank you for your proactive kindness, constant mockery and for helping me think through so many things; I hope you have a lifetime of perfectly adequate soup. Sarah, thanks for being such a reassuring and optimistic presence, even when the days were long and gloomy; I will always think of you when I see the Beaufort scale. Lolita, thanks for changing my perspective on moustaches forever, and for making the first year of my PhD so fun, even when fatigue and stress should have made that impossible. Becca, thank you for being the youngest old person I know, and simultaneously the oldest teenager, and for being one of the loveliest human beings I have had the pleasure of knowing (and sorry for my responses to your sneezes). Ewan, thank you for teaching me about James Vardy and Jordan Pickford, who I will forever remember in that utterly forgettable Croatian performance, and for WEEN misadventures with Mexican Shrek. Lucija, thanks for joining in on my “woohoos” in the Genomics Hub, sharing my frustration with a disintegrating shower, and sharing my enthusiasm for ground beetles. Jenny, thanks for your consistently upbeat disposition and

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Finally, I would like to thank Zac Winter, my dearest friend for many years, who taught me a colossal amount about myself and the wild and wonderful world that we experienced together growing up. Many of our conversations by a little stream in Corsham in late summer evenings truly built the foundations of the philosophy that I would ultimately apply to science. Zac taught me so much about adventure, boldness and humour, but also about introspection, humility and compassion. Beyond pandemics, fieldwork weather events and the innate scientific follies of a PhD, the greatest personal challenge of these 1508 days was surely the aftermath of this dear friend taking his own life. It is incredibly easy to lose one's self in the search of science and the excitement of experiments, but no event could serve as a greater reminder that we are all incredibly human. We must love, learn and live when we can, and hold close those we care for whenever we can. It takes seconds to check in, but a lifetime to mourn. I owe everything to the incredible collective of motivational, inspirational and encouraging people above, but that community will always feel emptier without Zac.

Lay Summary

Spiders help farmers by eating pests like aphids. We don't, however, understand how spiders choose what they eat. To make best use of spiders in farms, we must first study their dietary choices.

In this thesis, we designed a new way of measuring how much carbohydrate, fat and protein is in small invertebrates. We also created new tools to help detect what spiders have eaten using DNA in their guts, which helped us find out that spiders eat different things before and after the cereal crops that they live in are harvested.

Using the same tools, we looked into what 300 spiders had eaten and saw that what spiders eat changes over time and that, depending on the type of spider and its age, they eat different prey. We also found out that different spiders may be better at protecting farmers' crops than others, particularly young spiders and two specific types of spider (*Bathyphantes* and *Tenuiphantes*).

Using the new way of measuring the nutrients in spiders, we managed to group and rename the different prey that live near spiders based on what nutrients they have. We then saw that spiders eat groups of prey with different nutrients in them together to get a balanced diet, but that different spiders get their nutrients by eating different prey to one another. This is the first time that this has been shown in the wild and outside of a laboratory.

Summary

Spiders are abundant generalist predators in cereal crops and effective biocontrol agents, but the mechanisms underlying their prey choice are poorly understood. Nutrient-specific foraging suggests that predators choose their prey based on macronutrient content, but this has not been evidenced in the field. This thesis provides evidence that nutrient-specific foraging is both measurable and observable under field conditions, using spiders in cereal crops as a model system.

A streamlined protocol for the determination of macronutrient content from single microinvertebrates using rapid colorimetric plate assays was first developed. The first PCR primers purposed for analysing linyphiid spider diets were then designed and applied to pre- and post-harvest spiders in a prey choice analysis. This identified changes in prey choice following harvest, ultimately indicating the importance of consistent prey provision for generalist predators. These PCR primers were subsequently used for a dietary analysis of 300 spiders from barley fields. Spider diets differed over time and between genera and life stages of the predator. Similarly, predation of pests and predators differed between genera and life stages, identifying optimal candidates for biocontrol (i.e. *Bathyphantes*, *Tenuiphantes* and juvenile spiders) that can be encouraged through appropriate land management.

Finally, dietary data were analysed alongside the local abundance of prey and their macronutrient contents, the latter determined via the novel macronutrient analysis protocol. Prey taxa were grouped into “tropho-species” based on similarities in nutrient content, and evidence for nutrient-specific foraging was identified through tropho-species co-occurrence in the diet and prey choice null models. Individual spiders consumed prey rich in one macronutrient alongside prey with an average content of all three macronutrients. The overall spider population obtained balanced intake through predation of prey with different nutrient contents, those prey preferentially chosen differing between taxa, life stages and sexes. This is the first multi-faceted field-based evidence for nutrient-specific foraging.

Collaborators

This PhD was funded by the Biotechnology and Biological Sciences Research Council through the South West Biosciences Doctoral Training Partnership (grant BB/M009122/1). The overall project was proposed by the supervisory team, comprising William O.C. Symondson, Carsten T. Müller, Ian P. Vaughan, James R. Bell and Pablo Orozco-terWengel. These supervisors each contributed to the execution of every chapter of this thesis. Additional collaborators contributed toward the completion of this work, particularly for those works now published or under review.

Chapter 2: I led the study, including the lab work, analysis and writing of the manuscript/chapter. Shawn M. Wilder, initially through peer review of the first manuscript and subsequently as a co-author, advised on the protocol design and use. Maximillian P.T.G. Tercel, Paige S. Morley and Rafael A. Badell-Grau tested and assisted with calibration of the protocol. Rhiannon Hunt and Somoye Oluwaseun contributed experiments and samples for the initial testing of the protocol.

Chapter 3: I led the study, including the fieldwork, lab work, analysis and writing of the manuscript/chapter. Lorna E. Drake contributed toward the testing of the PCR primers and bioinformatic analysis. Maximillian P.T.G. Tercel assisted with invertebrate processing. Jennifer E. Stockdale advised on the design of the PCR primers.

Chapter 4: I led the study, including the fieldwork, lab work, analysis and writing of the chapter. Lorna E. Drake advised on the bioinformatic analysis. Maximillian P.T.G. Tercel advised on alternative entomological implications and analyses.

Chapter 5: I led the study, including the fieldwork, lab work, analysis and writing of the chapter. Maximillian P.T.G. Tercel contributed intellectually toward the tropho-species concept.

Chapter 6: I led the study, including the lab work, analysis and writing of the chapter. Megan Heppenstall, Jake O'Leary and Finn McCormick contributed toward the laboratory work for the volatile and cuticular hydrocarbon analysis of fruit flies. Somoye Oluwaseun contributed samples for the macronutrient analysis of cockroaches.

Publications

Publications directly arising from this project:

- Cuff *et al.* (2020). Money spider dietary choice in pre- and post-harvest cereal crops as revealed by metabarcoding. *Ecological Entomology.* **1st Author**
- Cuff *et al.* (2021). MEDI: a rapid, cheap and streamlined protocol for Macronutrient Extraction and Determination from Invertebrates. *Methods in Ecology and Evolution.* **1st Author**
- Cuff *et al.* (in prep.) The dynamics of cereal crop spider diet and biocontrol: a moment on the lips, a lifetime on the thrips. *Molecular Ecology*. **1st Author**
- Cuff *et al.* (in prep.) Nutrient-specific foraging identified in the field. *Nature*. **1st Author**
- Cuff *et al.* (in prep.) A comparison of prey survey methods for the analysis of spider prey choice: to stick or suck. *Methods in Ecology and Evolution*. **1st Author**

Publications arising from work completed alongside this project:

- Bajwa *et al.* (2019). Assessment of nematodes in Punjab Urial (*Ovis vignei punjabiensis*) Population in Kalabagh Game Reserve: Development of a DNA barcode approach. *European Journal of Wildlife Research.* **2nd Author**
- Lafage *et al.* (2019). A new primer for metabarcoding of spider gut contents. *Environmental DNA*. **3rd Author**
- Telfer *et al.* (2020). Neobisium simile (L. Koch, 1873) (Pseudoscorpiones: Neobisiidae): a new British species. *Arachnology*. **2nd Author**
- Ammann *et al.* (2020). Insights into aphid prey consumption by ladybirds: Optimising field sampling methods and primer design for High Throughput Sequencing. *PlosOne*. **5th Author**
- Cuff *et al.* (2020). 'Using DNA metabarcoding to analyse the gut contents of spiders' in Wheater et al. *Practical Field Ecology*. 2nd edition, Wiley-Blackwell, pp 121-124.
- Cuff *et al.* (2020). Home is where the heart rot is: violet click beetle, *Limoniscus violaceus* (Müller, 1821), habitat attributes and volatiles. *Insect Conservation and Diversity*. **1st Author**
- Badell-Grau & Cuff *et al.* (2020). The prevalence of reactive online searching in the COVID-19 pandemic. *Journal of Medical Internet Research.*
- Tercel *et al.* (submitted). The problem of omnivory: a synthesis on omnivory and DNA metabarcoding. *Molecular Ecology Resources*. **Last (3rd) Author**

- Co-wrote Cardiff University's campus-wide Biodiversity Action Plan as part of the Biodiversity Action Plan Working Group.
- Co-wrote Cardiff University's report for the Wales Environment Act Section 6.
- Informal articles for Mollusc World (2019), SEWBReC Gwent-Glamorgan Recorder's Newsletter (2016), The Conversation (2020) and the British Arachnological Society Newsletter (2020) alongside regular scientific social media engagement.

Student Supervision

- Elysia Davidson, 2017. Invertebrate communities present in heart--rot found in beech (*Fagus sylvatica*) trees. BSc Final Year Project.
- Lee Smith, 2018. Invertebrate communities present in heart--rot found in beech (*Fagus sylvatica*) trees. BSc Final Year Project.
- Jake O'Leary, 2019. Fat, sweet or meaty – how to detect? BSc Final Year Project.
- Finn McCormick, 2019. Fat, sweet or meaty – how to detect? BSc Final Year Project.
- Megan Heppenstall, 2019. Fat, sweet or meaty – how to detect? BSc Final Year Project.
- Paige Morley, 2019. Effect of land use on the nutrient content of freshwater macroinvertebrates and basal resources. MSci Final Year Project.
- Jamie Owen, 2020. Invertebrates of rot holes in Nant Bran woodland and the influence of habitat variables. BSc Final Year Project.

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- Genetics Society One-off Meeting Sponsorship Grant - £1,000
- Entomological Club Entomological Grant for biochemical analysis of virus vector aphids - £250
- British Ecological Society Training and Travel Grant - £500

Presentations

- "The dynamics of cereal crop spider diet and biocontrol: a moment on the lips, a lifetime on the thrips", TiBE Metabarcoding & Metagenomics 2020, 11/12/2020, Virtual Conference
- "Nutrient-specific foraging and the role of spiders as biocontrol agents", The Hebrew University of Jerusalem Arachnology Group Seminar, 06/12/2020, Zoom
- "Nature in Lockdown", Natural History Museum Nature Live Seminar Series, 08/09/2020, YouTube
    - **Invited panel member**
- "The BAS lockdown spider surveys", Swansea University StayHomeAndCountBugs Online Seminar Series, 27/08/2020, Zoom
    - **Invited speaker**
- "Symposium close with Thrips band", 1st Symposium on Thysanoptera in Brazil, 21/08/2020, YouTube
- *"Coccinella septempunctata* macrophotograph", AgriFoodBecause SCI Agri-Food Early Careers Photo Competition, 01/06/2020, Twitter
    - **1st place winner**
- "Home is where the heart-rot is", I3 (Innovation in Isolation), 29/07/2020, YouTube
- "The Great British Cellar Spider Survey", I3 (Innovation in Isolation), 01/04/2020, Discord
- "Browsing the web: prey choice in cereal crop spiders and their predation of aphids", Royal Entomological Society Postgraduate Forum, 20/02/2020, Bristol
    - **1st talk prize**
- "Every drop of crop", Cardiff University Images of Research Exhibition, 10/12/2020, Cardiff
- "Browsing the web: prey choice in cereal crop spiders and their predation of pests", Wales Ecology & Evolution Network, 30/11/2019, Centre for Alternative Technology, Machynlleth
- "Browsing the web: prey choice in cereal crop spiders and their predation of aphids", Rothamsted Research PhD Symposium, 20/11/2019, Harpenden
- "Browsing the web: spider prey choice in agroecosystems", University of Bristol Entomological Society Arachtober Event, 22/10/2019, Bristol
    - **Invited speaker**
- "Browsing the web: nutrient-specific foraging and the role of spiders as aphid predators", SWBio Annual Student Conference, 11/09/2019, Exeter
- "Home is where the heart-rot is", Royal Entomological Society Forest Insects and Their Allies Special Interest Group Meeting, 10/04/2019, BIFOR, Birmingham
- "A rapid streamlined protocol to determine macronutrient content in macroarthropods", Royal Entomological Society Postgraduate Forum, 21/03/2019, York
    - **1st talk prize**
- "Browsing the web: nutrient-specific foraging and the role of spiders as aphid predators", University of Nottingham Life Sciences Seminar Series, 29/11/2018, Nottingham
    - **Invited speaker**
- "Browsing the web: prey choice in cereal crop spiders and their predation of aphids", Rothamsted Research PhD Symposium, 28/11/2018, Harpenden
- "Giving snails a "whorl": my PIPS experience", SWBio Professional Internships for PhD Students Conference, 06/06/2018, Bath
    - **Talk prize**

- “Are spiders an effective pest control agent? An investigation of spider diet”, Wenvoe Farmer’s Union Monthly Meeting, 18/01/2018, Wenvoe
    - **Invited speaker**
- “Lipid la vida loca: determining macronutrient content of macroarthropods”, Wales Ecology & Evolution Network, 04/11/2017, Centre for Alternative Technology, Machynlleth
- “Browsing the Web: nutrient-specific foraging and the role of spiders as aphid predators”, Cardiff University Organisms and Environment Away Day, 14/05/2020, Cardiff
    - **Talk prize**
- “Browsing the web: design and evaluation of primers for the analysis of spider diets and predation on aphids”, Molecular Analysis of Trophic Interactions Conference, 14/09/2017, Uppsala, Sweden
- “Browsing the web: Nutrient-specific foraging and the role of spiders as aphid predators”, Cardiff University Organisms and Environment Away Day, 11/05/2017, Cardiff
    - **Poster prize**
- “Prey choice and the role of spiders as aphid predators in cereal crops”, 7th Spring Metabarcoding School, 28/04/2017, Gaia, Portugal
- “Home is where the heart-rot is: a pungent tale of beetles and trees” Cardiff Science Café,11/04/2017, Cardiff
- “Home is where the heart-rot is”, Wales Ecology & Evolution Network, 05/11/2016, Gregynog
    - **Best talk prize**

Outreach and Engagement

- I, along with three British Arachnological Society (BAS) committee members, launched four surveys of spiders in and around homes and gardens in Britain during the COVID-19 pandemic to facilitate community science recording of these spiders to extend their distributions and increase enthusiasm for arachnology. A review of the first few months of this was published in the BAS newsletter and I later discussed this for the Natural History Museum’s “Nature Live” lockdown YouTube series.
- I co-investigated a Wellcome Trust public engagement grant that led to me co-leading the resultant large public engagement portfolio project in which the core aim was to teach the public about biobanking alongside UK biobanking charity Frozen Ark. This involved public exhibitions that taught participants about DNA, conservation and how biobanking can help with mitigating the biodiversity crisis. The project migrated to an online platform following cancellation of the events due to the COVID-19 pandemic.
- Throughout the entire PhD, and shortly before, I co-organised a science café in Cardiff, bringing STEM presentations to lay audiences monthly, including one of my own talks. This was regularly attended by up to 70 people and received positive reviews.
- Given the popularity of the Science Café sessions, I was eager to continue this initiative during the COVID-19 pandemic, especially given the increased importance of science communication during healthcare crises. I thus co-founded Innovation in Isolation (I3) which provided STEM talks each week via Zoom during the first months of the pandemic.
- I led the design and deployment of an engagement activity for Green Man Festival 2018’s Einstein’s Garden. This was one of a group of activities funded by BBSRC’s South West Bioscience Doctoral Training Partnership themed around agricultural sustainability. Given the focus of my research on the control of crop pests, I took this opportunity to present a bespoke 3D-printed board game titled “Pest vs. Pesticide” to raise awareness of the many trade-offs between agricultural productivity and

sustainability. Combining “Mr. Potato Head” with “Top Trumps” and adding a few additional flourishes, families were eager to engage in competitively assembling the brightly coloured 3D-printed models.

Contents

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Abbreviations

Symbols/units

°C= Degrees Celsius

% = Percent

+ = Positive

− = Negative

cm = Centimetre

g = Gram

h = Hour

Hg = Inch of mercury

k = Thousand

kg = Kilogram

µL = Microlitre

µM = Micromolar

µmol = Micromole

m = Metre

min = Minute

M = Molar

mg = Milligram

ml = Millilitre

mm = Millimetre

nm = Nanometre

s = Second

A

agg. = Species aggregate

AIC = Akaike information criterion

ANOVA = Analysis of variance

B

BAS = British Arachnological Society

BBSRC = Biotechnology and Biological Sciences Research Council

BCA = Bicinchoninic acid

BGG = Bovine gamma globulin

BLAST = Basic Local Alignment Search Tool

BOLD = Barcode of Life Database

bp = Base pairs

BSA = Bovine serum albumin

BYDV = Barley yellow dwarf virus

C

CAP = Canonical analysis of principal components

CI = Confidence interval

CHC = Cuticular hydrocarbon

COI = Cytochrome *c* oxidase subunit I

D

Dev. = Deviance

DNA = Deoxyribonucleic acid

E

eDNA = Environmental DNA

e.g. = exempli gratia

G

GC-MS = Gas chromatography-mass spectrometry

GLM = Generalized linear model

H

HTS = High-throughput sequencing

I

i.e. = id est

IgG = Immunoglobulin G

L

LoB = Limit of blank

LoD = Limit of detection

LRT = Likelihood ratio test

M

MANOVA = Multivariate analysis of variance

MEDI = Macronutrient extraction and determination from invertebrates

MGLM = Multivariate generalized linear model

MID-tags = Molecular identifier tags

MLM = Multivariate linear model

mph = Miles per hour

MS = Microsoft

N

n = Number

NCBI = National Center for Biotechnology Information

NERC = Natural Environment Research Council

NMDS = Non-metric multidimensional scaling

n.s. = Non-significant

O

OTU = Operational taxonomic unit

P

PCR = Polymerase chain reaction

PerMANOVA = Permutational multivariate analysis of variance

R

RNA = Ribonucleic acid

RNAi = RNA interference

RPM = Revolutions per minute

RT = Room temperature

S

SD = Standard deviation

SDD = Smallest detectable difference

SE = Standard error

SES = Standardised effect size

SNB= Stick, net and bottle trap

sp. = Species (singular)

spp. = Species (plural)

SPF = Specific parasite-free

T

TD-GC-TOF-MS = Thermal desorption gas-chromatography time-of-flight mass-spectrometry

U

UK = United Kingdom

USA = United States of America

V

V = Volume

VOC = Volatile organic compound

Z

ZOTU = Zero-radius operational taxonomic unit

Taxonomic Authorities

Taxonomic authorities, orders and families are given for those species and genera directly mentioned in this thesis to avoid ambiguity. Names are presented in the main text at the first mention of each; if the name appears only in tables or figures, it is only listed here.

Acheta domesticus (Linnaeus, 1758; Orthoptera: Gryllidae)

Acrodactyla degener (Haliday, 1839; Hymenoptera: Ichneumonidae)

Aeolothrips intermedius Bagnall, 1934 (Thysanoptera: Aeolothripidae)

Agyneta rurestris (Koch, 1836; Araneae: Linyphiidae)

Alcedo atthis (Linnaeus, 1758; Coraciiformes: Alcedinidae)

Amaurobius similis (Blackwall, 1861; Araneae: Amaurobiidae)

Amischa sp. Thomson, 1858 (Coleoptera: Staphylinidae)

Anagrus sp. Haliday, 1833 (Hymenoptera: Mymaridae)

Anaphothrips obscurus (Müller, 1776; Thysanoptera: Thripidae)

Anchomenus dorsalis (Pontoppidan, 1763; Coleoptera: Carabidae)

Anopheles sp. Meigen, 1818 (Diptera: Culicidae)

Anotylus tetracarinatus (Block, 1799; Coleoptera: Staphylinidae)

Anthocoris nemorum (Linnaeus, 1761; Hemiptera: Anthocoridae)

Aphelinus sp. Dalman, 1820 (Hymenoptera: Aphelinidae)

Aphidius sp. Nees & Esenbeck, 1818 (Hymenoptera: Braconidae)

Bacillus thuringiensis Berliner, 1915 (Bacillales: Bacillaceae)

Bathyphantes nigrinus (Westring, 1851; Araneae: Linyphiidae)

Blattella germanica Linnaeus, 1767 (Blattodea: Ectobiidae)

Brachydesmus superus Latzel, 1884 (Polydesmida; Polydesmidae)

Bradysia urticae Mohrig & Menzel, 1992 (Diptera: Sciaridae)

Carduelis carduelis (Linnaeus, 1758; Passeriformes: Fringillidae)

Centromerita bicolor (Blackwall, 1833; Araneae: Linyphiidae)

Chernes cimicoides (Fabricius, 1793; Pseudoscorpiones; Chernetidae)

Chthonius ischnocheles (Hermann, 1804; Pseudoscorpiones; Chthoniidae)

Clubiona comta Koch, 1839 (Araneae: Clubionidae)

Coccinella septempunctata (Linnaeus, 1758; Coleoptera: Coccinellidae)

Copidosoma floridanum Ashmead, 1900 (Hymenoptera: Encyrtidae)

Corynoptera sp. Winnertz, 1867 (Diptera: Sciaridae)

Cryptops hortensis (Donovan, 1810; Scolopendromorpha: Cryptopidae)

Cylindroiulus punctatus (Leach, 1815; Julida; Julidae)

Drosophila melanogaster Meigen, 1830 (Diptera: Drosophilidae)

Drosophila suzukii (Matsumura, 1931; Diptera: Drosophilidae)

Dysdera crocata Koch, 1838 (Araneae: Dysderidae)

Elachiptera decipens (Loew, 1863; Diptera: Chloropidae)

Episyrphus balteatus (De Geer, 1776; Diptera: Syrphidae)

Eratigena atrica (Koch, 1843; Araneae: Agelenidae)

Erigone atra Blackwall, 1833 (Araneae: Linyphiidae)

Erigone dentipalpis (Wider, 1834; Araneae: Linyphiidae)

Erithacus rubecula (Linnaeus, 1758; Passeriformes: Muscicapidae)

Euproctis similis (Füssli, 1775; Lepidoptera: Erebidae)

Ficedula hypoleuca (Pallas, 1764; Passeriformes: Muscicapidae)

Folsomia candida Willem, 1902 (Entomobryomorpha: Isotomidae)

Frankliniella occidentalis Pergande, 1895 (Thysanoptera: Thripidae)

Frankliniella tenuicornis (Uzel, 1895; Thysanoptera: Thripidae)

Fringilla coelebs (Linnaeus, 1758; Passeriformes: Fringillidae)

Geophilus truncorum (Bergsoë & Meinert, 1886; Geophilomorpha: Geophilidae)

Gregarina blattarum von Siebold, 1839 (Apicomplexa: Eugregarinida)

Halotydeus destructor (Tucker, 1925; Trombidiformes: Eupodoidea)

Hypogastrura viatica (Tullberg, 1872; Poduromorpha: Hypogasturidae)

Isotoma anglicana (Schäffer, 1896; Entomobryomorpha: Isotomidae)

Isotomurus sp. Börner, 1903 (Entomobryomorpha: Isotomidae)

Javasella sp. Fennah, 1963 (Hemiptera: Delphacidae)

Lasius brunneus (Latreille, 1798; Hymenoptera: Formicidae)

Limothrips denticornis (Haliday, 1836; Thysanoptera: Thripidae)

Lithobius variegatus Leach, 1814 (Lithobiomorpha: Lithobiidae)

Loricera pilicornis (Fabricius, 1775; Coleoptera: Carabidae)

Macrosteles sexnotatus (Fallén, 1806; Hemiptera: Cicadellidae)

Melieria crassipennis (Fabricius, 1794; Diptera: Ulidiidae)

Metopolophium dirhodum (Walker, 1849; Hemiptera: Aphididae)

Microlinyphia pusilla Sundevall, 1830 (Araneae: Linyphiidae)

Micromus variegatus (Fabricius, 1793; Neuroptera: Hemerobiidae)

Neriene montana Clerck, 1757 (Araneae: Linyphiidae)

Nossidium pilosellum (Marsham, 1802; Coleoptera: Ptiliidae)

Nothodelphax sp. Fennah, 1963 (Hemiptera: Delphacidae)

Oedothorax fuscus (Blackwall, 1834; Araneae: Linyphiidae)

Oligotoma saundersii (Westwood, 1837; Embiidina: Oligotomidae)

Orchesella villosa (Linnaeus, 1767; Entomobryomorpha: Entomobryidae)

Oscinella sp. Becker, 1909 (Diptera: Chloropidae)

Oxychilus navarricus (Bourguignat, 1870; Gastropoda: Oxychilidae)

Pardosa amentata (Clerck, 1757; Araneae: Lycosidae)

Pardosa lugubris (Walckenaer, 1802; Araneae: Lycosidae)

Pardosa palustris (Linnaeus, 1758; Araneae: Lycosidae)

Pardosa prativaga (Koch, 1870; Araneae: Lycosidae)

Pardosa pullata (Clerck, 1757; Araneae: Lycosidae)

Passer domesticus (Linnaeus, 1758; Passeriformes: Passeridae)

Pheidole megacephala Fabricius, 1793 (Hymenoptera: Formicidae)

Philodromus sp. Walckenaer, 1826 (Araneae: Philodromidae)

Phylloscopus collybita (Vieillot, 1817; Passeriformes: Phylloscopidae)

Phylloscopus trochilus (Linnaeus, 1758; Passeriformes: Phylloscopidae)

Porcellio scaber Latreille, 1804 (Isopoda: Porcellionidae)

Promethes sulcator (Gravenhorst, 1829; Hymenoptera: Ichneumonidae)

Protophorura armata (Tullberg, 1869; Poduromorpha: Onychiuridae)

Psammotettix alienus (Dahlbom, 1850; Hemiptera: Cicadellidae)

Psilochorus simoni (Berland, 1911; Araneae: Pholcidae)

Pterostichus melanarius (Illiger, 1798; Coleoptera: Carabidae)

Reticulitermes lucifugus (Rossi, 1792; Blattodea: Rhinotermitidae)

Rhopalosiphum padi (Linnaeus, 1758; Hemiptera: Aphididae)

Scaptomyza pallida (Zetterstedt, 1847; Diptera: Drosophilidae)

Scatopsciara atomaria (Zetterstedt, 1851; Diptera: Sciaridae)

Sipha sp. Passerini, 1860 (Hemiptera: Aphididae)

Sitobion avenae (Fabricius, 1775; Hemiptera: Aphididae)

Sminthurinus aureus (Lubbock, 1836; Symphypleona: Katiannidae)

Sminthurinus elegans (Fitsch, 1863; Symphypleona: Katiannidae)

Sminthurus viridis (Linnaeus, 1758; Symphypleona: Sminthuridae)

Stegodyphus lineatus (Latreille, 1817; Araneae: Eresidae)

Sylvia atricapilla (Linnaeus, 1758; Passeriformes: Sylviidae)

Sylvia borin (Boddaert, 1783; Passeriformes: Sylviidae)

Tachyporus chrysomelinus (Linnaeus, 1758; Coleoptera: Staphylinidae)

Tachyporus hypnorum (Fabricius, 1775; Coleoptera: Staphylinidae)

Tapinopa longidens (Wider, 1834; Araneae: Linyphiidae)

Tasmanicosa leuckartii (Thorell, 1870; Araneae: Lycosidae)

Tenebrio molitor Linnaeus, 1758 (Coleoptera: Tenebrionidae)

Tenuiphantes tenuis (Blackwall, 1852; Araneae: Linyphiidae)

Trichopria sp. Ashmead, 1893 (Hymenoptera: Diapriidae)

Turdus merula Linnaeus, 1758 (Passeriformes: Turdidae)

Tvetenia calvescens (Edwards, 1929; Diptera: Chironomidae)

Utamphorophora sp. Knowlton, 1946 (Hemiptera: Aphididae)

Zeus faber Linnaeus, 1758 (Zeiformes: Zeidae)

Nutrient-specific Foraging and the Role of Spiders as Biocontrol Agents

“Words are like the spider's web: a shelter for the clever and a trap for the not-so-clever.”

- Madagascan proverb

Chapter 1 : Introduction and Literature Review

1.1 General Introduction

Insect pests substantially reduce crop yields, which is increasingly pressing given a rising demand for agricultural productivity in the face of an increasing global population. Many pest management strategies are environmentally-damaging, short-lived and increasingly ineffective, shifting attention to biological control. Conservation biocontrol, the use of naturally-occurring biota such as generalist predators to reduce crop pest populations, for example spiders that prey on aphids and other arthropod pests, is considered an effective and sustainable alternative to conventional synthetic insecticides, but the mechanisms underlying prey choice by these predators are poorly understood, precluding manipulation of these interactions to optimally benefit pest management. It is hypothesised that generalist predators selectively feed to redress nutritional deficiencies, termed nutrient-specific foraging, which is supported by evidence from lab-based studies, but no evidence has yet been uncovered in the field. This project aims to identify whether nutrient-specific foraging occurs in the field, using cereal crop spiders as a study system and employing DNA metabarcoding, biochemical analyses of macronutrient content and prey choice modelling.

1.2. Crop protection and sustainable intensification

1.2.1. Food security and agricultural intensification

Global food security is considered one of the greatest challenges of the 21st Century. With the global population predicted to rise to 7.8 billion by 2050 and a predicted increase in per capita food consumption, this challenge is increasingly urgent (United Nations Department of International Economic and Social Affairs 1992). The issue is compounded by insufficient supplies of inputs such as fertiliser and pesticides, and climate change affecting agricultural efficiency (Godfray and Garnett 2014). Food production must increase to meet these demands, but without compromising on biodiversity, viable farmland is in short supply where it is needed; this then requires a combination of sharing land between agriculture and nature by promotion of biodiversity on agricultural land, and sparing land from agriculture to further safeguard biota that are ecologically incompatible with agriculture (Grass *et al.* 2019; Meunier 2020). Instead, existing food production must become more efficient (Godfray and Garnett 2014; Gurr *et al.* 2016). Approximately 18% of cereal crops such as wheat are lost to pests in

Northwest Europe with current pest management strategies in place; these losses must be reduced for future food security (Oerke 2006).

Animal crop pests are responsible for massive crop losses, insects forming a large contingent of these. Traditional pest control strategies for insect pests typically involve application of chemical insecticides to the crop (Oerke 2006). These chemical applications are, however, washed away by precipitation, as well as being behaviourally avoided by pests, and it is difficult to predict exactly when applications will be optimally effective (Trumper and Holt 1998; McDonald *et al.* 1999; Feldman *et al.* 2000). Many insecticide treatments have become ineffective as resistance to the insecticides inadvertently, through natural selection, evolves in pests (Ripper 1944; Denholm *et al.* 1998), becoming one of the most serious threats to global agriculture given the longstanding dependence on synthetic inputs (Peterson *et al.* 2016). Alternative insecticides have been developed, but many of these have devastating off-target effects on beneficial organisms, such as pollinators and natural enemies of crop pests (Fountain *et al.* 2007; MacFadyen *et al.* 2009; Pekár 2013; Woodcock *et al.* 2016; Loetti and Bellocq 2017). Many synthetic insecticides elicit long-term damage to the environment, rendering them unsustainable (Gurr *et al.* 2016). For an effective increase in productivity without long-term negative consequences and without requiring a great increase in land, agriculture must sustainably intensify its output via alternative means, termed sustainable intensification (Oerke 2006; Godfray and Garnett 2014; Gurr *et al.* 2016).

1.2.2. Aphids and other crop pests

Aphid infestations in crops can cause losses of £70 million per year. Severe widespread aphid outbreaks on wheat alone can increase these losses to £120 million per year (Tatchell 1989), with similar losses estimated globally (Dedryver *et al.* 2010; Valenzuela and Hoffmann 2015). Aphids exist in two forms: an obligate asexual form which parthenogenetically reproduces all year, surviving harsh winter conditions (Bale *et al.* 2007), and a facultative form which produces sexual morphs when temperature and photoperiod decline (Loxdale and Balog 2018). The former give birth to clones already bearing an additional generation of clones, facilitating rapid population increases, reaching upwards of 16-22 aphids per wheat ear and reducing crop yields by 25-37% (Fletcher and Bardner 1969; Montandon *et al.* 1993; Butts *et al.* 1997). Asexual aphids can employ epigenetic switching (the activation/inactivation of genes) in overcrowded conditions or decreased host plant quality to produce alate (winged) forms which can migrate to new host plants, increasing their dispersal to unaffected crops (Srinivasan and Brisson 2012; Loxdale and Balog 2018).

Many aphid species are adapted to cereal crops, including common pest species such as the rose-grain aphid *Metopolophium dirhodum* (Walker, 1849; Hemiptera: Aphididae), the English grain aphid *Sitobion avenae* (Fabricius, 1775; Hemiptera: Aphididae) and the bird cherry-oat aphid *Rhopalosiphum padi* (Linnaeus, 1758; Hemiptera: Aphididae). Aphids cause direct damage to crops by feeding from plant stems, but also indirectly damage them by vectoring plant diseases (Tatchell 1989; Carter and Harrington 1991; Dedryver *et al.* 2010). Aphids vector around 275 plant viruses, the economic impact of which can far exceed the direct burden arising from aphids feeding on crops (Dedryver *et al.* 2010). Non-persistent viruses vectored by aphids are short-lived, remaining on the stylet and not being ingested by the aphid, whilst others manifest as semi-persistent or persistent viruses that can be transmitted to plants over a much longer period, increasing their impact (Carter and Harrington 1991). Aphids carry barley yellow dwarf virus (BYDV), the most economically-significant and widespread cereal crop virus (Domier 2008), causing average cereal yield losses of approximately 20%, but up to 80% (Dedryver *et al.* 2010). Other indirect effects of aphids on cereal crops include decreased root proliferation and plant size (Dedryver *et al.* 2010), contributing, alongside disease and direct feeding, to substantial losses.

The traditional insecticides employed against aphids have, in many cases, become ineffective or at least far less effective (Linda and Roger 2003; Dedryver *et al.* 2010; Bass *et al.* 2014; de Little and Umina 2017; Wang *et al.* 2017; Wang *et al.* 2018). Common cereal aphids like *Rhopalosiphum padi* have developed resistance to common commercial insecticides such as imidacloprid (Wang *et al.* 2018). Selective plant breeding has produced plant cultivars resistant to several aphid species, but production of wheat and barley cultivars resistant to common aphid species in Europe has proven difficult (Lowe 1984; Dedryver *et al.* 2010; Hu *et al.* 2016).

The pest fauna of cereal crops extends far beyond aphids though, with other insect taxa such as Thysanoptera (thrips) and Auchenorrhyncha (cicadas, hoppers, spittlebugs) including many species that cause substantial economic losses to a range of crop plants (Kharizanov 1970; Lewis 1973; Childers and Achor 1995; Denno and Peterson 2000). Hoppers are particularly problematic pests, many causing ‘hopperburn’ in which plant tissues are damaged by the interaction of toxic saliva, nutrient depletion and induction of plant wound response triggered by a unique stylet movement (Sogawa and Cheng 1979; Backus *et al.* 2005), but species such as *Psammotettix alienus* (Dahlbom, 1850; Hemiptera: Cicadellidae) can also vector debilitating diseases, such as wheat dwarf virus (Wang *et al.* 2014). Thrips include several hundred species considered agricultural and horticultural pests (Lewis 1973), notably the western flower thrips *Frankliniella occidentalis* Pergande, 1895 (Thysanoptera: Thripidae), which is increasingly widespread globally, and increasingly abundant (Frantz and Mellinger 2009). As with aphids, pest thrips species are developing insecticide resistance, necessitating

novel means of pest management (Bielza 2008). This is true for a large range of problematic pest species across a large taxonomic breadth. Given the large crop losses incurred by these pests and their increasing resistance to insecticides, alternative pest management strategies are increasingly favourable, including biological control, ideally as part of a diverse integrated pest management plan.

1.2.3. Biological control of crop pests

The most effective pest management strategies often employ an integrated approach including crop resistance, selective insecticides and promotion of biological control (Peterson *et al.* 2016). Biological control (biocontrol) is the use of biological agents, such as parasites, pathogens and predators, to control pests (DeBach and Schlinger 1965; Bale *et al.* 2008; Schetelig *et al.* 2017). Biocontrol is both economically and environmentally advantageous since it reduces the non-target effects and environmental damage incurred by many synthetic inputs whilst providing self-perpetuating control at little to no ongoing cost (Bale *et al.* 2008). Three forms of biocontrol are commonly used (Figure 1.1): inoculative, whereby a control agent, often of exotic origin, is introduced once in small numbers to suppress a pest population (DeBach and Schlinger 1965); augmentative, whereby control agents are periodically released, often inundatively (Van Lenteren 2000; Levie *et al.* 2005); and conservation, whereby populations of naturally-occurring control agents are promoted (Gurr *et al.* 2000; Bale *et al.* 2008). Insecticide resistance (Bass *et al.* 2014), a need for agricultural sustainability, and mounting food security pressures (Tilman *et al.* 2002) have shifted focus toward the use of biocontrol in pest management strategies (Symondson 2002; Symondson *et al.* 2006; Welch *et al.* 2016). Biocontrol has been increasingly exploited and techniques have been developed to improve the efficiency of biological systems for the control of crop pests (Riechert and Lockley 1984; Greenstone *et al.* 2010; Albert *et al.* 2017).

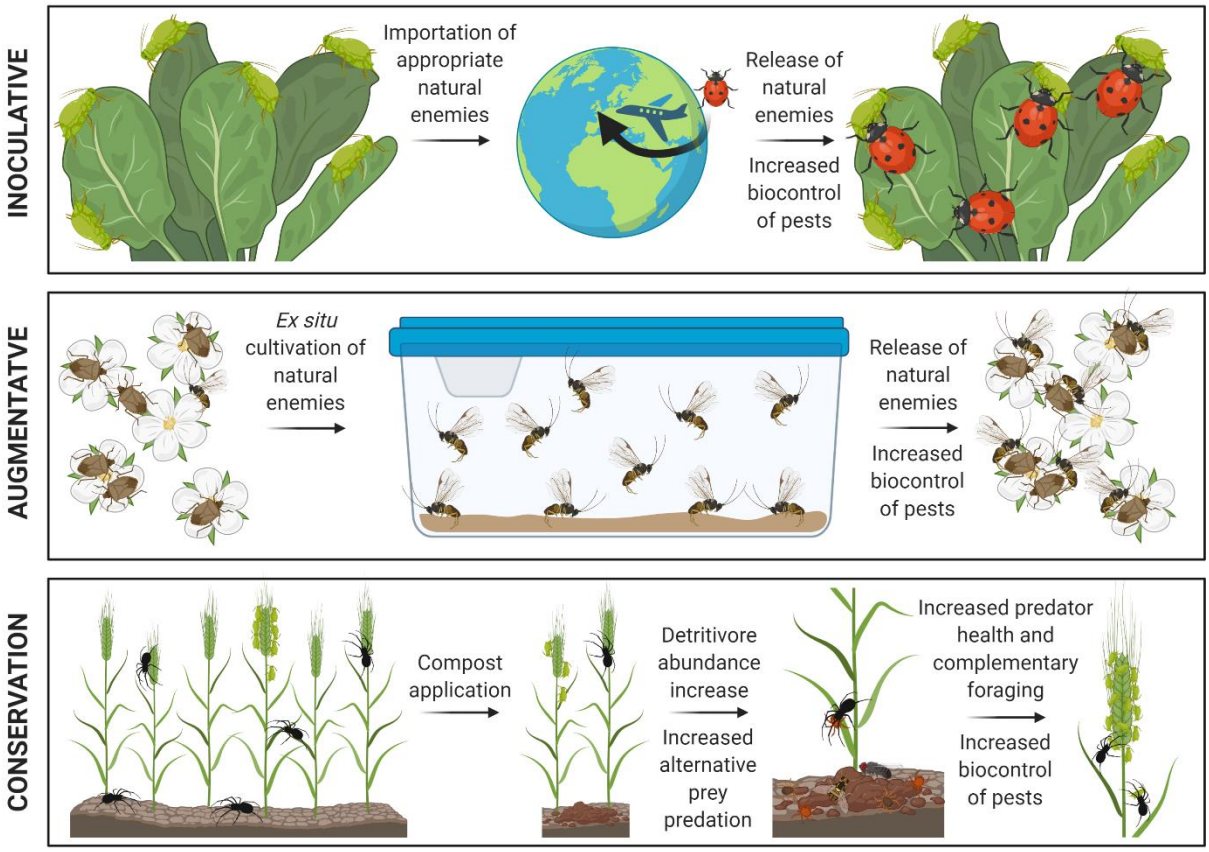


Figure 1.1: Examples of the three described biocontrol methods. Inoculative biocontrol involves the importation of novel biocontrol agents to increase predation pressure on native pests. Augmentative biocontrol involves artificially increasing native natural enemy abundances by means such as *ex situ* cultivation. Conservation biocontrol involves promotion of native natural enemy populations, such as by provision of alternative prey, often through habitat manipulation like compost application. Figure created in Biorender.

Advances in genetic modification have facilitated enhanced biocontrol techniques, arguably straying away from biocontrol in the strictest sense and into targeted application of systemic pesticides. Insertion of *Bacillus thuringiensis* Berliner 1915 (Bacillales: Bacillaceae) toxin genes into plants, for example, triggers *in vivo* production of pesticidal toxins for targeted pest suppression (Shelton *et al.* 2002). Furthering this concept, transgenic plants can be made to produce RNA molecules which fatally disrupt target pest species, termed RNA interference (RNAi; Zotti *et al.* 2017). Genes can be inserted into the males of some pest species to cause production of sterilising RNAi; these males can then be released to mate with females which then produce sterile eggs, thus reducing pest populations via this 'sterile insect technique' (Darrington *et al.* 2017; Schetelig *et al.* 2017; Zotti *et al.* 2017). Genetic modification is, however, scrutinised due to concerns surrounding the release of transgenes into wild populations, bioaccumulation of transgenic material in natural predators, and the ethical dilemmas associated with genetic modification (Mitchell and Sheehy 2000; Peterson *et al.* 2016). Natural pest management strategies (e.g. conservation biocontrol) are thus

preferentially used in many countries, such as manipulation of populations of naturally occurring predators. Habitat diversification, for example, provides suitable habitat for naturally-occurring generalist predators and their prey, increasing their fitness and promoting top-down control of pests (Sunderland and Samu 2000; Shayler 2005; Balmer *et al.* 2013; Gurr *et al.* 2016; Peterson *et al.* 2016; Gagic *et al.* 2018). Local habitats can also be modified for the benefit of natural predators, such as by application of compost to crop soil surfaces, which can increase generalist predator abundances through provision of alternative prey, thus promoting pest suppression through apparent competition between pests and alternative prey (Bonsall and Hassell 1997; Muller and Godfray 1997; Bell, Traugott, *et al.* 2008). Similarly, field margins (Mansion-Vaquie *et al.* 2017; Zhang *et al.* 2020) and beetle banks (MacLeod *et al.* 2004), through habitat diversification, provide refugia for alternative prey, facilitate niche separation of predators (in turn mitigating intraguild predation) and reduce the disruption of crop cycling (Sunderland and Samu 2000; Michalko *et al.* 2017). These techniques can be effective alone, but integration with other pest management strategies facilitates robust pest suppression, so long as the techniques are complementary (Bale *et al.* 2008; Mazzia *et al.* 2015; Tscharntke *et al.* 2016; Begg *et al.* 2017).

Predators of cereal crop systems provide a substantial baseline protection against crop pests (Lundgren and Fergen 2014; Drieu and Rusch 2017; Greenop *et al.* 2018). Naturally-occurring predators can suppress pest populations by direct consumption of pests and via nonconsumptive effects, such as by eliciting a 'fear response' in prey (Sitvarin *et al.* 2016). Predators will aggregate at sites of high prey abundance, providing biocontrol to areas of high pest density during rapid prey population increases (Winder *et al.* 2005; Bell *et al.* 2010). Predator diversity is important for efficient pest suppression, with different predator species exhibiting dissimilar pest suppression tendencies and efficacies in different seasons (Roubinet *et al.* 2018). The efficiency of predator-mediated biocontrol is, however, affected by abiotic variables, such as rainfall, which displaces pests from plant stems, and drought, which often reduces foraging (von Berg *et al.* 2008). Predator-mediated biocontrol can also be artificially increased by inundative release of natural enemies (Levie *et al.* 2005) or manipulation of the environment to promote naturally-occurring predators (Dedryver *et al.* 2010); however, introduction of additional predators often encourages intraguild predation (Jones *et al.* 2020) and can be economically inefficient (Dedryver *et al.* 2010). Introduced alien predators can often become a problem themselves, establishing populations, spreading novel diseases or consuming non-target species (Waldner *et al.* 2013; Camacho-Cervantes *et al.* 2017). Conservation biocontrol (using native predators), however, has fewer negative effects (Sunderland and Samu 2000; Dedryver *et al.* 2010; Gurr *et al.* 2016).

The dietary niche of a predator can have many implications for its efficacy as a biocontrol agent. Specialist predators and parasitoids depend on a single taxon for food or reproduction and have been studied extensively in the context of pest management (Sunderland *et al.* 1997; Levie *et al.* 2005; Ammann *et al.* 2020; Jordan *et al.* 2020). Stenophagous predators (predators that eat a small number of prey species) that target pest species, such as hoverflies (Diptera: Syrphidae), lacewings (Neuroptera) and ladybirds (Coleoptera: Coccinellidae), attack pests frequently given their focus on these taxa (Adams *et al.* 1987; Messina and Sorenson 2001; Michaud and Belliure 2001; Weber and Lundgren 2009); however, given their adapted metabolism, behaviour and nutrient extraction, specialists suffer losses to fecundity and survival when utilising other resources, thus requiring an abundance of the pest taxon for their proliferation (Pekár and Toft 2015; Líznarová and Pekár 2016; Petráková and Pekár 2017; Garcia *et al.* 2018). This dependence of specialists upon their prey or host to survive, and their inability to switch to another prey species if their specialism is affected by disease, decline in nutritional quality or population decline greatly detracts from their reliability as biocontrol agents (Peterson *et al.* 2016). Parasitoids are nonetheless efficient at suppressing populations of their host species, resulting in their frequent use in biocontrol of aphids and other pests (Levie *et al.* 2005; Traugott *et al.* 2008; Pook *et al.* 2017; Bale *et al.* 2008; Kruitwagen *et al.* 2018). The phenology of many such specialists can, however, reduce their effectiveness for biocontrol, particularly when targeting pests with short generation times such as aphids, since specialist species tend to emerge shortly after their prey or host, giving the pest populations ample time to establish and cause damage to crops (Pankanin-Francz and Ceryngier 1995). Pests can also acquire symbiont-mediated resistance to parasitoids (endosymbionts that actively or passively render the host less suitable for parasitoids), thus reducing their suppression by these biocontrol agents, sometimes to the extent of local parasitoid extinction (Symondson *et al.* 2002; Käch *et al.* 2018).

Polyphagous generalist predators are abundant in arable fields and have been regarded as beneficial biocontrol agents of insect crop pests such as aphids for decades (Riechert and Lockley 1984; Mark Alderweireldt 1994; Chapman *et al.* 2013). Generalist predators can complement the beneficial activities of specialist species for enhanced biocontrol (Sunderland *et al.* 1997), but can equally suppress specialists and one another via intraguild predation (Traugott and Symondson 2008; Moreno-Ripoll *et al.* 2012; Traugott *et al.* 2012; Davey *et al.* 2013; Moreno-Ripoll *et al.* 2014). Aphid biocontrol is widely based across many taxa, primarily including spiders, beetles and mites but also many specialist species (Sunderland 1975; Sunderland *et al.* 1987; Shayler 2005). Since generalist predators do not depend on a single species, they can emerge before pest species and sustain themselves on alternative prey, thus maintaining large populations and suppressing multiple pest species prior to crop damage

(Riechert and Lockley 1984; Chang and Kareiva 1999). Pest population densities can impact the frequency of predation though, with some predators only attacking aphids at high densities (Sunderland and Vickerman 1980). Consumption of alternative prey by generalist predators can, however, detract from their control of pests (Symondson *et al.* 2006; Gavish-Regev *et al.* 2009). Optimal predator fitness requires a diverse diet of pest and non-pest prey, with predators exclusively fed single species such as aphids suffering losses to growth rate and fecundity (Harwood *et al.* 2009). By promoting predator fitness, alternative prey may thus positively affect the biocontrol efficacy of predators (Ostman 2004; Roubinet *et al.* 2017), although these effects are taxon-specific (Symondson *et al.* 2006). To understand and refine how generalist predators can be used for biocontrol, the mechanisms underlying their prey choice must first be understood (Chapman *et al.* 2013).

1.3. Spider biocontrol and prey choice

Globally, spiders are among the most abundant predatory terrestrial arthropods, found across every life-supporting land mass (Turnbull 1973). Most spider species are polyphagous (broad dietary range) generalist predators, with only a few examples of stenophagy (narrow dietary range; Gajski *et al.* 2020) and monophagy (single species diet; Petráková *et al.* 2015). A large number of spiders are naturally abundant in cereal crops, reaching densities of 200-600 m^{-2} in UK crops (Nyffeler and Sunderland 2003; Shayler 2005). Pest suppression is reduced in regions of lower spider density (Greenstone 2001). Spiders represent a diversity of foraging techniques, including sit-and-wait and active hunting (Turnbull 1973; Riechert and Lockley 1984), which influence food webs via strategy-specific prey capture (Michalko and Pekár 2016). Different prey species are active at different times in the diel cycle, resulting in spiders foraging both diurnally and nocturnally (Bollinger *et al.* 2015). Over-wintering adult spiders are present at the inception of pest emergence and population increase and are the dominant winter predator in many systems (Juen *et al.* 2002; Korenko *et al.* 2010; Boreau De Roincé *et al.* 2013). Spiders will often attack prey on encounter regardless of hunger, termed superfluous killing; this may relate to the time necessary for external digestion of the prey after the spider has immobilised it. The spider might not return to consume the prey, resulting in more prey killed than are required by the spider, which is advantageous for pest management (Riechert and Lockley 1984; Sunderland 1999). Spiders do, however, scavenge on dead prey, somewhat balancing out their superfluous killing (von Berg *et al.* 2012).

Spider webs indicate investment in foraging by individual spiders and can be easily simulated for studies of prey choice (Welch *et al.* 2016). Spiders will rapidly colonise favourable web locations with high prey densities, sometimes engaging in territorial contests (Riechert and

Lockley 1984; Samu *et al.* 1996; Harwood *et al.* 2001; Bollinger *et al.* 2015). Spiders can indirectly suppress pest populations via capture and subsequent death of pests trapped in abandoned webs due to the low escape rate of pests from webs established in high prey density sites (Sunderland 1999; Harwood and Obrycki 2005). Prey availability can also directly affect web structure, with different structures used to attach the web for different prey (e.g. soil-surface-attached webs for springtails and plant-stem-attached webs for aphids; Welch *et al.* 2016).

Different spider species, even within the same family, will often build webs of different sizes and locations, sometimes varying additionally with maturity (Harwood *et al.* 2001; Harwood *et al.* 2003). For example, Erigoninae spiders will often build small webs near to the ground which they leave regularly to hunt itinerantly, whilst Linyphiinae spiders produce larger sheet webs several centimetres higher in the vegetation (Sunderland *et al.* 1986; Figure 1.2). The spatial separation of the webs of different species may facilitate their complementary biocontrol activity through niche separation (Harwood *et al.* 2003) but it also separates the spiders, reducing instances of intraguild predation (Opatovsky *et al.* 2016). In fact, many spiders are thought to avoid building webs in the proximity of other active webs, further enhancing this separation (Opatovsky *et al.* 2016).

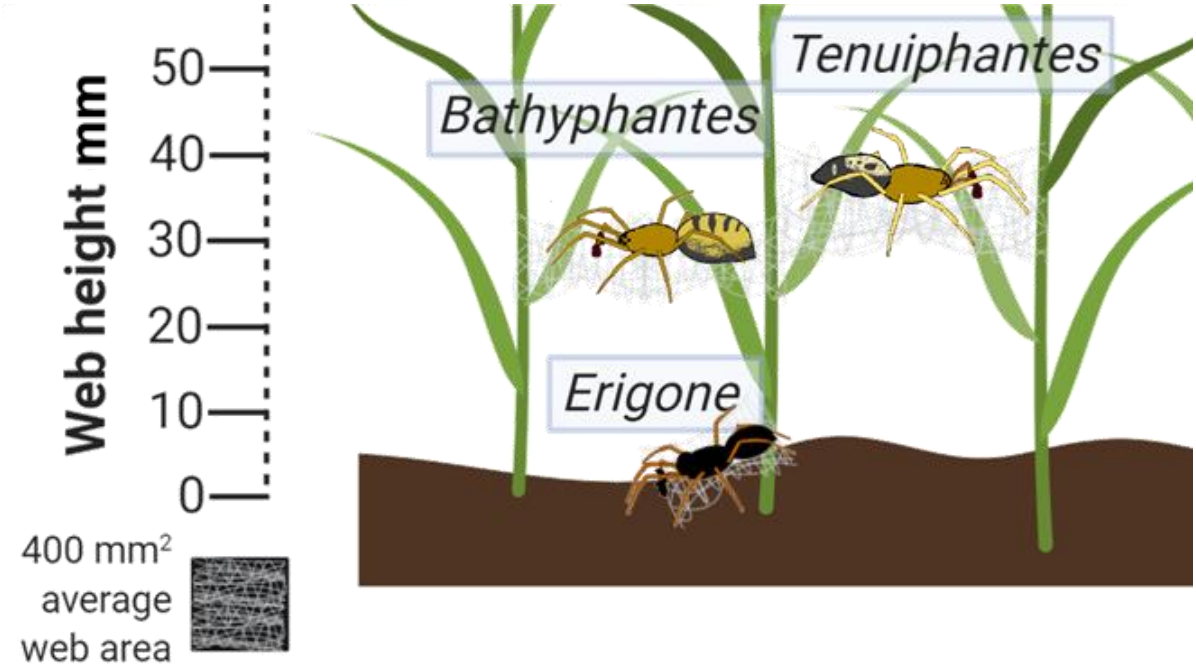


Figure 1.2: A visual representation of the different web height of three common linyphiid genera. *Bathyphantes* and *Tenuiphantes* are from the subfamily Linyphiinae, and *Erigone* of subfamily *Erigoninae*. Figure created in Biorender with spiders imported from custom MS paint drawings.

Many spiders disperse large distances via ballooning, which is the use of silk to sail through the air on currents (Greenstone 1990; Suter 1999; Bell *et al.* 2005). Many theories suggest that ballooning depends on thermal currents, but a recent study also suggests an important role of electrostatic potential (Morley and Robert 2018), although this study simulated extreme weather conditions far from 'fair weather' (i.e. 6250 Vm^{-1} vs. 'fair weather' 50-300 Vm^{-1}). Ballooning nonetheless requires suitable meteorological conditions (Greenstone 1990; Weyman 1993) and conspecifics tend to employ similar dispersal strategies, with increased ballooning occurring in autumn and winter (Woolley *et al.* 2016). Via ballooning, spiders quickly populate crop fields early in the year, providing additional biocontrol when pest populations are still establishing (Bishop and Riechert 1990; Weyman 1993; Suter 1999). Immigrant spiders contribute substantially to pest suppression, sometimes more so than existing spider populations (Opatovsky *et al.* 2016), proving particularly beneficial following the ecologically disruptive process of harvest and crop cycling (Opatovsky and Lubin 2012).

The diet of cereal crop spiders is commonly known to predominantly include springtails, flies and aphids (Toft 1995; Agustí *et al.* 2003; Piñol *et al.* 2014). Spiders have been considered biocontrol agents for decades (Riechert and Lockley 1984; Sunderland *et al.* 1997; Sunderland 1999), predating large numbers of pests such as aphids (Sunderland *et al.* 1986; Beck and Toft 2000; Mayntz and Toft 2000; Bilde and Toft 2001; Nyffeler and Sunderland 2003), planthoppers (Wang *et al.* 2016; B. Wang *et al.* 2017), psyllids (Petráková *et al.* 2016), medflies (Monzó *et al.* 2010), lepidopteran pests (Quan *et al.* 2011; Pérez-Guerrero *et al.* 2013; Senior *et al.* 2016), and weevils (Vink and Kean 2013). Whereas many generalist predators are disrupted by crop cycling, spiders have similar generation times to typical crop cycles, with peak abundances occurring at critical early phases in pest population establishment (Riechert and Lockley 1984; Welch *et al.* 2011). Several spider species have been shown to consume disproportionately high numbers of aphids (Sunderland *et al.* 1986; Harwood *et al.* 2005).

Aphids are of low nutritional value to spiders (Toft 1995; Bilde and Toft 2001). Spiders reared exclusively on aphids produce fewer eggs and develop much slower than those reared on flies; however, spiders reared on a diet of flies and aphids together produce larger offspring than those fed only flies, indicating a nutritional benefit to aphid consumption (Bilde and Toft 2001). Spiders have been observed preferentially seeking out aphids over alternative prey, such as springtails, possibly due to additional sensory cues from aphids, but alternatively suggesting a nutritional benefit to consuming aphids (Welch *et al.* 2016). In fact, Sunderland *et al.* (1986) found that aphids formed 38-63% of the diet of common linyphiids. Some aphid species, such as *S. avenae*, are thought to have a toxic effect on spiders, reducing the spider's fecundity, whilst others, such as *M. dirhodum* have fewer negative effects (Bilde and Toft

2001). Regardless of any toxic effect, spiders are often the most frequent predators of aphids among common polyphagous predators of cereal crops (Sunderland *et al.* 1987). Predation of aphids by spiders can vary between sexes, with female spiders tending to eat more aphids than males, and between taxa, with the subfamily Linyphiinae feeding on more aphids than spiders of Erigoninae (Harwood *et al.* 2004); however, itinerant Erigoninae spiders may encounter more aphids by actively searching for prey on the ground (Harwood *et al.* 2004; Gavish-Regev *et al.* 2009). It is difficult, however, to predict the efficacy of aphid predators via functional traits, with taxonomy, life stage and morphology serving as poor predictors of predation rates (Bell, Mead, *et al.* 2008).

The functional response of predators to prey, effectively determining prey choice, is determined by encounter rates, the search and handling time required, predator hunger, innate behaviours, current physiology and past experience with the prey (Holling 1966; Peterson *et al.* 2016; Welch *et al.* 2016). Generalist invertebrate predators are thought to employ prey switching, which is disproportionate feeding on the most common prey to increase foraging efficiency (Cornell 1976). Spiders have been shown to forgo abundant prey in favour of prey less locally abundant, indicating prey choice beyond density-dependent predation (Agustí *et al.* 2003; Welch *et al.* 2016). The determination of the diet of *in situ* predators necessary for an investigation of the complex underlying mechanisms of foraging in the field has, however, traditionally involved techniques such as direct observation that would introduce sources of error or bias (Sheppard and Harwood 2005). With the advancement of molecular techniques and the use of null models to analyse prey choice, increasingly ambitious experiments can now be carried out *in situ* to elucidate the mechanisms underlying prey choice (Clare 2014; Vaughan *et al.* 2018).

1.4. Metabarcoding for trophic ecology

1.4.1. Molecular analysis of trophic interactions

Trophic interactions underlie most ecosystem processes and understanding the diet of an animal is useful in determining ecosystem health, response to environmental change, conservation strategies and prey choice (Murray *et al.* 2011; Piñol *et al.* 2014; Peterson *et al.* 2016). Assessing an animal's diet via direct observation is both labour-intensive and prone to bias (Symondson 2002; Birkhofer *et al.* 2017). Many alternative techniques, such as hard-parts analysis, provide insufficient taxonomic resolution and render the detection of soft-bodied prey and the prey of fluid-feeding predators impossible (Symondson 2002; Pompanon *et al.* 2012; Birkhofer *et al.* 2017). The trophic interactions of fluid-feeding predators, which comprise most terrestrial arthropod predators, must therefore be studied via molecular

methods (Greenstone *et al.* 2007). Serological techniques, such as monoclonal antibody-based analyses of diet, allow detection of a specific taxon in the gut of a predator and can be designed to detect specific stages of development, but these techniques are laborious, expensive and difficult to apply to the diet of generalist predators (Symondson 2002; Greenstone *et al.* 2007).

Through advances in DNA sequencing over the last few decades, it is possible to identify species by short fragments of their DNA, termed DNA barcoding (Hebert, Ratnasingham, *et al.* 2003; Hebert, Cywinska, *et al.* 2003). By using specific polymerase chain reaction (PCR) primers, which are short oligonucleotides complementary to phylogenetically-conserved regions of DNA flanking variable regions, short fragments of DNA can be selectively amplified, even in minute concentrations (Symondson 2002; Paula *et al.* 2015). Assuming the DNA fragment is sufficiently long to account for the mutation rate of the targeted gene region, species can then be distinguished from one another by their barcode and can be identified by comparison of the resultant sequence against a reference library of DNA barcodes (Hebert, Cywinska, *et al.* 2003). The use of several taxon-specific primer pairs to each detect a single taxon from dietary DNA allows compilation of the species present in a sample taxon-by-taxon but is laborious and expensive (Harper *et al.* 2005) and requires a comprehensive prior knowledge of the taxa consumed, unless targeting a specific taxon. Multiplex PCR, which is the simultaneous amplification of different DNA targets via parallel use of multiple primer pairs, circumvents the need to conduct a multitude of PCRs with no apparent loss of represented biodiversity (Harper *et al.* 2005; King *et al.* 2011). The different efficiencies of primers used in multiplex PCR must, however, be considered when interpreting the results and prior knowledge of the species expected to be present is still required to select primers (Sint *et al.* 2012). Instead, ‘universal’ PCR primers can be used, which amplify a broad spectrum of potential prey species, including non-target organisms, but at the cost of preferential amplification of those species with sequences most complementary to the primer site (Folmer *et al.* 1994; Sharma and Kobayashi 2014; Piñol *et al.* 2018).

For DNA samples taken from the environment without first isolating specific target organisms, termed environmental DNA (eDNA), barcoding is slightly more complicated due to natural degradation over time and DNA being present in a mixed community (Taberlet *et al.* 2012; Bohmann *et al.* 2014). Extraction and amplification of eDNA has facilitated sensitive non-invasive biological surveys of material with low concentrations of DNA, including air (Folloni *et al.* 2012), water (Thomsen *et al.* 2012), soil (Andersen *et al.* 2012) and faeces (King *et al.* 2008; Bohmann *et al.* 2014; Clare 2014). Environmental DNA was first used in microbial studies (Ogram *et al.* 1987; Rondon and Al 2000; Handelsman 2005; Taberlet *et al.* 2012), but has been increasingly adopted in macroecological research. Barcoding gut or faecal DNA for

dietary analysis facilitates accurate identification of those prey consumed by a predator (King *et al.* 2008; Clare 2014; Pompanon *et al.* 2012). Barcoding-based studies of diet can be performed *ex situ* on samples collected *in situ* or post-mortem and, via faecal analysis, allow non-invasive dietary analysis (King *et al.* 2008; Pompanon *et al.* 2012; Birkhofer *et al.* 2017). Molecular methods for dietary studies reduce labour whilst increasing identification accuracy and detectability of target taxa, when compared to traditional techniques (Soininen *et al.* 2009; Deagle *et al.* 2013; Nielsen *et al.* 2018).

High-throughput sequencing (HTS) now facilitates the sequencing of millions of DNA strands in parallel from samples containing many taxa. When combined with barcoding, many species can be identified in parallel from eDNA, termed metabarcoding (Pompanon *et al.* 2011; Taberlet *et al.* 2012; Bohmann *et al.* 2014; Cristescu 2014; Deiner *et al.* 2017). Direct DNA shotgun-sequencing (the parallel sequencing of long fragments of DNA without need for amplification, usually to assemble genomes) circumvents issues with amplification bias and quantification, and has been used with some success (Paula *et al.* 2015; Coissac *et al.* 2016; Paula *et al.* 2016; Bista *et al.* 2018). Metabarcoding via PCR amplification of target species is, however, still predominantly used in eDNA studies due to the high costs associated with achieving the sequencing depth (i.e. the number of sequencing reads per sample) necessary for shotgun-sequencing of eDNA, the paucity of reference data (i.e. the databases used to taxonomically identify sequenced DNA) suitable for shotgun sequencing, and the low prey read counts obtained, which are susceptible to false positives through contamination or error (Paula *et al.* 2016). In recent years, increasingly novel sequencing platforms, now including nanopore sequencing (sequencing by pulling DNA strands through small protein pores and identifying sequences by differential detection of electric currents), can be used for the generation of increasingly viable and cost effective metabarcoding data (Baloğlu *et al.* 2020). Metabarcoding has been successfully used for biodiversity surveys (Ji *et al.* 2013; Deiner *et al.* 2017), biomonitoring (Ji *et al.* 2013; Beng *et al.* 2016; Stat *et al.* 2017), disease monitoring (Batovska *et al.* 2018), ecological network construction (Evans *et al.* 2016) and dietary analysis (Pompanon *et al.* 2012; Clare 2014).

Metabarcoding has been used to study the diet of carnivores (Deagle *et al.* 2009; Birkhofer *et al.* 2017; Galan *et al.* 2018), herbivores (Soininen *et al.* 2009; Kartzinel *et al.* 2015) and omnivores (Barba *et al.* 2014; Robeson *et al.* 2018). Whilst such DNA-based techniques cannot differentiate predation from scavenging or secondary predation (Calder *et al.* 2005; Foltan *et al.* 2005; Sheppard *et al.* 2005; von Berg *et al.* 2012) and cannot distinguish between life stages, they do provide an accurate method for dietary analysis. Given the reliance of metabarcoding on amplification of DNA, the selection of appropriate PCR primers is possibly the most critical step (Piñol *et al.* 2018). Primers must be designed to amplify the DNA of all

target species simultaneously from mixed communities, for which an appropriate marker must be selected to optimise the output of data (Deagle *et al.* 2014; Elbrecht and Leese 2016a; Elbrecht and Leese 2016b; Elbrecht and Leese 2017).

1.4.2. Markers and primers in dietary metabarcoding

Most animal barcoding thus far has been based on the mitochondrial cytochrome *c* oxidase subunit I (COI) gene, which encodes the cytochrome c oxidase enzyme, the terminal component of the electron transport chain involved in mitochondrial respiration (Anderson *et al.* 1981). The COI gene is universally present across most taxa of interest to macro-ecological dietary studies, with the exception of a few protozoa (Folmer *et al.* 1994). In barcoding, COI is commonly used due to the ideal mutation rate in animals for species-specific identification (Hebert, Cywinska, *et al.* 2003) and the extensive sequence databases available for many taxa (Deagle *et al.* 2014; Porter and Hajibabaei 2018). Alternative markers (i.e. genes used for barcoding), whilst circumventing the bias associated with highly-variable COI primer sites, often lack the extensive reference libraries of barcode sequences, and less variable amplicons are difficult to resolve to species level (Deagle *et al.* 2014; Elbrecht *et al.* 2016). The creation of a comprehensive reference library for the barcodes of prey species of a generalist predator can be very laborious and expensive, rendering markers like COI favourable for the ease of designing, testing and using primers (Deagle *et al.* 2014).

Context-specific PCR primers can be designed for metabarcoding within COI for detection of predetermined target taxa, allowing simultaneous assessment of many prey in dietary DNA samples (Littlefair and Clare 2016). Metabarcoding primers are often shorter than those typically used for standard barcoding to account for degradation of DNA in environmental samples (Zaidi *et al.* 1999; Symondson 2002; Paula *et al.* 2015); for example, primers typically used for animal barcoding produce a 658 bp amplicon (Folmer *et al.* 1994), whereas metabarcoding primers tend to be 100-350 bp long to facilitate detection of degraded DNA, including semi-digested prey DNA in the guts and faeces of predators (Zeale *et al.* 2011; Leray *et al.* 2013; Elbrecht and Leese 2016a; Vamos *et al.* 2017). The half-life of DNA in the gut can be estimated empirically to approximate the length of time for which a given length of DNA can be detected, through which semi-quantitative predation rates can be calculated (Greenstone *et al.* 2007; Egeter *et al.* 2015; Uiterwaal and DeLong 2020); this is complicated in spiders, which have highly variable metabolic rates (Sheppard *et al.* 2005; Greenstone *et al.* 2014).

Metabarcoding primers for dietary analysis must amplify the DNA of a full range of the prey species of interest, ideally without amplifying the DNA of the predator. Given the degraded

quality of the prey DNA and the intact DNA of the predator, amplification of the predator is much more efficient and is likely to comprise a large contingent of the PCR product (Waldner *et al.* 2013; Paula *et al.* 2015). To circumvent this issue, blocking probes were developed, which prevent amplification of the DNA of specific taxa (Vestheim and Jarman 2008; Figure 1.3) but these have been found to introduce biases of their own and can have unpredictable non-target effects (Murray *et al.* 2011; Piñol *et al.* 2015). Instead, primers can be designed carefully with a comprehensive reference database to amplify only target species, or amplification of the predator must be accepted and bioinformatically filtered out (Piñol *et al.* 2014; Figure 1.3). Primers can be tested *in silico* (i.e. computationally through simulated PCR reactions) to evaluate the likelihood of amplifying a large number of target species prior to investing in reagents and locating and extracting DNA from a broad range of target species (Ficetola *et al.* 2010; Clarke *et al.* 2014; Elbrecht and Leese 2016b; MacDonald and Sarre 2016). Results from *in vitro* (i.e. lab-based) testing, which can differ substantially from *in silico* results, can then be used to confirm the range of taxa amplified by the primer pair.

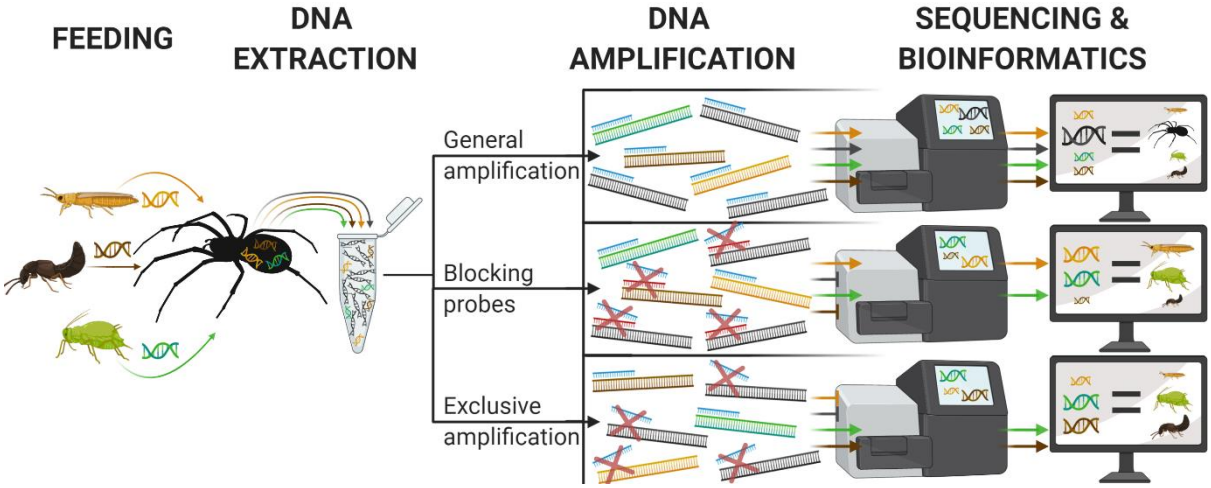


Figure 1.3: The dietary metabarcoding process, from feeding through to bioinformatics, with three types of DNA amplification. The focal predator ingests the DNA of its prey. Extraction of DNA from gut contents will capture degraded prey DNA from the guts, and longer undegraded DNA of the predator. Amplification using general primers (primers shown in blue) will amplify the prey of the predator alongside all prey, which is sequenced and represented in data output, sometimes outcompeting the DNA of prey taxa. Blocking probes (red primers) can prevent predator DNA amplification, but also exhibit bias against some prey taxa, causing their absence or reduction in sequencing and data output. Exclusive amplification, the use of primers that do not amplify DNA of the predator, can similarly neglect amplification of some prey DNA, causing their absence or reduction in the data output. Figure created in Biorender.

Appropriate use of these molecular techniques can generate answers to long-standing ecological questions, many of which would not be currently possible otherwise. Through application of these methods to the understanding of prey choice, the mechanisms underlying

biocontrol of crop pests by naturally-occurring predators can be elucidated, including theories suggesting that predators will diversify their diet to redress nutritional deficiencies by seeking prey rich in those macronutrients lacking in their diet (Bilde and Toft 2001; Mayntz *et al.* 2005; Welch *et al.* 2016).

1.5. Nutrient-specific foraging

Caloric restriction was previously thought to dictate lifespan and fitness, but macronutrients have an even greater influence (Fanson *et al.* 2017). Dietary macronutrients, comprising proteins, carbohydrates and lipids, are necessary for the development and survival of all animals, each performing a distinct set of vital roles, from energy provision to cell signalling (Cheng *et al.* 2011; Roeder and Behmer 2014). Invertebrates are often intimately involved in the cycling of nutrients in the environment and their community structure is largely dependent upon these processes (Atkinson *et al.* 2016). Changes to local nutrient abundances manifest in bottom-up effects on invertebrate community structure and, ultimately, top-down effects via increased predator abundance and fitness (Fountain *et al.* 2008; Fountain *et al.* 2009). For invertebrates, a balanced macronutrient intake is necessary for many functions, and deficits can impact fitness, survival, body composition, behaviour, immunity, reproductive performance and development, among other effects (Woch *et al.* 2009; Barry and Wilder 2013; Neeson *et al.* 2013; Bong *et al.* 2014; Rho and Lee 2015; Bunning *et al.* 2016; Littlefair *et al.* 2016; Srygley 2017). Macronutrient intake has also been shown to affect behaviour, including prey choice via compensatory feeding to redress dietary deficiencies, termed nutrient-specific foraging (Mayntz *et al.* 2005).

Nutrient-specific foraging has been demonstrated in controlled experiments spanning a large range of predatory taxa (Mayntz *et al.* 2005; Kohl *et al.* 2015; Fanson *et al.* 2017). These include invertebrates such as spiders (Araneae), beetles (Coleoptera), flies (Diptera) and ants (Hymenoptera: Formicidae; Mayntz *et al.* 2005; Raubenheimer *et al.* 2007; Christensen *et al.* 2010; Jensen *et al.* 2011; Jensen *et al.* 2012; Mooney *et al.* 2016; Fanson *et al.* 2017), and vertebrates, both marine and terrestrial, such as cats (Carnivora: Felidae), dogs (Carnivora: Canidae), mink (Carnivora: Mustelidae), carp (Cypriniformes: Cyprinidae), sole (Pleuronectiformes: Soleidae), trout (Salmoniformes: Salmonidae) and bass (Perciformes; Yamamoto *et al.* 2001; Rubio *et al.* 2003; Mayntz *et al.* 2009; Rubio *et al.* 2009; Hewson-Hughes *et al.* 2011; Hewson-Hughes, Hewson-Hughes, Colyer, Miller, Hall, *et al.* 2013; Hewson-Hughes, Hewson-Hughes, Colyer, Miller, McGrane, *et al.* 2013; Jensen *et al.* 2014; Kohl *et al.* 2015). Spiders have been observed selecting theoretically nutritionally suboptimal

prey despite the availability of alternatives, suggesting a benefit to dietary diversification, thought to be macronutrient-based (Welch *et al.* 2016).

Nutrient-specific foraging can occur at any stage of predation, from the selection of specific prey, to the consumption of different quantities of different prey or the extraction of specific nutrients (Kohl *et al.* 2015; Pekár *et al.* 2010). Vertebrate predators, for example, have been observed consuming specific organs such as the liver, thought to relate to tissue-specific macronutrient content (Kohl *et al.* 2015). The primary macronutrient sought by nutrient-specific foraging predators may change seasonally and with life stage to reflect the different macronutrients required for growth and development, reproduction and overwintering (Bressendorff and Toft 2011). Cereal crop systems could be manipulated based on an understanding of the mechanisms underlying prey choice, for example by using the habitat manipulation and diversification methods discussed above, to increase crop productivity by aiding native predators and facilitate sustainable intensification of agriculture (Gurr *et al.* 2016).

1.6. Thesis outline

1.6.1 Study system

This project primarily focuses on money spiders (Araneae: Linyphiidae) as a model for prey choice in polyphagous generalist predators of agricultural systems and secondarily includes wolf spiders (Araneae: Lycosidae) in the same capacity. Spiders are a large component of the epigeal predators of cereal crops, mostly comprised of species in the families Araneidae, Linyphiidae, Lycosidae, Salticidae, Tetragnathidae, Theridiidae and Thomisidae; of these, linyphiids are typically most abundant in European cereal crops (Nyffeler and Sunderland 2003).

Comprising the largest family of the over 650 spider species in the British Isles (Merrett *et al.* 2014), many linyphiid species are associated with cereal crops (Nyffeler and Sunderland 2003; Welch *et al.* 2011). Common linyphiid genera of cereal crops include *Erigone*, *Tenuiphantes*, *Oedothorax* and *Bathyphantes* (Sunderland *et al.* 1986), together comprising a large contingent of cereal crop spiders (Nyffeler and Sunderland 2003; Welch *et al.* 2011). Web-building spiders are a good model of generalist arthropod predators for prey choice studies given the ease with which prey encounter rates can be estimated by simuation of webs via sticky traps (Welch *et al.* 2016). Webs also provide an indication of foraging behaviour and investment which can be monitored and recorded (Welch *et al.* 2016). The webs of linyphiids are typically horizontal sheets varying in size and location based upon species and maturity

(Harwood *et al.* 2001; Harwood *et al.* 2003). Erigoninae spiders (e.g. *Erigone atra*) will typically produce small ground-based webs which they will abandon regularly to hunt on the ground, whilst Linyphiinae spiders (e.g. *Tenuiphantes tenuis*) will build larger sheet webs several centimetres from the ground (Sunderland *et al.* 1986).

The high rate of ballooning in linyphiids can be a confounding variable to consider when analysing their recent diet, since individuals may be recent arrivals from afar (Suter 1999). Whilst it is possible to account for ballooning rates (Woolley *et al.* 2007; Woolley *et al.* 2016), a second model group, wolf spiders (lycosids), was selected for this project given their reduced propensity for ballooning. The analysis of two groups will support a broader taxonomic relevance of any results and will lessen any experimental issues relating to the ecology of a single group. Lycosids, whilst still capable of ballooning, tend to do so as juveniles and less regularly than linyphiids (Richter 1970). Whilst web-predators such as linyphiids will detect prey via web vibrations at any time, active-hunting spiders, such as lycosids, forage on the ground, mostly at night (Shayler 2005). Lycosids can reach densities of up to 60-90 individuals per m^2 (Wagner and Wise 1996). Whilst less efficient aphid predators than linyphiids, lycosids still eat many aphids, with 20% of their diet thought to be comprised of aphids (Sunderland *et al.* 1986; Kuusk *et al.* 2008).

1.6.2. Thesis aim and objective

The primary aim of this project was to identify whether nutrient-specific foraging occurs in the field. The overall objective was to metabarcode spider gut contents, comparing these against local prey densities and prey nutrient contents in prey choice models. The design of appropriate PCR primers was first required for the metabarcoding of spider prey, and the determination of the macronutrient content of spider prey species was necessary, for which a novel streamlined protocol was needed. The overall dietary dynamics of cereal crop spiders were broadly investigated to determine optimal candidates for spider-mediated biocontrol among those spiders commonly found in British cereal crops.

1.6.3. Thesis hypotheses

From the aims outlined, six hypotheses are drawn: (i) macronutrients can be extracted and quantified from a single small invertebrate; (ii) PCR primers can be designed for the broad metabarcoding of spider prey whilst reducing amplification of undegraded spider DNA; (iii) spider diet will vary between genera and with time; (iv) spider prey choice is not based solely on the relative abundance of their prey; (v) closely-related invertebrates will have similar

macronutrient contents, whilst distant taxa will be dissimilar, allowing taxonomy-based generalisation of macronutrient contents; (vi) nutrient-specific foraging occurs in the field.

1.6.4. Chapter outlines

To address the hypotheses detailed above, this thesis will form six chapters:

i. **Chapter 1** has introduced the major themes and knowledge gaps that this thesis will address, establishing the background for the overall narrative.

ii. **Chapter 2**, “MEDI: Macronutrient Extraction and Determination from Invertebrates, a rapid, cheap and streamlined protocol”, details the development of a protocol for the determination of macronutrient content in small invertebrates.

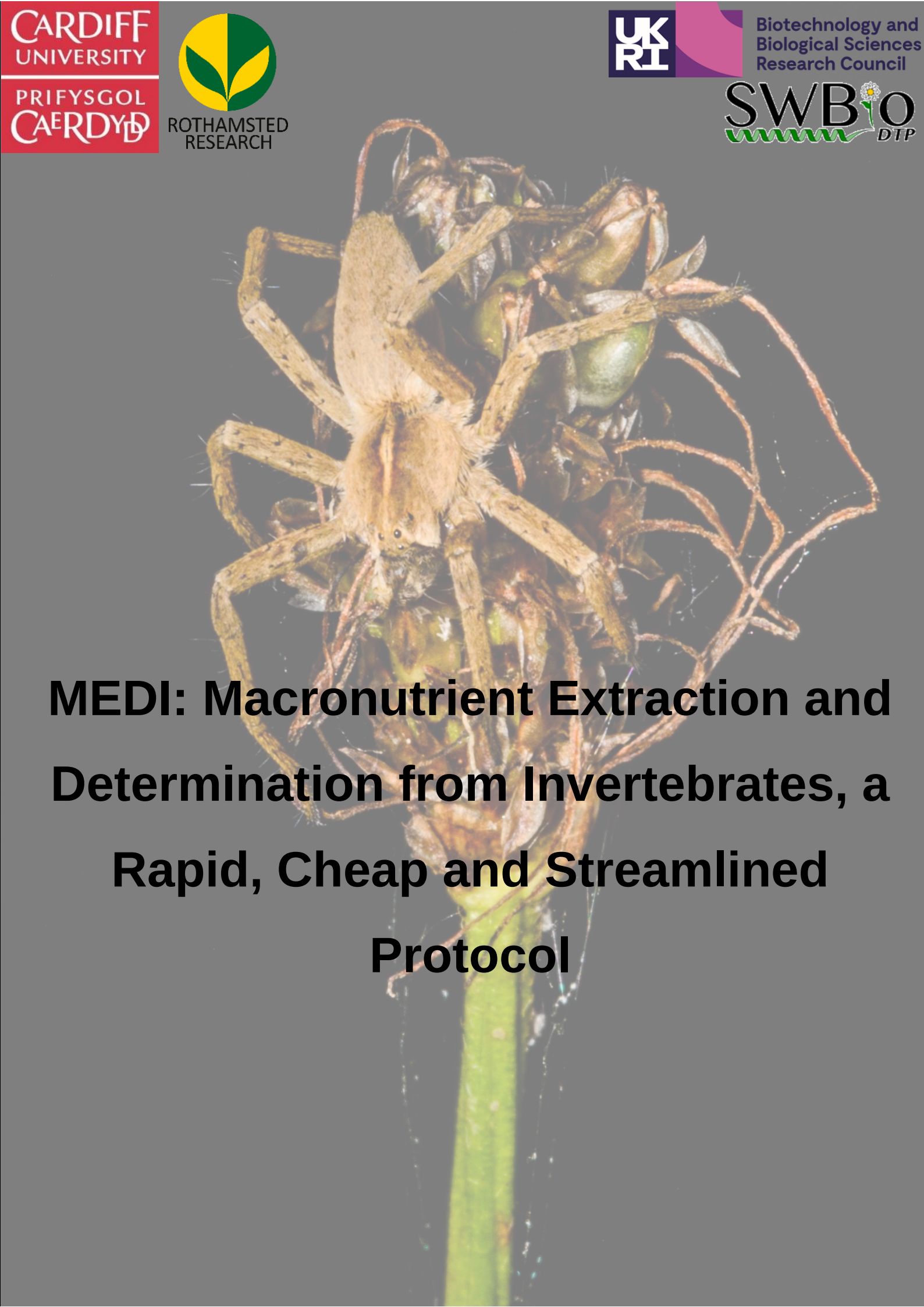
iii. **Chapter 3**, “Browsing the web: the design and evaluation of PCR primers for the analysis of linyphiid prey choice using metabarcoding”, details the design and development of novel PCR primers for spider dietary analysis via DNA metabarcoding, and provides a proof-of-concept for the analysis of spider prey choice in the field.

iv. **Chapter 4**, “A moment on the lips, a lifetime on the thrips: an analysis of the diet and biocontrol potential of spiders in cereal crops”, determines the diet of a large cohort of cereal crop spiders and assesses the factors affecting spider diet with a focus on biocontrol and intraguild predation.

v. **Chapter 5**, “Dude, where’s my carbs? Nutrient-specific foraging by spiders in cereal crops”, investigates dietary, macronutrient and prey availability data to identify evidence for nutrient-specific foraging in the field.

vi. **Chapter 6**, “A synthesis of investigations into nutrient-specific foraging and the role of spiders as biocontrol agents”, synergistically discusses the findings of each of these chapters with reference to a broader scientific context.

vii.



MEDI: Macronutrient Extraction and Determination from Invertebrates, a Rapid, Cheap and Streamlined Protocol

“The spider and the fly can't make a deal.”

- Jamaican proverb

Chapter 2 : MEDI: Macronutrient Extraction and Determination from Invertebrates, a rapid, cheap and streamlined protocol

This chapter has been published in manuscript form under the same title in Methods in Ecology and Evolution.

2.1. Abstract

1. Macronutrients, comprising carbohydrates, proteins and lipids, underpin many ecological processes, but their quantification in ecological studies is often inaccurate and laborious, requiring large investments of time and bulk samples, which make individual-level studies impossible. This study presents MEDI (Macronutrient Extraction and Determination from Invertebrates), a protocol for the direct, rapid and relatively low-cost determination of macronutrient content from single small macroinvertebrates.
2. Macronutrients were extracted by a sequential process of soaking in 1:12 chloroform:methanol solution to remove lipid and then solubilizing tissue in 0.1 M NaOH. Proteins, carbohydrates and lipids were determined by colorimetric assays from the same specimens.
3. The limits of detection of MEDI with the equipment and conditions used were 0.067 mg ml^{-1}, 0.065 mg ml^{-1} and 0.006 mg ml^{-1} for proteins, carbohydrates and lipids, respectively. Adjusting the volume of reagents used for extraction and determination can broaden the range of concentrations that can be detected. MEDI successfully identified taxonomic differences in macronutrient content between five invertebrate species.
4. MEDI can directly and rapidly determine macronutrient content in tiny (dry mass ~3 mg) and much larger individual invertebrates. Using MEDI, the total macronutrient content of over 50 macroinvertebrate individuals can be determined within around three days of collection at a cost of ~£1.02 per sample.

2.2. Introduction

The macronutrient content of invertebrates, comprising proteins, carbohydrates and lipids, underpins many ecological processes, including biodiversity maintenance (Asmus *et al.* 2018), crop yield losses (Behmer 2009) and trophic interactions (Mayntz *et al.* 2005). Unbalanced macronutrient intake can have substantial effects on fecundity, survival, and behaviour (Woch *et al.* 2009; Barry and Wilder 2013; Bong *et al.* 2014). The macronutrient content of prey species is thus thought to affect a predator's choice of prey (Mayntz *et al.* 2005; Raubenheimer *et al.* 2007; Jensen *et al.* 2012; Kohl *et al.* 2015).

Despite the relevance of macronutrients to a broad range of applications, few ecological studies quantify them. Many studies concerned with the macronutrient content of invertebrates use analogues, such as nitrogen as a surrogate for protein (e.g., crude protein = nitrogen x 6.25; Jones 1931; Finke 2005; Pekár and Mayntz 2014; Bryer *et al.* 2015). This allows broad-scale studies of ecological stoichiometry in trophic networks, focusing on the ratios of analogous elements such as carbon, nitrogen and phosphorous (Anderson and Hessen 2005; Frost *et al.* 2005; Raubenheimer *et al.* 2009). Whilst broadly useful, these analogues can produce inaccurate results since, for example, nitrogen is present in many non-protein constituents of invertebrates, including exoskeleton (Jones 1931; Raubenheimer *et al.* 2009; Janssen *et al.* 2017). Correction factors may circumvent these issues, but one correction factor is unlikely to work for all species given the vast diversity of invertebrates (Janssen *et al.* 2017). Additionally, some analyses of macronutrient content use gravimetric methods (e.g. Pekár and Mayntz 2014) which require either bulk samples (~1 kg insect material for Finke (2013)) or very fine, often expensive, scales for the determination of macronutrient mass, long waiting times, and often still rely on analogues. Bulk samples are laborious to collect and process, impeding multi-taxon or individual-level analyses (Bryer *et al.* 2015).

Methods have previously been developed for determining the macronutrient content of small single macroinvertebrate samples (e.g. Lu *et al.* 2008), but these are standalone protocols each tailored to only one macronutrient, tripling the collection effort necessary to determine the content of each macronutrient from a population and making individual-level studies impractical. By implementing a uniform extraction method and streamlining a protocol to determine all three macronutrient contents from a single specimen, data output would increase whilst reducing sampling effort. A protocol has yet to be published which uses direct measures of all three macronutrients taken in parallel from single small invertebrate specimens. Standardised adoption of such a protocol would also ultimately benefit future meta-analyses. For individual-level determination of macronutrient content, or studies involving particularly

small or scarce invertebrates, there is need for a standardised approach to directly determine macronutrient content in parallel from single macroinvertebrate specimens.

2.2.1. Objectives and hypotheses

This chapter concerns the development of a streamlined protocol for the determination of macronutrient content from small invertebrate specimens. The aims of this protocol are to: (i) determine the content of all three macronutrients from the same individual specimen; (ii) directly determine macronutrients (i.e. without using analogues) via colorimetric assays; and (iii) rapidly and cost-effectively produce macronutrient data from samples to facilitate high-throughput screening. This protocol will enhance the study of macronutrient content in invertebrates and other small samples in contexts including trophic interactions, parasitology and developmental biology. This will be applied in **Chapter 5** to the first analysis of nutrient-specific foraging in the field.

2.3. Methodology

2.3.1. Materials

All materials, unless stated otherwise, were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Flat bottom, 96-well microplates (Sterilin Microplate F Well), Pierce BCA Protein Assay reagents, Pierce Modified Lowry Protein Assay reagents and Pierce Coomassie Plus (Bradford) Assay reagents were obtained from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Ribbed, skirted 1.5 ml screwcap microtubes and caps were obtained from STARLAB (Hamburg, Germany). Sulfuric acid (95%) and phosphoric acid (85%) were obtained from Fisher Scientific (Pittsburgh, Pennsylvania, USA).

2.3.2. Macronutrient extraction

Macronutrient extraction is a two-step process that first involves extracting lipid and then solubilizing the remaining tissue for carbohydrate and protein analysis (Figure 2.1-2.2). Details of the methods will vary depending on the size of arthropod used. There are many important considerations when analysing the macronutrient content of arthropods (Table 2.1).

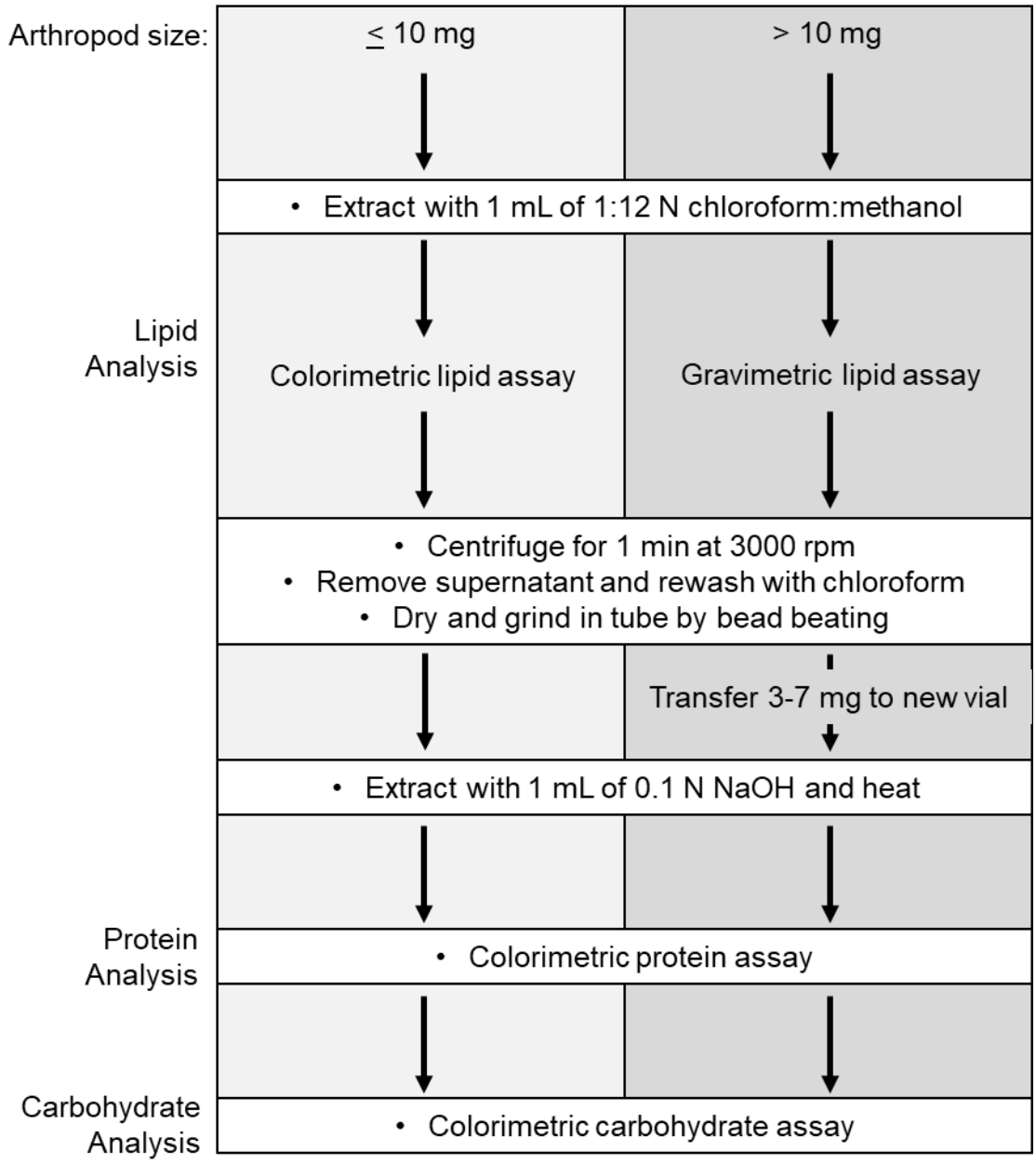


Figure 2.1: Workflow of MEDI for specimens of different sizes.

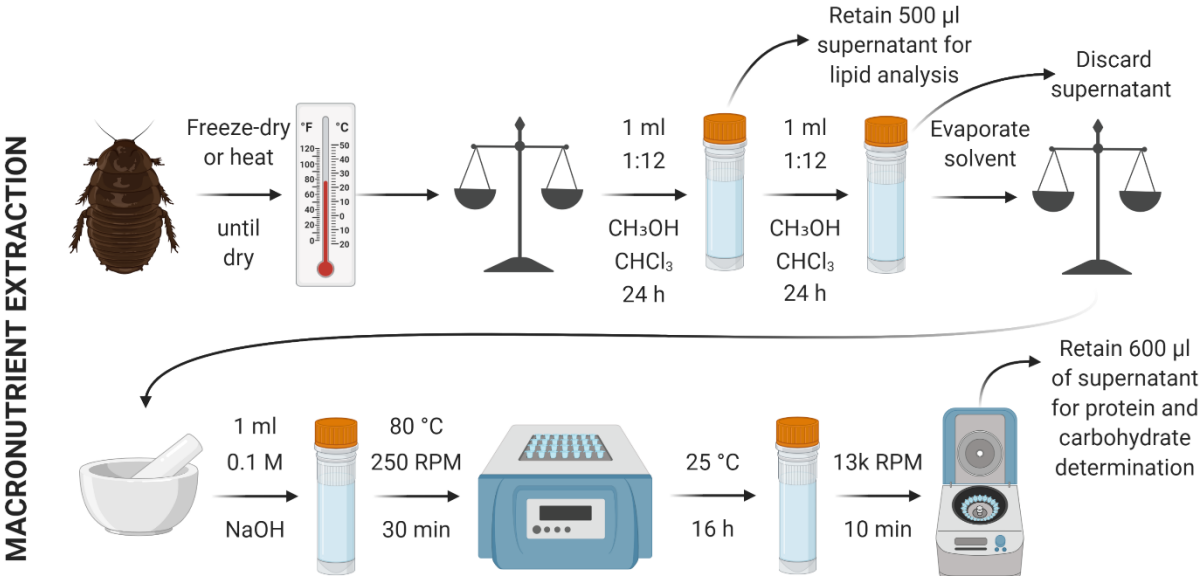


Figure 2.2: Protocol for the extraction of macronutrients from invertebrate bodies. Figure created using Biorender.com.

Table 2.1: Important considerations when analysing the macronutrient content of arthropod samples.

Technique	Options	Information	Best Practice Suggestion
Protein Assay	Crude protein (6.25 x % nitrogen)	Assumes that all nitrogen in a sample is in the form of protein with 16% nitrogen.	The estimated protein content of a sample will vary depending on the method used and each has biases. Ideally, analysis of hydrolysed amino acids could be used to determine which assay is most appropriate for a group of organisms. Alternatively, users can measure samples using multiple assays and take the average of those estimates.
	Bradford	Primarily reacts with arginine, lysine, and histidine.	
	BCA	Primarily reacts with cysteine/cystine, tyrosine, and tryptophan.	
	Lowry	Primarily reacts with cysteine/cystine, tyrosine, and tryptophan.	
	Hydrolysed amino acid analysis	Considered one of the most accurate measures of protein and provides measures of amino acid composition of samples but is far more expensive.	
Protein Standard	BSA vs IgG vs BGG	Protein standards differ in amino acid content. Given that protein assays primarily react with only several amino acids, the choice of protein standard will affect the estimate of protein measured with the assay.	Most protein assays note conversion factors that can be used to convert protein measures estimated with one standard to an estimate based on another standard. Users could take the average of the estimate from BSA and IgG rather than choosing to present data based on one or the other standard.

Lipid Assay	Colorimetric	Some will only, or primarily, measure certain types of lipids (e.g. the sulfo-phospho-vanillin assay only detects unsaturated lipids).	First consider the size of the invertebrate. Colorimetric assays are the most practical solution for very small invertebrates (e.g., < 5 mg dry mass). Then consider what lipids you want to measure to address the goals of your study (i.e. a specific type or all lipids).
		May be better for life history studies in which users are interested in measuring specific types of lipids.	
		May be used on any size of invertebrate, including individual collembolans or aphids.	
	Gravimetric	Measures total lipid content, which can include triglycerides and phospholipids. This is a very easy assay, especially on larger invertebrates. This can be a better measure of nutrients available to consumers of an arthropod.	
Carbohydrate Assay	Simple sugars	Not a common form of carbohydrate in insects, mainly found in sap or nectar feeding insects. Choice of standard (e.g., glucose vs. sucrose) may be important.	The user must consider the goals of the study, particularly the reason for measuring carbohydrates and which carbohydrates are most relevant to addressing the study question. The anthrone assay will detect simple sugars and will break down glycogen and trehalose, but other assays could be considered on a case-by-case basis for further applications.
	Glycogen and trehalose	These are common forms in which carbohydrates are stored in insects.	
Exoskeleton Determination		This assay measures the mass of exoskeleton present in an arthropod.	This may be useful to measure in studies of arthropod morphology or when measuring the quality of arthropods as food for predators since exoskeletal chitin is indigestible to most consumers.

The aphid *Metopolophium dirhodum*, house cricket *Acheta domesticus* (Linnaeus, 1758; Orthoptera: Gryllidae), German cockroach *Blattella germanica* Linnaeus, 1767 (Blattodea: Ectobiidae), mealworm larvae *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae) and springtail *Folsomia candida* Willem, 1902 (Entomobryomorpha: Isotomidae) were used to test the protocol's limits of detection, given their ease of cultivation and range of dry masses (in this study, *F. candida* mean 1.14 ± 0.55 mg, *M. dirhodum* 3.10 ± 0.65 mg, *A. domesticus* 22.20 ± 5.83 mg, *B. germanica* 22.53 ± 4.96 mg, *T. molitor* 36.20 ± 22.30 mg).

Samples were first weighed and lipids extracted by soaking whole arthropods in 1 ml of 1:12 chloroform:methanol for 24 hours (smaller specimens such as those <0.5 mg dry mass could be soaked in 0.5 ml for increased detectability, and larger specimens in larger volumes ~5x their body volume to ensure full submersion and to prevent saturation of the solvent). Half of the added volume of supernatant was then pipetted into a fresh tube for later lipid determination, the rest of the supernatant discarded, and any residue allowed to evaporate. This procedure for soaking arthropods was repeated for another 24 hours, but discarding all supernatant, to ensure all lipids were removed from the sample prior to protein and carbohydrate extraction. The change in dry mass of a sample before and after soaking in the solvent can also be used as an estimate of the lipid content of samples where practicable (i.e. gravimetric assay).

Following the extraction of lipids, the soft tissue of samples was digested to facilitate quantification of protein and carbohydrates. This procedure only measures the macronutrient content of the soft tissue of arthropods and not any protein that may be bound in the chitinous matrix of the exoskeleton during sclerotization. Whole arthropods were weighed, added to a microcentrifuge tube along with a stainless-steel bead (~3-7 mm diameter) and lysed at room temperature using a TissueLyser II (Qiagen, Hilden, Germany) for eight minutes at 30 Hz in two-minute increments. Larger samples can be ground (e.g., bead beating in an electronic bead mill, or mortar and pestle) and an approximately 5 mg subsample weighed into a clean tube. To each tube was added 1 ml of 0.1 M NaOH (or 0.5 ml for smaller specimens, e.g. <1 mg). Tubes were placed in a thermo-shaker at 80 °C and 250 RPM for 30 min, then removed and left at room temperature overnight (~16 h). Samples were centrifuged for 10 min at 13,000 RPM and 600 µl of supernatant pipetted into a separate tube for protein and carbohydrate determination. Supernatant was diluted prior to assaying such that the concentration of lean tissue (approximately 25–75 % protein for arthropods) was approximately 1 – 2 mg/ml to allow protein values to fall within the range of the protein assay kit (most commercial protein assay kits can measure 0.025 – 2 mg ml^{-1} protein). Dilution of supernatant or change in volume of NaOH used, along with the mass of sample used, must be accounted for in subsequent calculations of protein content.

2.3.3. Exoskeletal mass determination

The exoskeleton content of samples can also be measured, which may be of interest in morphological studies or those concerned with the nutritional quality of arthropods for consumers (Figure 2.3). A separate sample is required for this measurement. First, lipid should be completely extracted from the sample as described above. Then, the exoskeleton of the

sample should be lightly cracked (i.e. the exoskeleton split to facilitate entry of NaOH into the internal tissues) and 0.1 M NaOH (a volume approximately 5–10 times that of the sample) should be added to a vial with the sample. Samples should be heated for 2 hours at 80 °C and then allowed to soak overnight after which the NaOH should be removed and discarded. Centrifugation may help move the exoskeleton to the bottom of the vial. An additional volume of NaOH is added to the tubes and allowed to soak for 24 hours at room temperature, after which the NaOH can again be removed and discarded. Similar volumes of water should then be added to samples and removed twice to rinse any remaining NaOH from samples. Exoskeleton content is then the dry mass of sample remaining in the vial.

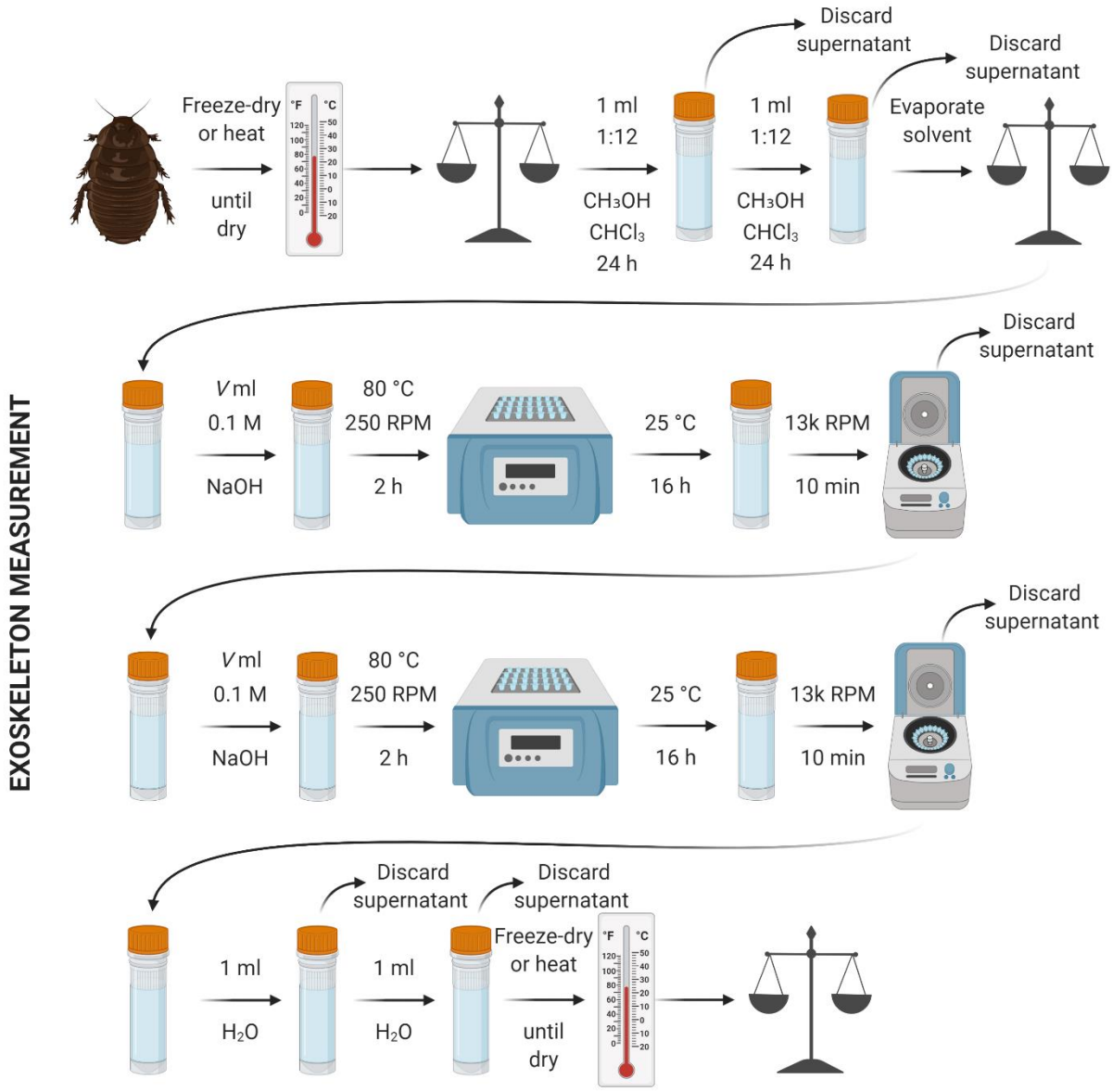


Figure 2.3: Protocol for the determination of exoskeletal mass. The volume (*V*) of NaOH used will vary depending on the amount of material, with 1 ml probably being sufficient for most ~5 mg specimens. Figure created using Biorender.com.

2.3.4. Macronutrient determination

Colorimetric assays were selected for the determination of macronutrients, given their ease-of-use and capacity for high-throughput assaying of samples in 96-well plates (Rodrı *et al.* 2008; Cheng *et al.* 2011; Supplementary Information 2.1). All absorbance measurements were obtained from a Tecan Infinity M200 Pro plate reader (Tecan Life Sciences, Männedorf, Switzerland) with Magellan v.7.1 software (Tecan 2011). For all assays, standard dilution series for calibration of absorbance readings consisted of 0-2 mg ml^{-1} in nine increments (0, 0.025, 0.125, 0.25, 0.5, 0.75, 1, 1.5 and 2 mg ml^{-1}; Table S2.1), with corn starch diluted in water, lard oil diluted in chloroform/methanol and bovine serum albumin (BSA) diluted in water for carbohydrates, lipids and proteins, respectively. For each assay, three repeats were taken from each sample and standard.

For determination of lipids, a sulfo-phospho-vanillin method adapted from Cheng et al. (2011) was used (Figure 2.4). This was adapted from a microplate colorimetric assay to estimate invertebrate lipid content, originally described by Van Handel (1985) and considered reliable for small samples (Lu *et al.* 2008). This method determines unsaturated lipid content; for total lipid content, gravimetric methods are the most appropriate option, but difficult for small invertebrates without using specialised scales. A standard dilution series was prepared with lard oil diluted with chloroform/methanol. Samples for lipid analysis comprised the initial supernatant taken after chloroform/methanol extraction. From each sample and standard, three repeats of 50 µl were placed in a heating block at 100 °C for approximately 10 min to evaporate the solvent, after which 10 µl concentrated sulfuric acid was added to each, vortexed and incubated at 100 °C for 10 min. The samples were cooled to room temperature and 240 µl vanillin reagent (1.2 mg dissolved in 0.2 ml hot water and 0.8 ml 85% phosphoric acid) was added and vortexed. After 5 min, 200 µl of each sample and standard were loaded into a 96-well microplate and absorbance at 490 nm was measured.

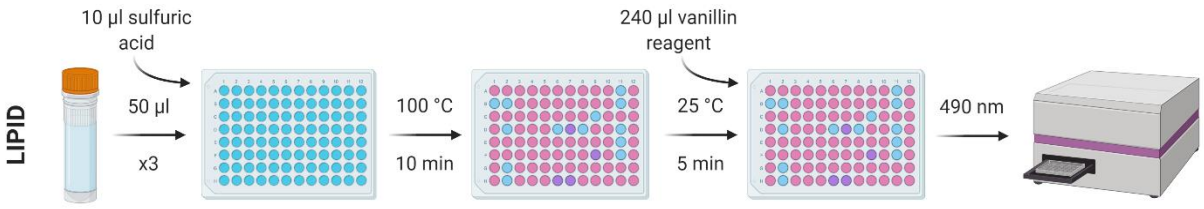


Figure 2.4: Protocol for the determination of lipid content using the sulfo-phospho-vanillin method. Figure created using Biorender.com.

Given the range of available protein assays, each with different benefits, the same samples from the five species analysed were put through three different protein-based colorimetric assays: bicinchoninic acid (BCA), Bradford and Lowry assays (Figure 2.5). These assays followed the manufacturer protocols for each. Samples for protein analysis comprised the supernatant taken after NaOH extraction.

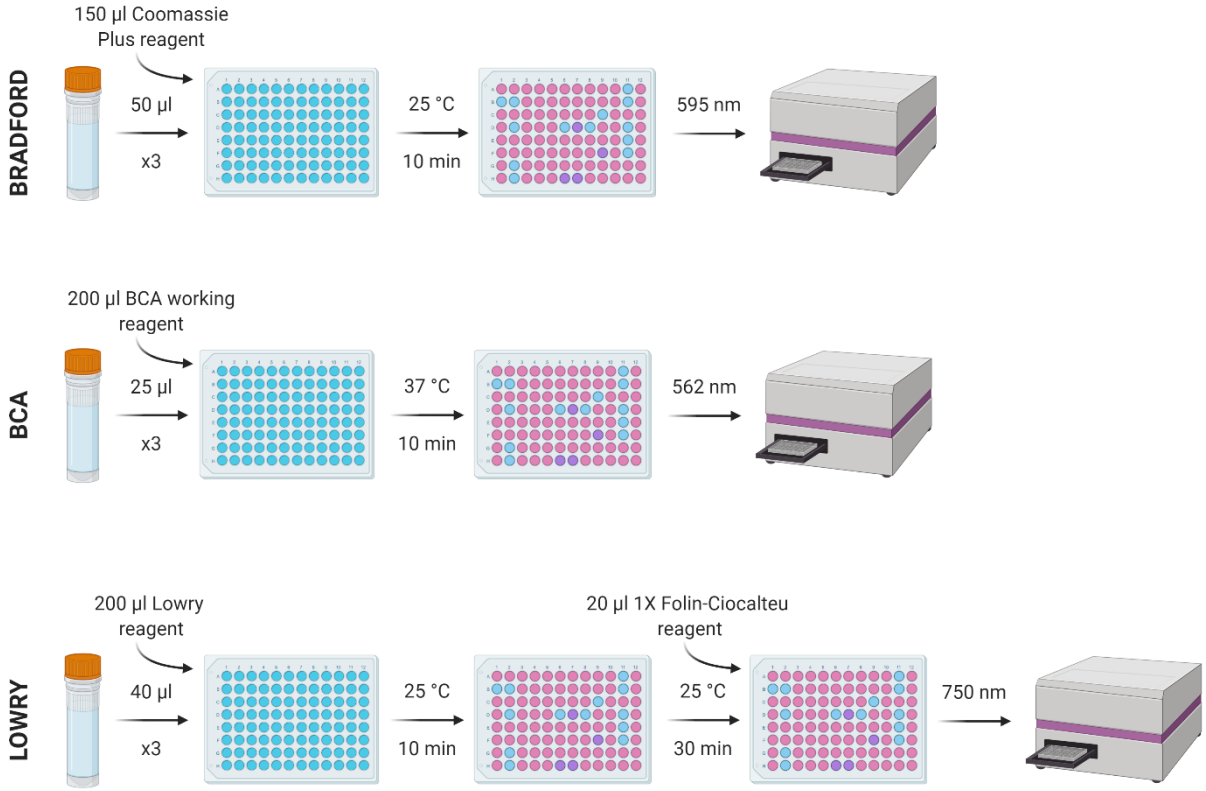


Figure 2.5: Protocol for the determination of protein content using the Bradford, BCA and Lowry methods. Figure created using Biorender.com.

First, the BCA protein assay, originally proposed by Smith *et al.* (1985) was used. This method colorimetrically detects a purple product formed by the chelation of two molecules of BCA to a cuprous ion produced via a biuret reaction. A standard dilution series was prepared with BSA diluted in polished water. Other standards such as IgG can be used and conversion factors exist for any discrepancy between standards; choice of a standard should ideally rely on proximity of the standard to the proteins of the focal species. For each sample and standard, 600 µl (200 µl per repeat) of BCA working reagent was prepared by mixing 50 parts BCA reagent A with 1 part BCA reagent B (Fisher Scientific). Of each sample and standard, three repeats of 25 µl were added to a 96-well microplate with 200 µl of the working reagent; this was mixed in a thermo-mixer at room temperature for 30 s at 450 rpm before incubation

at 37 °C for 10 min. The plate was cooled to room temperature and the absorbance at 562 nm measured.

The Pierce Coomassie Bradford protein assay, originally proposed by Bradford (1976) was also used. This method colorimetrically detects a blue product formed by the noncovalent binding of Coomassie Brilliant Blue G-250 dye to the carboxyl group of the protein present. The same standard dilution series was prepared as for the BCA assay. From each standard and sample, three replicates of 50 µL were mixed with 150 µL of the Coomassie Plus Reagent. The plate was incubated at room temperature for 10 minutes and absorbance at 595 nm measured.

Finally, the Pierce modified Lowry protein assay, originally proposed by Lowry *et al.* (1951) was used. This method colorimetrically detects heteropolymolybdenum Blue, a molecule formed by the reaction of the Folin-Ciocalteu reagent with Cu^{+} produced by peptide bond oxidation. The same standard dilution series was prepared as for the BCA assay. From each standard and sample, three replicates of 40 µl were mixed with 200 µl of the Modified Lowry Reagent and incubated at room temperature for 10 minutes. To each well, 20 µl of 1X (1N) Folin-Ciocalteu reagent was added, incubated at room temperature for 30 minutes and absorbance at 750 nm measured.

For carbohydrate determination, the anthrone method, originally proposed by Dreywood (1946), was adapted (Figure 2.6). Samples for carbohydrate analysis comprised the final supernatant taken after NaOH extraction. This method colorimetrically detects a blue-green complex formed by condensation by anthrone with furfural produced by hydrolysis of carbohydrates with acid. The method is best applied to sugars, although complex carbohydrates should be detectable in smaller components following hydrolysis in the protocol. A standard dilution series was prepared with corn starch diluted with polished water. The anthrone reagent was prepared by dissolving 1 mg of anthrone in 1 ml of concentrated (>95 %) H_2SO_4. From each standard and sample, three repeats of 40 µl were added to a 96-well microplate and each mixed with 160 µl anthrone reagent before mixing in a thermo-mixer at room temperature for 30 s at 450 rpm before. The plate was incubated at 92 °C for 10 min and cooled to room temperature. Absorbance at 620 nm was measured.

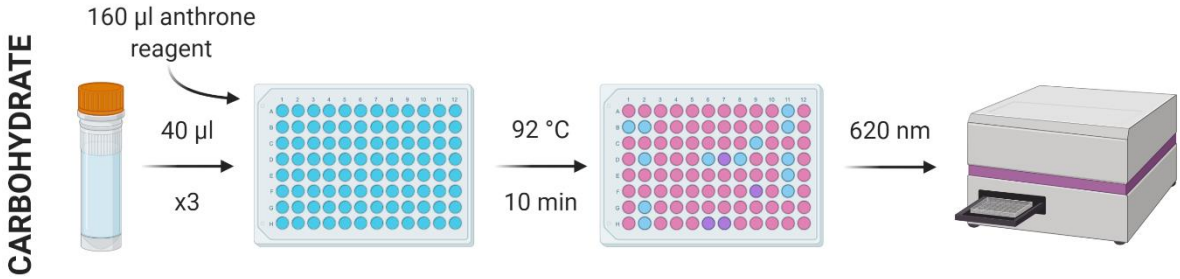


Figure 2.6: Protocol for the determination of carbohydrate content using the anthrone method. Figure created using Biorender.com.

For each assay, the absorbance measurement of the blank standard was subtracted from all other absorbance measurements and a standard curve prepared by plotting the blank-corrected measurement of the standards against their known concentrations. The regression equation of the standard curve was used to determine macronutrient concentration in each sample in mg ml^{-1}, which was then used to calculate the total macronutrient concentration in the sample based on the sample weight used for analysis and any dilution that was applied to the sample.

2.3.5. Limits of MEDI

Limit of blank (LoB) and limit of detection (LoD) describe the largest apparent concentration of analyte expected for blank samples and the lowest concentration likely to be detected and distinguished from a blank sample, respectively. The smallest detectable difference (SDD) is the smallest variance of measurement required to deem two measurements distinct. The LoB and LoD were determined as discussed by Armbruster and Pry (2008; Clinical and Laboratory Standards Institute, 2004), while the SDD was determined as outlined by Kropmans *et al.* (1999). Calculations used the below equations where '*B*', '*SD*' and 'SE' denote concentration readings for 60 blank methanol samples taken from the same plate, standard deviation of those readings, and standard error of those readings, respectively.

$$LoB = mean_B + 1.645(SD_B)$$

$$LoD = LoB + 1.645(SD_B)$$

$$SDD = 1.96(\sqrt{2(SE)})$$

2.3.6. Statistical analysis

All statistical analyses were performed using R version 3.5.3 (R Core Team 2020). To compare the macronutrient content of the species analysed, multivariate linear models

(MLMs) were fitted using the 'manylm' function of the 'mvabund' package (Wang *et al.* 2012). Ternary plots were produced via 'ggtern' (Hamilton and Ferry 2018) and 'ggplot2' (Wickham 2016).

2.4. Results

2.4.1. Calculation of methodological boundaries

MEDI successfully determined protein, carbohydrate and lipid content directly in parallel from a range of invertebrates, with a turnaround time from sample to data of three days and at a cost of ~£1.02 per individual invertebrate sample using standard laboratory equipment (heating block, shaker, bead beater and plate reader). Limits of detection using normal standard curve concentrations, reagent ratios and solvent volumes facilitate analysis of all but carbohydrate (for which half solvent volumes and six or more aphids pooled would be sufficient) in an invertebrate as small as an aphid, although differences between single aphids may not be accurately detectable (Table 2.2).

Table 2.2: MEDI limit of blank (LoB), limit of detection (LoD) and smallest detectable difference (SDD). Calculated from repeat methanol blanks, and single *Metopolophium dirhodum* aphid macronutrient content (mean ± SD).

	Protein (mg ml^{-1})	Carbohydrate (mg ml^{-1})	Lipid (mg ml^{-1})
LoB	0.067	0.065	0.006
LoD	0.133	0.130	0.011
SDD	0.321	0.317	0.093
Single aphid content	0.17 ± 0.09	<0.01 ± <0.01	0.17 ± 0.03

MEDI successfully detected significant differences in proportional macronutrient content between species (MLM: $F_{4,35}$ = 38.91, p = 0.002; Figure 2.7, Table 2.3).

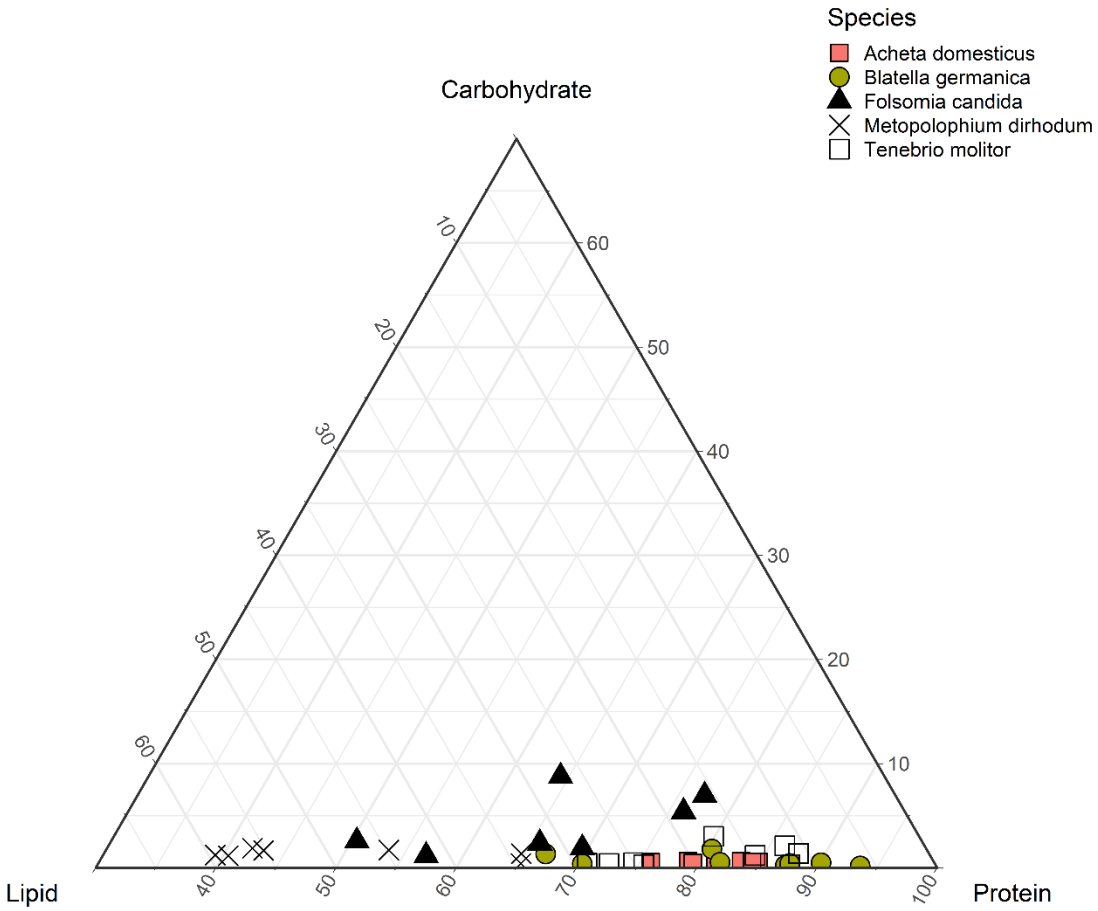


Figure 2.7: Ternary plot of the macronutrient content of the five species analysed. Each value is expressed as a proportion of total macronutrient mass, with the placement of points indicating proportional content of each of the three macronutrients (i.e. proximity to each point of the triangle indicates a greater proportional content of that macronutrient).

Table 2.3: Macronutrients determined from each of the five analysed species. Values are expressed as absolute macronutrient mass (mass mg; mean ± SD), percentage of body mass (%mass; mean ± SD) and percentage of total macronutrient mass (%macronutrients; mean ± SD). Values were calculated from eight individuals of each species (except body mass-related values for one specimen of *F. candida*). The presented protein values were determined via the Lowry assay (Table S2.2).

Species	Carbohydrate			Lipid			Protein		
	Mass (mg)	%mass	%macronutrients	Mass (mg)	%mass	%macronutrients	Mass (mg)	%mass	%macronutrients
Acheta domesticus	0.05 ± 0.01	0.23 ± 0.02	0.54 ± 0.07	1.62 ± 0.41	7.41 ± 1.16	17.49 ± 3.53	8.05 ± 3.30	35.35 ± 5.15	81.97 ± 3.55
Blattella germanica	0.07 ± 0.06	0.29 ± 0.20	0.67 ± 0.55	1.71 ± 0.48	7.76 ± 2.16	17.15 ± 8.60	10.25 ± 4.56	48.34 ± 26.18	82.18 ± 8.86
Folsomia candida	0.01 ± <0.01	0.43 ± 0.22	3.90 ± 2.61	0.05 ± 0.02	5.88 ± 3.70	35.35 ± 17.07	0.010 ± 0.05	8.75 ± 3.98	60.76 ± 15.68
Metopolophium dirhodum	<0.01 ± <0.01	0.14 ± 0.05	1.33 ± 0.42	0.17 ± 0.03	5.66 ± 1.32	51.67 ± 12.12	0.17 ± 0.09	5.62 ± 2.63	47.01 ± 12.26
Tenebrio molitor	0.18 ± 0.18	0.43 ± 0.38	1.16 ± 0.91	2.08 ± 0.41	7.31 ± 2.67	19.93 ± 6.76	9.99 ± 5.18	28.74 ± 6.50	78.91 ± 6.16

The gravimetric lipid mass and exoskeletal mass were determined for the three of the focal species for which body mass measurements could be accurately measured (Table 2.4).

Table 2.4: Body mass, exoskeletal mass, and gravimetric lipid mass for *Acheta domesticus*, *Blattella germanica* and *Tenebrio molitor*. Body mass and gravimetric lipid mass values were calculated from eight individuals of each species (seven for *A. domesticus* and *B. germanica* gravimetric lipid mass), while exoskeletal mass values were calculated from a separate five individuals of each species.

Species	Body mass (mg)	Exoskeletal mass (% body mass)	Gravimetric lipid mass (% body mass)
Acheta domesticus	22.20 ± 5.83	13.03 ± 1.93	16.75 ± 6.20
Blattella germanica	22.53 ± 4.96	19.75 ± 1.44	12.78 ± 8.65
Tenebrio molitor	36.20 ± 22.30	14.34 ± 1.85	28.81 ± 6.06

2.5. Discussion

MEDI successfully measured macronutrient content directly and rapidly from the same macroinvertebrate, even as small as a single aphid or collembolan, or as large as a mealworm or German cockroach. Aphid macronutrient content exceeds the LoDs except for carbohydrate (for which pooling, reduced solvent volumes or different reagent volumes could facilitate detectability), confirming a sensitivity broadly appropriate for small arthropods and other samples. The relatively low concentration of lipid and carbohydrate estimated in many invertebrate bodies may result in difficulties quantifying at least carbohydrates in such invertebrates (Finke 2005; Bryer *et al.* 2015), but the extraction procedure could overcome this by using smaller solvent volumes (e.g. 0.5 ml) to increase the solution concentration, leaving enough material to complete all three assays, or altering the plate incubation times, reagent concentrations and standard concentrations. Directly comparing the macronutrient contents of small invertebrates at an individual level via MEDI could prove difficult without taking such measures given a relatively high SDD relative to the content of the specimens tested. For larger samples, care should be taken to keep readings within the calibration curve; for this, sample dilutions are recommended following an initial test. Increased standard concentrations are not recommended due to likely saturation of the reagents and resultant loss of accuracy.

Of the protein assays compared, Lowry was selected as the preferred assay, at least for the specimens tested. The Bradford assay estimated protein concentrations far lower than expected from arthropod bodies (i.e. <10% body mass, vs. expected values of 25-75%) and, whilst the results from the BCA assay were not greatly dissimilar to those of Lowry, the values for German cockroaches regularly exceeded the entire mass of the cockroach, indicating some inaccuracy. This issue may result from German cockroaches storing nitrogen as uric acid in their bodies (Patiño-Navarrete *et al.* 2014). Uric acid is known to interfere with the BCA assay, as per the manufacturer notes. In fact, there are many chemicals that can interfere with the BCA assay (Vashist and Dixit 2011) and indeed most assays. Such inhibitors could be eliminated by introducing a purification step such as trichloro acetic acid protein precipitation, but this is unlikely to be necessary in most cases. Rather than highlighting an optimal assay, this emphasizes the importance of selecting assays and standards to best match the context of the work being carried out. The detection of different amino acids by each assay, their consequently differential relevance to protein standards and their variable performance in the presence of inhibitory compounds thus warrants a case-by-case consideration of the optimal assay to use, or the averaging of values from a range of assays or standards.

The large disparity in colorimetric and gravimetric measurements of lipids could highlight that these assays measure different pools of lipids with the sulfo-phospho-vanillin method only measuring unsaturated lipid content while the gravimetric method measures total lipid content. There were inaccuracies in the weighing of these specimens, with one specimen returning a negative body mass and two negative gravimetric lipid values (these were thus removed from any calculations relying on these values). The large variability in overall body mass (due to differences in growth stage and possibly body condition, e.g. parasitism, disease, loss of limbs) of the tested organisms may have impacted their similarity in macronutrient content due to the variable structure of organisms at different developmental stages. Particularly for the smaller invertebrates, for which body mass measurements are difficult, the proportional content of macronutrient content can be used as an effective proxy for studies concerned with a given taxon's nutritional quality. Alternatively, several specimens can be pooled, as is done in many existing protocols, if only to weigh them together to calculate an average individual mass. Length-mass relationships could be determined from many individuals, but the accuracy, particularly for smaller invertebrates, could be poor. Such pooling, if maintained for assay preparation, could ensure sufficient concentrations to overcome the limits of detection for smaller invertebrates.

Micronutrients (e.g. vitamins, calcium, iron) were not considered in this protocol, despite their biological importance (Jing *et al.* 2014), as they do not comprise a single detectable or quantifiable group. Without considering a specific micronutrient, or a subset of them, their quantification can be extremely laborious and, given the expectedly minute content of micronutrients in each invertebrate, detection, much less quantification, of micronutrients may be unfeasible for all but the largest macroinvertebrates without specialised equipment.

MEDI accurately detects macronutrients for a broad range of potential experimental applications involving invertebrates and other tissues, improving upon existing protocols for macronutrient determination. The protocol is relatively cheap, fast and simple and could present a uniform standard to be used across ecological studies. This protocol will be applied to a broad range of agricultural invertebrates for an analysis of nutrient-specific foraging in **Chapter 5**.

Browsing the Web: The Design and Evaluation of PCR Primers for the Analysis of Linyphiid Prey Choice Using Metabarcoding

“The spider taketh hold with her hands and is in kings' palaces.”

- Proverbs 30:28

Chapter 3 : Browsing the web: the design and evaluation of PCR primers for the analysis of linyphiid prey choice using metabarcoding

This chapter has been published in manuscript form in Ecological Entomology, entitled "Money spider dietary choice in pre- and post-harvest cereal crops using metabarcoding".

3.1. Abstract

1. Money spiders (Linyphiidae) are an important component of conservation biocontrol in cereal crops, but they rely on alternative prey when pests are not abundant, such as between cropping cycles. To optimally benefit from these generalist predators, prey choice dynamics must first be understood.
2. Money spiders and their locally available prey were collected from cereal crops two weeks pre- and post-harvest. Spider gut DNA was amplified with two novel metabarcoding primer pairs designed for spider dietary analysis, and sequenced.
3. The combined general and spider-exclusion primers successfully identified prey from 15 families in the guts of the 46 linyphiid spiders screened, whilst avoiding amplification of DNA of the linyphiid genus *Erigone*. The primers show promise for application to the diets of other spider families such as Agelenidae and Pholcidae.
4. Distinct invertebrate communities were identified pre- and post-harvest, and changes in spider diet and, to a lesser extent, prey choice reflected this. Spiders were found to predate one another more than expected, indicating their propensity toward intraguild predation, but also predated common pest families.
5. Changes in spider prey choice may redress prey community changes to maintain a consistent dietary intake. Consistent provision of alternative prey via permanent refugia should be considered to sustain effective conservation biocontrol.

3.2. Introduction

As fully introduced in **Chapter 1**, the polyphagous and generalist trophic nature of spiders, alongside their abundance (Nyffeler and Sunderland 2003; Shayler 2005), highlights their prospective positive contribution toward integrated pest management (Bale *et al.* 2008; Peterson *et al.* 2016). The longstanding acknowledgement that spiders are an effective biocontrol agent (Riechert and Lockley 1984; Sunderland *et al.* 1997; Sunderland 1999) is partly due to their year-round suppression of pests. Whilst crop rotation disrupts biocontrol by many generalist predators through habitat disturbance, spider generation times often coincide with crop cycles, with early pest population establishment coinciding with peak spider abundances in Spring, thus facilitating early pest suppression (Riechert and Lockley 1984; Symondson *et al.* 2002; Harwood and Obrycki 2005; Welch *et al.* 2011).

Harvest, akin to mass deforestation at the scale of a spider, changes the fundamental structure of macro- and micro-habitats, causing major changes to invertebrate community composition and interactions through immigration/emigration and potential exposure to other predators (Opatovsky and Lubin 2012; Davey *et al.* 2013). The large degree of turnover in invertebrate communities following harvest profoundly affects the diet of generalist predators, with the changes in spatial co-occurrence of predator and prey fundamentally influencing predation events (Bell *et al.* 2010). Given that many spiders over-winter in the field, field margins and hedgerows (Sunderland and Samu 2000), the post-harvest provision of prey for these predators will influence their abundance and ability to suppress early pest populations in the subsequent crop cycle (Symondson *et al.* 2002). To understand more precisely how harvest affects spider behavioural dynamics and how to optimise prey availability to support over-wintering and early-season spiders, the prey choice and dietary dynamics of spiders during this period must first be analysed.

Web-building spiders are effective models of prey choice, with webs providing a proxy for foraging investment (Welch *et al.* 2016). Spiders are known to forgo abundant prey in favour of less locally abundant taxa (Agustí *et al.* 2003; Welch *et al.* 2016). Studies of spider prey choice have mostly consisted of laboratory feeding trials (e.g. Mayntz *et al.* 2005; Rendon *et al.* 2019). Given that spiders are fluid feeders, morphological analysis of gut contents is impossible, thus field studies are restricted to direct observation and molecular methods (Symondson 2002; Harwood *et al.* 2004; Bell *et al.* 2010; Pompanon *et al.* 2012; Chapman *et al.* 2013; Birkhofer *et al.* 2017). Field-based analyses of spider diet have used DNA metabarcoding in recent years, but relatively few have yet been published (e.g. Piñol *et al.* 2014; Lafage *et al.* 2019). A major limiting factor for metabarcoding-based analyses of spider diet is the dearth of suitable PCR primers.

Primer selection for DNA metabarcoding requires identification of a target gene containing a region which is variable between target species (the barcode) but flanked by two conserved regions (the primer sites) based on the full range of target taxa. For animal metabarcoding, this is commonly in the cytochrome c oxidase subunit I (COI) gene due to its mutation rate and the extensive reference libraries available (Hebert, Cywinska, *et al.* 2003; Deagle *et al.* 2014). These short sections of DNA, typically of 200-400 base pairs, can then be amplified using polymerase chain reactions (PCRs) with primers acting as the initial catalytic scaffold. Primers are short synthetic oligonucleotides which are complementary to the regions either side of the barcode that are conserved across the target taxa; these catalyse the amplification of the short sections of DNA (Folmer *et al.* 1994; Piñol *et al.* 2018). Using HTS, such PCR-amplified short sections of DNA are then identified in parallel from a single sample, such as the prey in a spider's gut. This ultimately provides an efficient and accurate method for analysing the prey range of predators (Pompanon *et al.* 2012; Piñol *et al.* 2018). Cases in which ideal primers do not already exist may, however, warrant the design of entirely new bespoke primers.

Efficient primer design must follow a strict framework (MacDonald and Sarre 2016) and, ultimately, all known primers fail to amplify some taxa (Mao *et al.* 2012; Brandon-Mong *et al.* 2015), so compromises must always be made. The efficacy of primers in amplifying target taxa DNA is largely dependent on their complementarity with the target DNA. There are, however, many factors which can affect the efficiency with which the primer anneals to the target DNA which, if unaddressed, can prevent amplification entirely or result in amplification bias (Piñol *et al.* 2018). Many existing metabarcoding primers are affected by biases, resulting in a non-linear relationship between starting and amplified concentrations of DNA of each species (Paula *et al.* 2015). Mismatches are the greatest contributor to such biases and wholly inhibit any potential for quantitative results, regardless of other biases (Stadhouders *et al.* 2010; Piñol *et al.* 2018). Novel methods for the design of PCR primers facilitate batch downloads of available sequence data for the design of primers based on mass alignments, which alleviates issues with bias at the design stage (Elbrecht and Leese 2016b). *In silico* evaluation of primers allows prediction of their efficacies against reference databases larger than would be practicable via *in vitro* testing, and prior to investment in reagents or lab time (Ficetola *et al.* 2010; Elbrecht and Leese 2016b). All software capable of *in silico* analyses do, however, over-simplify the PCR process by neglecting the nuances of small molecule interactions and results must thus be validated *in vitro* prior to confident use in metabarcoding applications (Piñol *et al.* 2018).

Primers must be designed carefully with particular focus on their 3' end given its importance in binding to template DNA (Bru *et al.* 2008; Stadhouders *et al.* 2010). Different mismatch

types may also alter the effect on amplification success, with purine-purine mismatches less detrimental than pyrimidine-pyrimidine mismatches which are less debilitating again than purine-pyrimidine mismatches (Kwok *et al.* 1990; Stadhouders *et al.* 2010; Wright *et al.* 2014; Piñol *et al.* 2018). Degenerate bases (bases designed to vary within a primer solution; e.g. Y represents C or T, thus 50% of the primer population will contain a C base there, and 50% a T base) partly circumvent the issue of mismatches by splitting the population of oligonucleotides for each primer to represent multiple possible nucleotide sequences, increasing the number of taxa to which the primers are complementary, and with little impact on performance or bias (Piñol *et al.* 2018). Degeneracies must, however, be limited to prevent over-dilution of the primer population, resulting in no amplification of template DNA; typical degenerate primers rarely exceed a degeneracy value of 128 (calculated by multiplying the degenerate bases in a primer together in which two-fold degenerate bases are represented by two, three-fold by three and "N", the only four-fold degenerate base, by four; e.g. Y = C or T, and D = A, G or T, so if a primer contained only these two degenerate bases, its value would be 6 (2 X 3); Najafabadi *et al.* 2008). Inosine bases (denoted by 'I' in IUPAC code) theoretically bind to all bases whilst circumventing the dilution issue associated with degeneracies, and are thus used in many universal primers to encourage amplification of target taxa; however, even inosine has a different affinity for different bases and can incur primer bias (Martin *et al.* 1985; Piñol *et al.* 2018), reducing its utility without even considering its far greater financial cost. Given the inevitable biases, the application of multiple primer sets in a single study and attainment of high sequencing depths are relatively innocuous methods for the reduction of taxonomic biases (Alberdi *et al.* 2017).

Two additional factors must be overcome if HTS and metabarcoding are to be used for dietary analysis of arthropod-consuming arthropods: (1) if the predator is an arthropod, then there is a high probability that existing general arthropod PCR primers will amplify the predator as well as the prey in its guts; (2) tissue from the spider predator will be undegraded and will hence swamp amplification of the prey (Vestheim and Jarman 2008; Piñol *et al.* 2014). Applying universal primers and accepting a loss of data to amplification of the predator is feasible with sufficient read depth (i.e. the number of sequences attributed to a sample) and facilitates analysis of interactions between closely-related species (Piñol *et al.* 2014). Blocking primers can be used alongside universal primers for the prevention of amplification of the predator whilst still amplifying the prey (Vestheim and Jarman 2008; Deagle *et al.* 2009), but these can introduce strong taxonomic biases (Piñol *et al.* 2015; Piñol *et al.* 2018). Primers can be designed to exclude amplification of the predator whilst still amplifying a broad range of prey species and such primers have been designed for wolf spiders (Lycosidae; Lafage *et al.* 2019). However, these primers amplify money spider DNA and are thus not appropriate for analysing

linyphiid gut contents. Taxonomically-similar predators and prey, such as intraguild spider-spider interactions, may also be undetected when using primers that exclude amplification of the predator DNA (Vestheim and Jarman 2008; Piñol *et al.* 2014).

3.2.1. Objectives and hypotheses

In this chapter, the diets and prey choices of linyphiids were analysed in cereal crops pre- and post-harvest using DNA metabarcoding. To facilitate this, novel PCR primers were developed for the analysis of spider diet using high-throughput sequencing, with a specific focus on the diet of linyphiids. Linyphiid prey availability and diet were hypothesised to change following harvest, with diet largely reflecting prey assemblage turnover. The prevalence of some prey species in the diet of the linyphiid predators was, however, expected to be disproportionate to their availability, reflecting prey choice. The dietary and prey choice analysis workflows, and the primers designed in this chapter will be applied to subsequent analyses in **Chapters 4 & 5**.

3.3. Methodology

3.3.1 Field site

The fieldwork detailed throughout this thesis was carried out at Burdons Farm, Wenvoe, Wales (51° 44'N, -3° 27'E), a 235-hectare farm managed under the Glastir Welsh government agricultural scheme which encourages the maintenance and enhancement of biodiversity. The farm is comprised of both arable and livestock farming, the arable component dominated by barley. Among these fields are several small meadows and copses, with woodland bordering much of the farm, and game cover strips bordering several fields. The present study was carried out exclusively in the arable fields of the farmland containing cereal crops, with all concerted collections taken from barley fields. Burdons Farm has been used in several studies of predator-prey interactions in agricultural systems (Agustí *et al.* 2003; Foltan *et al.* 2005; Shayler 2005; Davey *et al.* 2013; Piñol *et al.* 2014).

3.3.2. Primer development and testing

Existing PCR primers were tested and ultimately redesigned to better match the target taxa of this study. Two novel primer pairs were used for amplification of DNA for the dietary analysis of spider gut contents to overcome the problems associated with the taxonomic proximity of spiders and their prey (particularly other spider species). Novel PCR primers were adapted for

the exclusion of all spider DNA, with a focus on linyphiids (henceforth spider exclusion primers, titled TelperionF-LaurelinR), based upon a novel primer site adjacent to that of the general animal barcoding primer LCO1490 (forward primer Folmer *et al.* 1994), and mICOIintR (Leray *et al.* 2013). A second primer pair was employed for broad amplification of both spiders and their prey (henceforth general primers, titled BerenF-LuthienR), based upon mICOIintF (Leray *et al.* 2013) and HCO2198 (Folmer *et al.* 1994). Both primer pairs were adapted via base changes designed with reference to mass alignments of invertebrate COI sequences and tested *in silico* and *in vitro*. The spider-exclusion primers were designed to overcome the loss of reads to predator DNA, whilst the general primers were designed to avoid the taxonomic biases associated with the exclusion primers.

Mass-alignments of COI sequences were batch-downloaded from GenBank (NCBI) and BOLD (Ratnasingham and Hebert 2007) using PrimerMiner (Elbrecht and Leese 2016b) in R v.3.3.4 (R Core Team 2020) to aid visual inspection of existing and novel primer sites. PrimerMiner clusters batch downloads into operational taxonomic units (OTUs) based on sequence similarity and visualises mass alignments of sequence data for primer design. By merging overrepresented and duplicate sequences through taxonomy-independent clustering, PrimerMiner accounts for within-species variation and cryptic species whilst ignoring rare haplotypes (Elbrecht and Leese 2016b). Sequences were downloaded for all terrestrial invertebrate orders available, and consensus sequences were created by clustering these into OTUs for each order. The COI sequences were trimmed to include only the Folmer region (Folmer *et al.* 1994) using Geneious R10 (Kearse *et al.* 2012) for subsequent use in PrimerMiner. Alignments of prey sequences created via PrimerMiner included cereal crop spiders, in order to find primer sites conserved between a wide range of potential prey, but different for spiders. Where these sites were 100-400 base pairs apart on sequences from one another or from existing primer sites, they were paired, and primers designed (Table 3.1, Figures S3.2-S3.3). Existing general invertebrate primer sites were compared against the PrimerMiner alignments to identify any potential improvements to the primers for the amplification of cereal spider prey. The coverage of primers (% amplified) was determined via PrimerMiner using the same mass alignments used for primer design. PrimerMiner uses a taxonomy-independent database and accounts for adjacent base mismatches and the position of each base in the primer. Primers were also analysed using the online ThermoFisher Scientific Multiple Primer Analyzer tool. After the primers were deemed successful *in silico*, they were tested *in vitro*.

Table 3.1: COI primers designed via PrimerMiner.The designed primer pairs, TelperionF – LaurelinR and BerenF – LuthienR, with amplicon sizes of 302 bp and 314 bp, respectively.

Primer	Sequence (5' – 3')	Source	3' Location	Direction (forward/ reverse)	BP
TelperionF (spider excluding)	GGAACWHTATAYTTWATWTTYGG	This study	1535	F	23
LaurelinR (spider excluding)	GGRTAWACWGTTCAWCCWGT	Adapted from mICOIintR (Leray *et al.* 2013)	1837	R	20
BerenF (general)	CAGGWTGAACWGTWTAYCCYCC	Adapted from mICOIintF (Leray *et al.* 2013)	1859	F	22
LuthienR (general)	ACTTCWGGRTGWCCAAARAAYCA	Adapted from HCO2198 (Folmer *et al.* 1994)	2173	R	23

The primer pairs were tested *in vitro* against a wide range of extracted invertebrate DNA including spiders, common spider prey and additional invertebrates. For this, invertebrate samples included those collected from the field site at Burdons Farm, Wenvoe, South Wales, the study site used for subsequent ecological analysis. Invertebrates were found via manual searching, collected via aspirator and placed in microcentrifuge tubes filled with 100% ethanol. Invertebrates were identified at 20-50X magnification using a light stereomicroscope and taxonomic keys (Goulet and Huber 1993; Roberts 1993; Unwin 2001; Ball 2008; Barber 2008; Duff 2012; Dallimore and Shaw 2013). Additional invertebrates and DNA were taken from existing archived collections within Cardiff University. Extraction of DNA used DNeasy Blood & Tissue Kits (QIAGEN Inc., Chatsworth, CA, USA) following the manufacturer's protocol for animal tissue. For predatory invertebrates, DNA was extracted from the lower legs, excluding the femur, to avoid the inclusion of prey DNA in the gut diverticulae and leg coxae (Macıas-Hernández *et al.* 2018). To verify successful extraction, the DNA and negative controls were amplified via PCR with the Qiagen PCR Multiplex Kit (Qiagen) with 95 °C for 15 minutes to activate the HotStarTaq® DNA polymerase, 35 cycles of 95 °C for 30 seconds, 40 °C for 90 seconds and 72 °C for 90 seconds, respectively, followed by a final extension at 72 °C for 10 minutes using universal invertebrate primers LCO1490 and HCO2198 (Folmer *et al.* 1994). PCR reactions comprised 25 µl reaction volumes containing 12.5 µl Qiagen PCR Multiplex kit, 0.2 µmol (0.5 µl of 10 µM stock) of each primer, 6.5 µl DNase-free water and 5 µl template DNA. Amplification was confirmed by gel electrophoresis.

Primers were initially tested against a small selection of spider and non-spider DNA. Temperature-gradient PCRs were used to determine the optimal annealing temperatures for the primer pairs selected, with temperatures between 40 °C and 60 °C considered and initial tests starting 5 °C lower than the mean melting temperature of both primers. Inclusion of the Q reagent supplied with Multiplex Kits was trialled for each pair to ascertain whether this could improve performance but was ultimately excluded in all cases. PCR conditions were: 95 °C for 15 minutes to activate the HotStarTaq® DNA polymerase, 35 cycles of 95 °C, the annealing temperature (BerenF-LuthienR: 52 °C; TelperionF-LaurelinR: 42 °C) and 72 °C for 30, 90 and 90 seconds, respectively, and a final extension at 72 °C for 10 minutes. PCR reactions comprised 25 µl reaction volumes containing 12.5 µl Qiagen PCR Multiplex kit, 0.2 µmol (0.5 µl of 10 µM stock) of each primer, 6.5 µl DNase-free water and 5 µl template DNA. Successful amplification was confirmed by gel electrophoresis. Once optimised to amplify a range of non-spider species whilst amplifying few spiders, or amplify a broad range of all species included, primers were further tested on a broader range of DNA (Table S3.3).

The TelperionF–LaurelinR primer pair has well-conserved sites, facilitating broad coverage with few degenerate bases necessary. The terminal base at the 3' end of Laurelin, being a

thymine base, critically mismatches with the guanine base present for most spider taxa tested; this should theoretically prevent or at least severely reduce amplification of spiders with little cost to amplification breadth otherwise. The BerenF–LuthienR pair similarly makes use of conserved primer sites employed in other studies but adapted for universal amplification of the focal taxa of this study.

3.3.3. Field collection and identification

Linyphiids were visually located on transects through two adjacent spring barley fields at Burdons Farm, Wenvoe in South Wales (51°26'24.8"N, 3°16'17.9"W), and collected from occupied webs and the ground, in August and September 2017. Each transect comprised 4 m^2 searching areas at least 10 m apart and all observed linyphiids were collected. Spiders were taken from 20 locations along the aforementioned transects, 10 pre-harvest and 10 post-harvest. Spiders were collected two weeks prior (7-13th August) to harvest (~20th August) of the crop and two weeks after harvest (4-8th September) in crop stubble and placed in 100% ethanol using an aspirator. Ground-active linyphiid spiders were collected when webs were not abundant. Spiders were taken to Cardiff University, transferred to fresh 100% ethanol, adults identified to species-level and juveniles to genus, and stored at -20 °C until subsequent DNA extraction.

Invertebrate prey communities were collected for the measurement of invertebrate community composition (i.e. not for molecular analysis) using a converted McCulloch GBV 325 G-vac leaf blower as a suction sampler for 1 minute per sample over 4 m^2 areas near to those from which spiders for DNA analysis were collected. Samples were taken in transects, with 10 samples each pre- and post-harvest (20 total), split evenly between two adjacent fields. Invertebrate prey community samples were taken approximately 10 m apart, in sites near to those from which spiders were collected, with different sites used pre- and post-harvest. Invertebrates were killed with ethyl acetate and stored in 70% ethanol at -20 °C. All invertebrates were identified to family level under an Olympus SZX7 stereomicroscope using morphological keys for Araneae (Roberts 1993), Diptera (Ball 2008), Coleoptera (Duff 2012), Hymenoptera (Goulet and Huber 1993), Hemiptera (Unwin 2001), Collembola (Dallimore and Shaw 2013) and Chilopoda (Barber 2008). The only exceptions were springtails of Sminthuroidea (Sminthuridae and Bourletiellidae, which were often indistinguishable following vacuum sampling and preservation due to damage to the fine features necessary to distinguish them) which were left at super-family, and mites (many of which were immature or in poor condition), which were identified to order level.

3.3.4. Extraction, amplification and sequencing of spider gut DNA

Erigone atra Blackwall, 1833 and *E. dentipalpis* (Wider, 1834; Erigoninae), and *Tenuiphantes tenuis* (Blackwall, 1852; Linyphiinae) were the focus of this study, although a few juveniles were included from other genera due to the difficulties associated with morphological identification of linyphiid juveniles; these misidentifications were confirmed in the subsequent metabarcoding. In total, 66 individual spiders were screened (Table S3.2), unevenly split across the 20 corresponding prey sampling sites. Spiders were washed in and transferred to fresh 100 % ethanol to reduce external contaminants prior to identification using Roberts (1993) morphological key. Abdomens were removed from spiders and washed again in fresh 100 % ethanol. Only abdomens were used for molecular analysis of their gut contents given their higher concentration of prey DNA than that of the cephalothorax (Krehenwinkel *et al.* 2017; Macıas-Hernández *et al.* 2018). To ascertain optimal extraction technique, samples were split into two groups. From one group, DNA was extracted from the abdomens via Qiagen TissueLyser II as per the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) manufacturer's protocol and abdomens kept in the lysis buffer during incubation (Figure 3.1). From the other group, DNA was extracted by splitting the abdomen with a sterile micropestle, swilling it in the lysis buffer and then removing the bulk tissue (Figure 3.2). Neither method ultimately afforded a significantly greater proportion of prey DNA reads post-amplification (Figure S3.1), so were combined for analysis. Post-lysis, all extractions followed the DNeasy Blood & Tissue Kit (Qiagen) manufacturer's protocol but with an extended lysis time of 12 h (recommended: 1-3 h) to account for the complex and branched gut system in spider abdomens (Krehenwinkel *et al.* 2017). Per 12 spiders, each DNA extraction session included at least one negative control consisting of an empty tube treated identically to the samples.

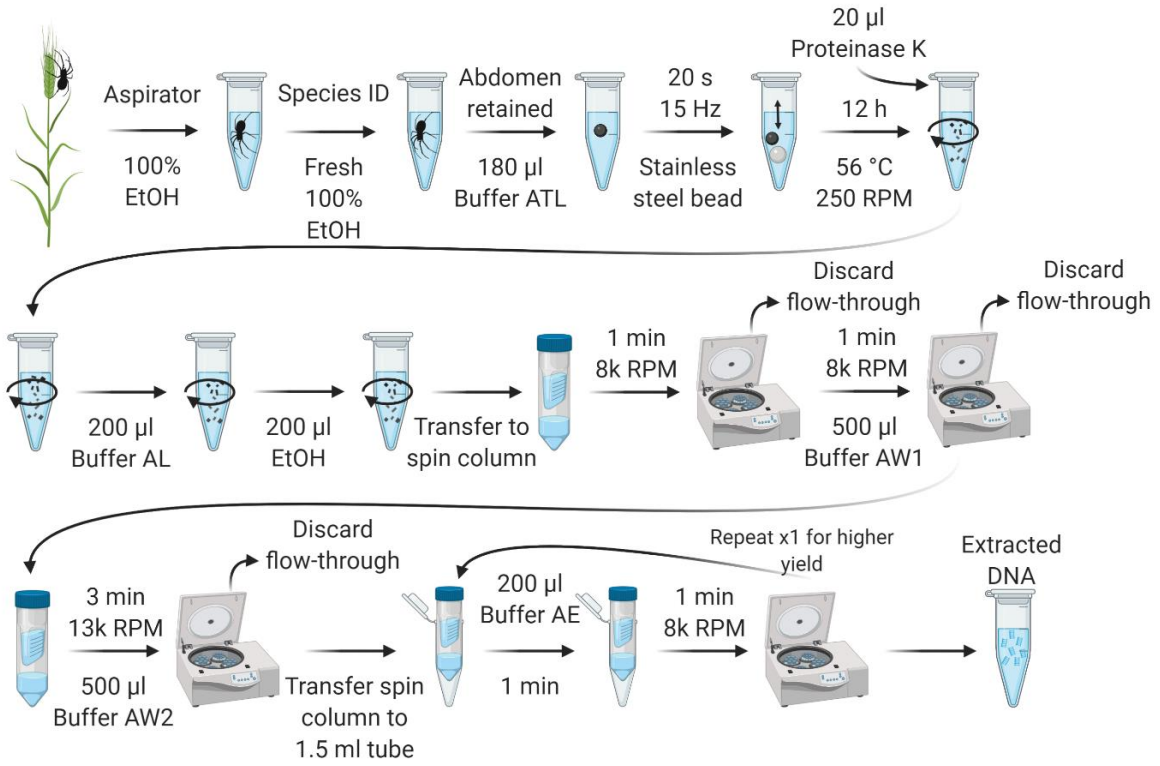


Figure 3.1: "Crush" DNA extraction protocol. Figure created with BioRender.com.

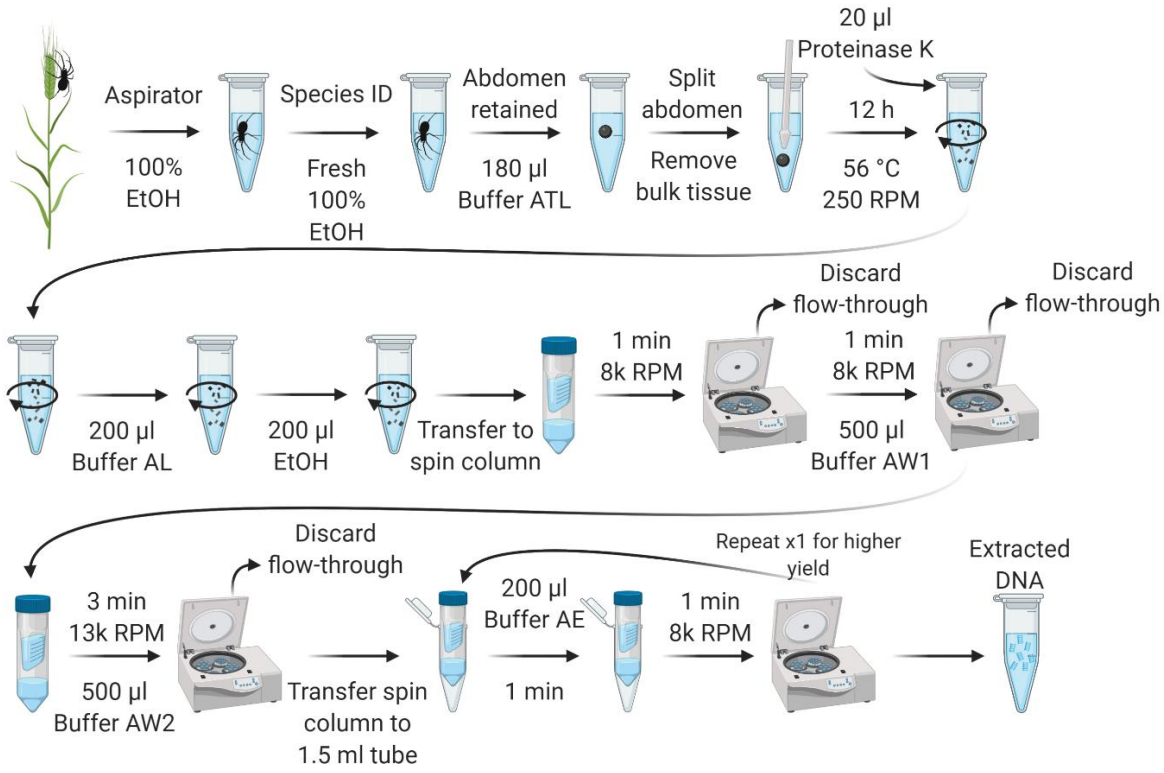


Figure 3.2: "Flush" DNA extraction protocol. Figure created with BioRender.com.

Primers were labelled with unique 10 bp molecular identifier tags (MID-tags) and samples had a unique pairing of forward and reverse tags for identification of each sample post-sequencing. PCR reactions of 25 µl reaction volumes contained 12.5 µl Qiagen PCR Multiplex kit, 0.2 µmol (2.5 µl of 2 µM) of each primer, 2.5 µl DNase-free water and 5 µl template DNA (Figure 3.3). Reactions were carried out in the same Veriti Thermal Cycler (ThermoFisher Scientific, Waltham, USA), with annealing temperatures optimised via temperature gradient PCRs in the same machine. PCRs comprised 15 min at 95 °C, followed by 35 cycles of: 95 °C for 30 seconds, the primer-specific annealing temperature for 90 seconds, and 72 °C for 90 seconds; followed by a final extension at 72 °C for 10 minutes. The new primers, designated BerenF-LuthienR (universal) and TelperionF-LaurelinR (spider-excluding), used annealing temperatures of 52 °C and 42 °C, respectively.

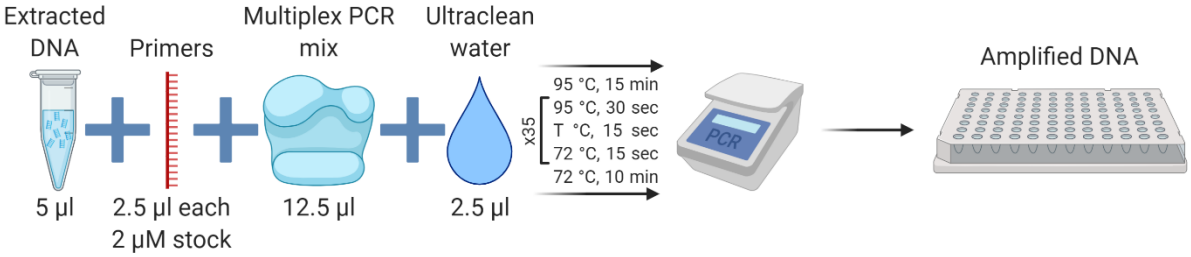


Figure 3.3: PCR protocol. T is the annealing temperature for each primer (BerenF-LuthienR: 52 °C; TelperionF-LaurelinR: 42 °C). Figure created with BioRender.com.

Within each PCR 96-well plate, 12 negative (either extraction or PCR) and two positive controls were included following Taberlet *et al.* (2018). Negative PCR controls consisted of DNase-free water. Positive controls comprised mixtures containing known concentrations of the invertebrate DNA used for primer testing, detailed above, quantified using Qubit dsDNA High-sensitivity Assay Kits (ThermoFisher Scientific) to ascertain any effects of primer bias. All DNA concentrations were standardised at 0.1 ng μl^{-1} by diluting the DNA in DNase-free water. Five mixtures of different species richness and proportions were prepared (Table S3.1). A negative control was present for each MID-tag to identify any contamination of primers. Each plate was pooled according to concentrations determined by Qiaxcel Advanced System (Qiagen; Figure 3.4). Each pool was cleaned via SPRIselect beads (Beckman Coulter, Brea, USA), with a left-side size selection using a 1:1 ratio (retaining ~300-1000 bp fragments). The concentration of the pooled DNA was determined via Qubit dsDNA High-sensitivity Assay Kits, quality-checked via TapeStation 2200 (Agilent, Santa Clara, USA) and all pools sharing the same primer pair were pooled again into a 'super pool', thus forming one pool per primer pair. Library preparation for Illumina sequencing was carried out on these cleaned 'super pools' via NEXTflex Rapid DNA-Seq Kit (Bioo Scientific, Austin, USA) and samples were sequenced on

an Illumina MiSeq via a Nano chip with 2x250 bp paired-end reads (expected capacity ≤1,000,000 reads).

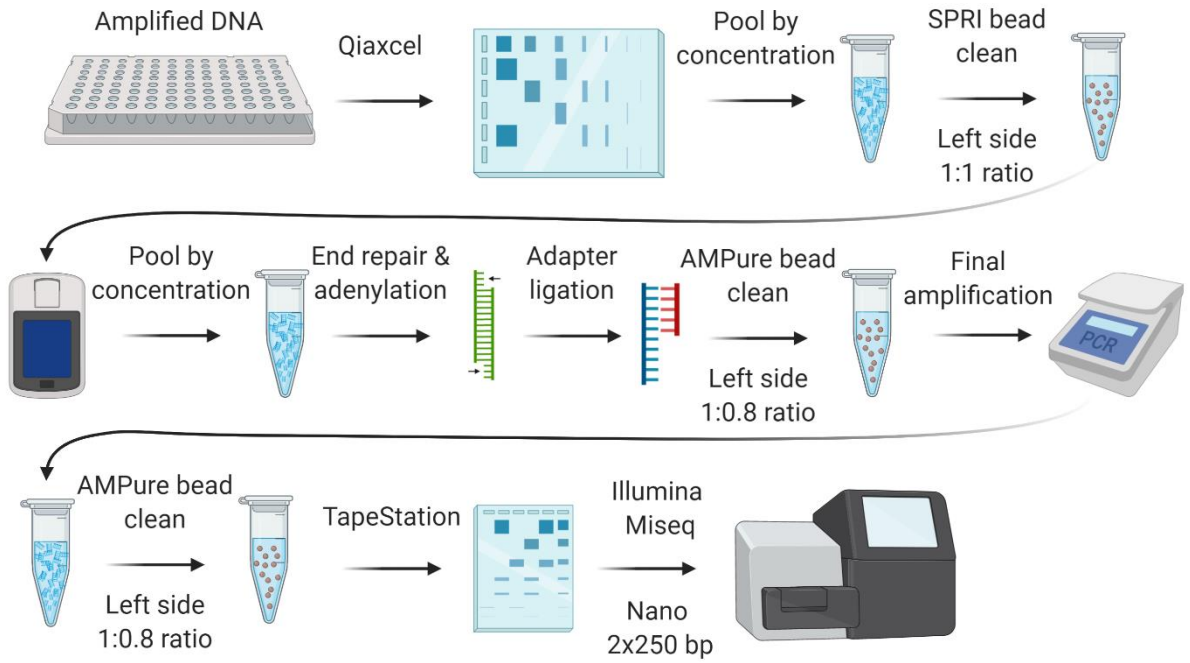


Figure 3.4: Pooling and cleaning of DNA and sequencing library preparation. Figure created with BioRender.com.

3.3.5. Bioinformatic analysis

The Illumina run generated 405,270 and 482,249 reads using BerenF-LuthienR (universal) and TelperionF-LaurelinR (spider-excluding), respectively. All reads were quality-checked and trimmed in Trimmomatic v0.38 (Bolger *et al.* 2014) with a minimum quality score and sliding window of 20 and 4 bp, respectively, and a minimum length of 135 bp. The read pairs were aligned via FLASH v1.2.11 (Magoč and Salzberg 2011) and demultiplexed via Mothur v1.39.5 (Schloss *et al.* 2009), removing the MID and primer sequences. Replicates were removed, and denoising and clustering to zero-radius OTUs (ZOTUs; clustered without % identity to avoid multiple species represented within a single OTU) completed via Unoise3 in Usearch11 (Edgar 2010). The resultant sequences were assigned a taxonomic identity from GenBank via BLASTn v2.7.1. (Camacho *et al.* 2009) using a 97% identity threshold (Alberdi *et al.* 2017). The BLAST output was analysed in MEGAN v6.15.2 (Huson *et al.* 2016). Where the top BLAST hit, determined by lowest e-value, was resolved at a higher taxonomic level than species-level, the results were checked by blasting the sequence manually in GenBank and comparing the results; where possibly erroneous entries were preventing species-level assignment (e.g. poorly-resolved identifications on GenBank), finer resolution was considered.

Where ZOTUs were assigned the same taxon, these were aggregated. Given the prevalence of family-level assignments (e.g. Chloropidae), the data were eventually converted to family-level, but were retained at their respective output assignments for clean-up.

To clean data prior to statistical analysis, all read counts less than the maximum read count present in blanks (negative controls and unused MID-tag combinations) for its respective ZOTU were removed. Instances of non-positive control taxa (i.e. taxa not included in the positive control mock communities) present in positive controls were calculated as a percentage of the maximum read count for that taxon. The greatest of these percentages was used to guide a universal percentage of the maximum read within each taxon to be removed. This accounts for tag-jumping and "bleeding" (i.e. misassignment of sample IDs or erroneous displacement of tags between samples) of over-represented taxa into other samples during sequencing. For BerenF-LuthienR, 0.54 % was optimal, whilst there were no obvious instances for TelperionF-LaurelinR, so the conservative 0.54 % was also applied for that library.

Simultaneously, known lab contaminants (e.g. German cockroach *Blattella germanica*) and various species for which molecular analysis was recently undertaken in the same lab (i.e. likely contaminants) that could be differentiated from the target taxa in this study, such as tropical species) were identified and the percentage of these occurrences of the total read count for their respective samples was calculated. The highest of these percentages was used as a guide for the universal percentage of each total sample read count to be removed. This accounts for environmental and lab contamination, and artefacts and errors of the sequencing process, which for BerenF-LuthienR and TelperionF-LaurelinR were 0.43 % and 0.45 %, respectively. The data from the two libraries were then aggregated together, first removing non-target taxa (e.g. fungi) and instances in which predator DNA was amplified (i.e. ZOTUs matching the individual spider's morphological identity). All taxa were converted to family-level to standardise the taxonomic level since many ZOTUs could not be resolved further; this also increases evenness for subsequent analyses. Whilst all conspecific reads were removed to account for predator amplification, interspecific linyphiid interactions were still retained, thus any counts of linyphiids in the diet exclusively represent the consumption of other species. Finally, read counts were converted to presence-absence data.

3.3.6. Statistical analysis

All analyses were conducted in R v.3.3.4 (R Core Team 2020). Invertebrate communities were compared between pre- and post-harvest using multivariate generalized linear models (MGLMs) via 'manyglm' in the 'mvabund' package (Wang *et al.* 2012) with a binomial error

distribution and Monte Carlo resampling (the latter to increase confidence in the results by ascertaining their prevalence in repeated simulations). Spider diets were compared between pre- and post-harvest, and spider life stage (juvenile, sub-adult and adult) and sex, using MGLMs that included all two-way interactions between these variables. Whilst the samples selected for sequencing primarily comprised *T. tenuis* and *E. atra/dentipalpis agg.* with the intention of comparing these taxa, sample sizes were not sufficient for taxonomic comparisons due to sample drop-out in the sequencing process and the misidentification of some juvenile linyphiids (pre-harvest: *Tenuiphantes* = 19, *Erigone* = 5, other = 3; post-harvest: *Tenuiphantes* = 16, *Erigone* = 1, other = 2). Models were simplified using 'step' from the base R 'stats' package to determine an optimal model based on the lowest AIC value by removing variables. Dietary differences were also visualised by non-metric multidimensional scaling via metaMDS in the 'vegan' package (Oksanen *et al.* 2016) using Jaccard distance to estimate the dissimilarity projected onto two dimensions (stress = 0.082, indicating an effective two-dimensional projection). Spider plots were created with nMDS results via 'ordispider', plotted through 'ggplot2' (Wickham 2016) to illustrate the distinction in communities between categories. Spider prey choice was analysed using network-based null models in the 'econullnetr' package (Vaughan *et al.* 2018) with the 'generate_null_net' command, visually represented with the 'plot_preferences' command. This detects whether prey taxa are consumed more or less frequently than expected based on their relative abundance in the community.

3.4. Results

3.4.1. Novel primer performance

Both primer pairs amplified a broad range of prey *in silico* and *in vitro* (Figures S3.5-3.6, Table S3.3). BerenF-LuthienR outperformed both the widely-used animal barcoding primers LCO1490-HCO2198 (Folmer *et al.* 1994) and ZBJ-ArtF1c-ZBJ-ArtR2c (Zeale *et al.* 2011) *in silico* for most taxa, with the exceptions of Lepidoptera, Coleoptera and Thysanoptera (Figure S3.5). TelperionF-LaurelinR performed comparably to ZBJ-ArtF1c-ZBJ-ArtR2c but with far greater coverage of several taxa. The only taxa for which TelperionF-LaurelinR did not outperform ZBJ-ArtF1c-ZBJ-ArtR2c were Araneae (intentionally so) and Thysanoptera (Figure S3.5). The primers were similarly successful *in vitro*, BerenF-LuthienR amplifying all but *Psilochorus simoni* (Berland, 1911; Araneae: Pholcidae) and a nudibranch (Table S3.3). TelperionF-LaurelinR avoided amplification of spiders in the families Agelenidae, Pholcidae and Clubionidae, but did amplify representatives of Amaurobiidae, Dysderidae, Philodromidae and Lycosidae, as well as some Linyphiidae, but not the two focal genera of this study.

TelperionF-LaurelinR otherwise amplified a very broad range of prey, but exhibited a reduced coverage compared to BerenF-LuthienR, failing to amplify 6/45 of the non-spider taxa tested (Table S3.3). In the mock community samples, BerenF-LuthienR exhibited some bias toward Lepidoptera and Diptera, whereas TelperionF-LaurelinR exhibited a stronger bias toward Hemiptera, Collembola, Hymenoptera and Neuroptera (Figure S3.6).

3.4.2. Invertebrate community comparison

Identified invertebrates comprised 67 families: 45 pre- and 51 post-harvest (Table S3.4). Of the 67 families, 29 were recorded in both periods. Distinct invertebrate communities were associated with pre- vs. post-harvest crops (MGLM: LRT = 227.8, d.f. = 18, p = 0.001; Figure 3.5). Specifically, Ephydridae (shore flies, Diptera; LRT = 34.301, d.f. = 18, p = 0.001) and Isotomidae (Entomobryomorpha; LRT = 18.761, d.f. = 18, p = 0.001) were significantly more abundant pre-harvest, whilst Eupelmidae (Hymenoptera; LRT = 11.728, d.f. = 18, p = 0.014), Microphysidae (Hemiptera; LRT = 12.957, d.f. = 18, p = 0.006), Parasitiformes (Acari; LRT = 11.879, d.f. = 18, p = 0.012) and Thripidae (Thysanoptera; LRT = 16.821, d.f. = 18, p = 0.002) were significantly more abundant post-harvest.

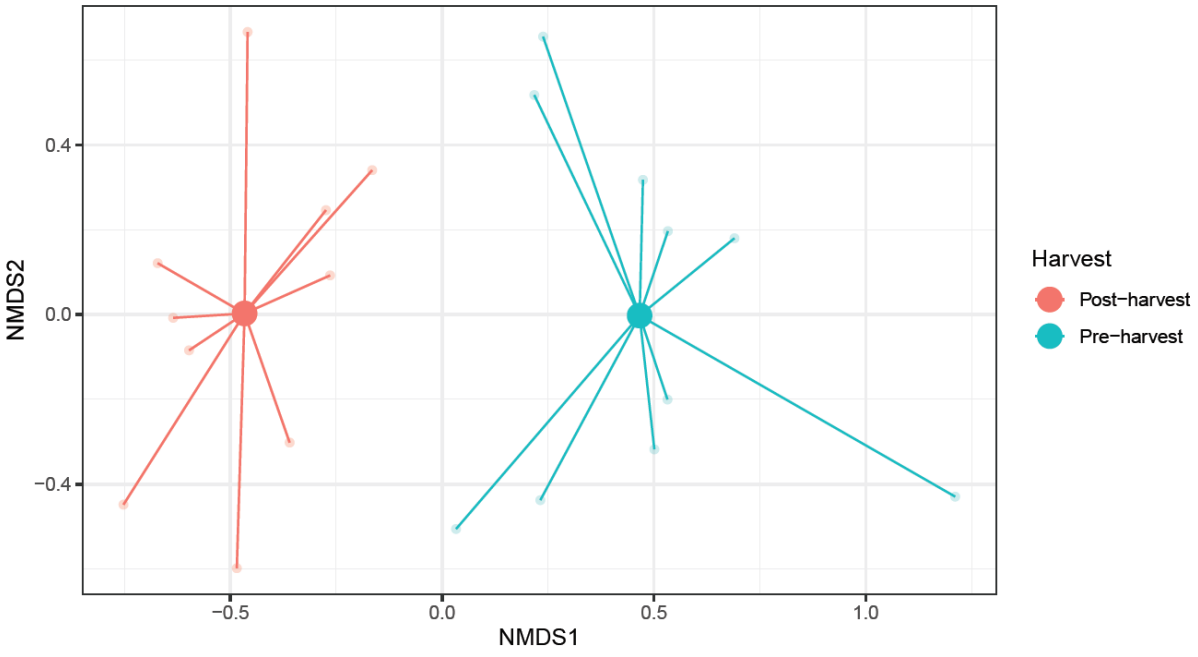


Figure 3.5: Spider plot derived from non-metric multi-dimensional scaling of invertebrate communities pre- and post-harvest. Community samples (smaller nodes) and centroids of community samples (larger nodes, mean coordinates of the samples for each category) are given for both categories. Species plots align with the distinctions between pre- and post-harvest communities (Figure S3.6).

3.4.3. Dietary analysis

The general primers (BerenF-LuthienR) recovered an average of 4204 reads (8.39% reads were prey) and 1.93 prey taxa per spider, and the spider-exclusion primers (TelperionF-LaurelinR) recovered 3530 reads and 0.72 prey taxa per spider from those spiders from which prey were recovered by at least one of the primer pairs. The spider-exclusion primers successfully avoided amplifying any *Erigone* DNA but did amplify the DNA of the other spiders studied (mostly *Tenuiphantes tenuis*; in those cases, 2.99% reads were prey, whereas 100% reads were prey for *Erigone* spp.).

Of the 66 spiders screened, dietary data was recovered for 46 (Table 3.2), comprising 15 families (Tables S3.5 and S3.6). Several pest taxa were identified from the spider gut DNA, including aphids (*Sitobion avenae*), true flies (Cecidomyiidae sp., *Oscinella* sp. Becker 1909), hoppers (*Macrosteles sexnotatus* (Fallén, 1806), *Javasella* sp. Fennah, 1963, *Nothodelphax* sp. Fennah, 1963) and thrips (*Anaphothrips obscurus* (Müller, 1776), *Frankliniella tenuicornis* (Uzel, 1895)). Distinct spider diets were associated with pre- and post-harvest (MGLM: LRT = 27.93, d.f. = 36, p = 0.027; Figure 3.6), but this was affected by the life stage of the spider (MGLM: LRT = 27.43, d.f. = 33, p = 0.001); however, no specific prey were associated with these differences (i.e. overall diets were significantly different but this was not due to a specific prey taxon/taxa). Five families were only found in one of the two periods: Aphididae (aphids, Hemiptera), Cecidomyiidae (gall midges, Diptera) and Cicadellidae (leaf hoppers, Hemiptera) were only detected in spider diets pre-harvest, whilst Chironomidae (non-biting midges, Diptera) and Ephydridae (shore flies, Diptera) were only detected post-harvest.

Table 3.2: The 46 spiders from which dietary data was recovered.

Pre-harvest	**Erigoninae**	Adult	Female	3
			Male	2
		Sub-adult	Female	0
			Male	0
			Juvenile	2
	Linyphiinae	Adult	Female	5
			Male	4
		Sub-adult	Female	0
			Male	6
			Juvenile	5
Post-harvest	**Erigoninae**	Adult	Female	0
			Male	1
		Sub-adult	Female	0
			Male	0
			Juvenile	1
	Linyphiinae	Adult	Female	3
			Male	7
		Sub-adult	Female	0
			Male	7
			Juvenile	0

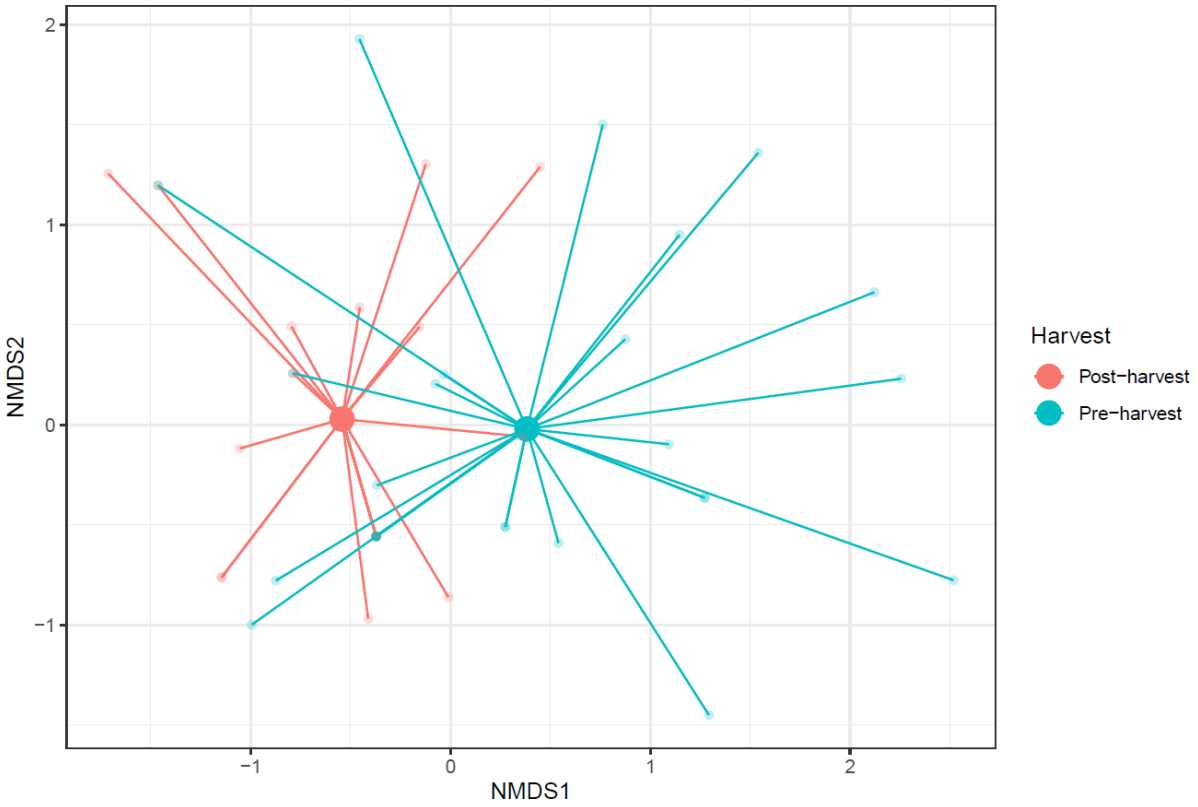


Figure 3.6: Spider plot derived from non-metric multi-dimensional scaling of spider diets pre- and post-harvest. Dietary samples (smaller nodes) and centroids of diets (larger nodes) are given for both categories. Species plots align with the distinctions between pre- and post-harvest communities (Figure S3.7).

3.4.4. Prey choice analysis

Spiders exhibited prey choice (prey consumed by predators at a higher or lower relative frequency than expected based on availability) in both pre- and post-harvest periods (Figure 3.7; Figure S3.8). Pre-harvest spiders predated Cicadellidae (leaf hoppers, Hemiptera), Linyphiidae, Phoridae (humpbacked flies, Diptera) and Sminthuroidea (globular springtails) significantly more than expected, and Chloropidae (frit flies, Diptera), Ephydridae (shore flies, Diptera) and Isotomidae (Entomobryomorpha) significantly less than expected. Post-harvest spiders predated Entomobryidae (Entomobryomorpha), Ephydridae (shore flies, Diptera), Linyphiidae and Delphacidae (plant hoppers, Hemiptera) significantly more than expected, and Thripidae (Thysanoptera) significantly less than expected. All other taxa were consistent with the null models and were consumed at the rates expected from their density.

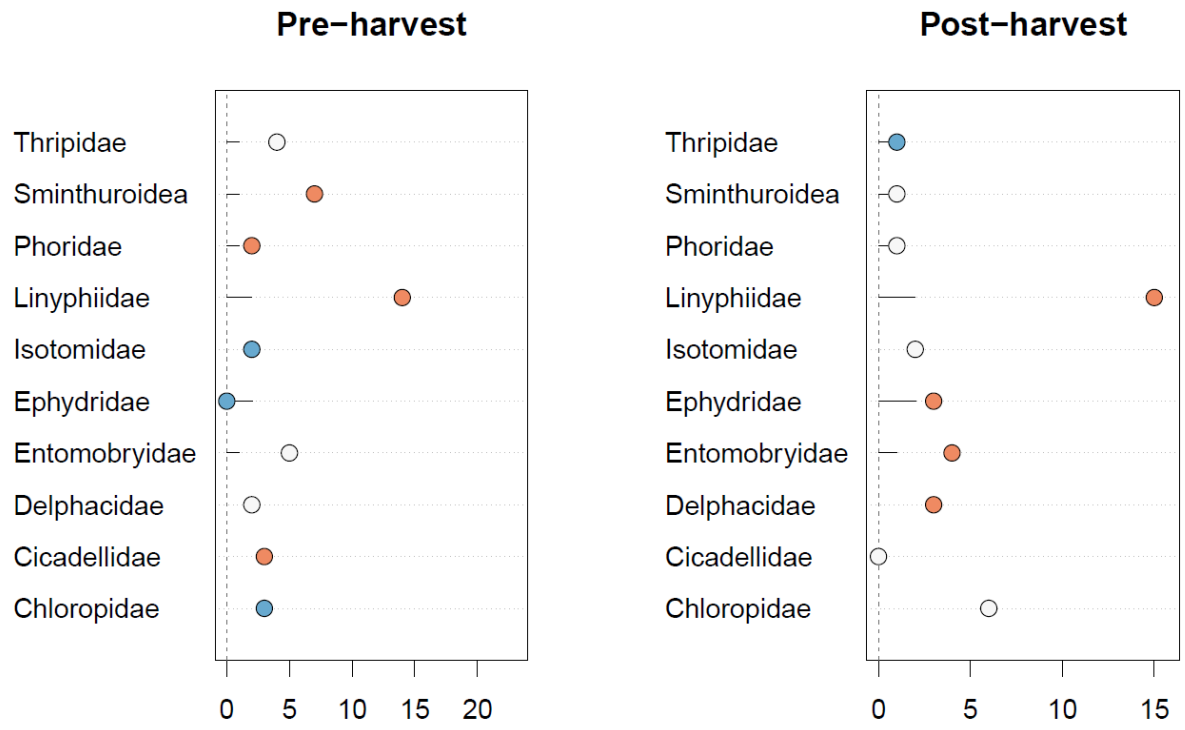


Figure 3.7: Significant deviations from expected frequencies of trophic interactions. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), white = as expected (in proportion to relative abundance), red = higher than expected (consumed more frequently than predicted from relative abundance).

3.5. Discussion

Through the application of novel PCR primers, linyphiid diet was shown to differ following harvest, primarily due to changes in prey availability (and apparent changes in prey choice).

The primers presented in this study are the first designed specifically for gut content analysis of linyphiid spiders and provide an effective means of dietary analysis for a range of species. Both novel primer pairs performed well both *in silico* and *in vitro*, ultimately successfully detecting prey in the guts of field-captured linyphiid spiders with complementary coverage and overlap of detected prey taxa. The broad range of species amplified by the primers suggests applicability to other species and study systems. In the case of TelperionF-LaurelinR, this is particularly true for dietary analysis of spiders in the families Agelenidae, Pholcidae and Clubionidae at least, as well as some success with ants and other predatory invertebrates given their lack of amplification. Whilst TelperionF-LaurelinR did ultimately amplify the DNA of *Tenuiphantes*, these primers facilitated effective gut content analysis for *Erigone* spiders, likely further Erigoninae species and possibly the other taxa listed above. Whilst no significant difference was found between the two extraction methods trialled, the mean percentage of

prey reads per sample was slightly greater when the abdomen was left in the lysis buffer for the longer incubation period, aligning with the findings by Krehenwinkel *et al.* (2016).

This study provides an example of the use of general and predator-exclusion PCR primers together for the same dietary samples. That both primers exhibited taxonomic biases is unsurprising given this widespread phenomenon in metabarcoding (Piñol *et al.* 2018), but their complementary biases, determined via mock communities, is promising for their combined use, ultimately providing a more complete snapshot of diet. Given the increasing understanding of the effects of primer bias in metabarcoding (Piñol *et al.* 2018; Braukmann *et al.* 2019; Elbrecht *et al.* 2019), further studies should consider employing this approach to begin mitigating these effects without relying on the heavier biases associated with other methodologies, such as the use of blocking probes (Piñol *et al.* 2015).

The low annealing temperature used for Telperion-Laurelin (42 °C) was selected due to the exclusion of several prey species at higher temperatures, but could present issues such as dimerization (i.e. the primers binding to one another rather than target DNA) or non-specific binding, the latter of which can create differently-sized amplicons in some cases, including in the subsequent application of these primers in **Chapter 4**. Given the melting temperatures of around 54 °C for these primers, other users could consider higher annealing temperatures when testing against their target organisms to avoid any associated issues, but, as highlighted by the data of this study, the primers will successfully amplify HTS-appropriate DNA from the guts of spiders even at these lower temperatures. Care must be taken, however, to ascertain whether the focal spider species is amplified prior to analysis. Modifications to the primer sequences may increase their specificity for the study of other spider species diets. These spider-exclusion primers, alongside NoSpi2 (Lafage *et al.* 2019), provide a complementary suite of primers for the metabarcoding of the diets of many spider taxa. The dietary analysis workflow used here shows great promise for application to a larger cohort of spiders in **Chapters 4 & 5**, as do the novel PCR primers. The amplification of *T. tenuis*, the most common linyphiid at this field site, by TelperionF-LaurelinR is, however, suboptimal and warrants a return to the primer sequences to ascertain any possible amendments to the primer sequences that may prevent this by more selectively avoiding amplification of spiders (successfully addressed in **Chapter 4**).

That distinct invertebrate communities were identified pre- and post-harvest indicates a substantial ecological impact of harvest on community composition and dynamics, and thus the provision of alternative prey for generalist predators. The significant decrease in isotomid abundance following harvest is noteworthy given that linyphiids regularly predate springtails such as *Isotoma anglicana* (Schäffer, 1896; Agustí *et al.* 2003; Harwood *et al.* 2003; Piñol *et al.* 2014). The loss of a major prey species could be detrimental to linyphiids immediately prior

to winter, especially for those that overwinter as adults. The relative avoidance of isotomids pre-harvest, however, suggests that this may not be the case and that reduced abundance of isotomids may still be sufficient for linyphiid populations. Continuous provision of alternative prey not only reduces intraguild predation (Athey *et al.* 2016), but likely supports over-winter and early-season predation of pests, which is critical in curbing pest populations with short generation times, such as aphids (Symondson *et al.* 2002; Korenko *et al.* 2010; Pekár *et al.* 2015). A situational understanding of community ecology is, however, critical in managing conservation biocontrol (Chailleux *et al.* 2014); whilst alternative prey can sustain generalist predator-mediated biocontrol (Agustí *et al.* 2003; Bell, Traugott, *et al.* 2008), some taxa may possibly detract from net biocontrol activity (Symondson *et al.* 2006). Whilst linyphiid abundance was relatively similar pre- and post-harvest, Opatovsky and Lubin (2012) report post-harvest declines in agrobiont linyphiid species in a Mediterranean system (Opatovsky and Lubin 2012). Climatic differences may account for this contrast, but the importance of immigrant spiders in rapidly recolonising fields at the start of each cropping cycling and the substantial pest suppression that they provide cannot be overlooked (Opatovsky *et al.* 2012), possibly suggesting a reduced importance of over-winter prey provision for early season spider abundance.

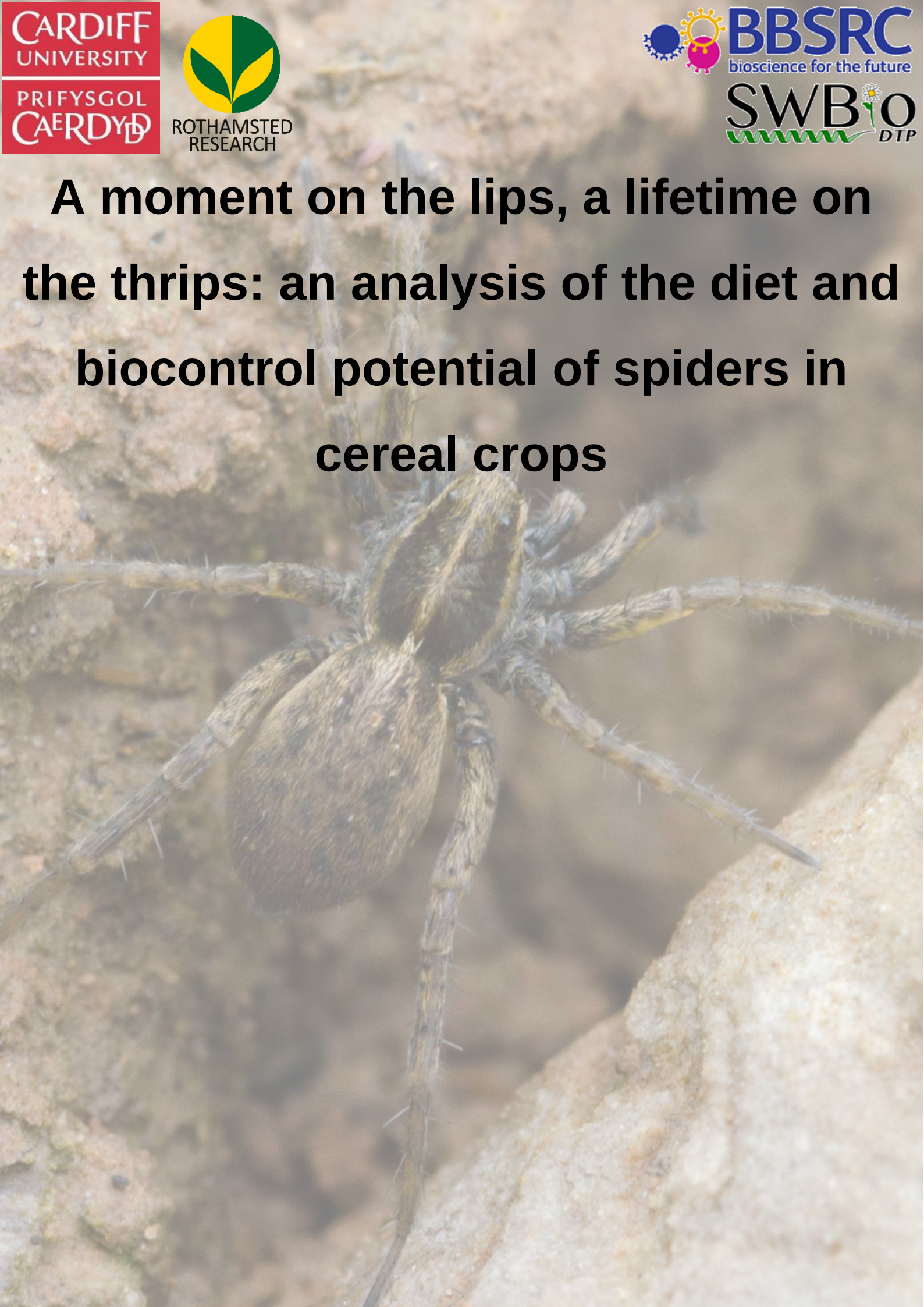
The difference in diet between pre- and post-harvest spiders reflects the community turnover following harvest, with many crop-dependent species such as aphids disappearing from the diet post-harvest. This finding is consistent with Bell *et al.* (2010) in that community turnover, and thus changes in co-occurrence, facilitate dietary changes. Whilst only an overall significant difference was found, not specific taxonomic associations, some taxa were only found in diets in one of the two periods. Regardless of dietary differences, spiders differentially selected from the prey taxa available to them both pre- and post-harvest, inferring differences in prey choice between the two periods. A larger number of significant deviations from expected trophic interaction frequencies were found pre-harvest than post-harvest (7 *vs*. 4), suggesting that spiders can be more selective regarding their dietary intake prior to harvest, possibly due to greater prey abundance in this period.

Isotomid springtails were predated less frequently than expected pre-harvest when their abundance was greatest, but not post-harvest, following the significant reduction in their abundance. Pre-harvest, spiders may choose alternative prey over isotomids to diversify their dietary intake despite the ease of consuming isotomids, possibly redressing nutritional deficiencies (including accumulation of prey toxins) that would result from consuming only one species (Mayntz *et al.* 2005). The greater-than-expected frequency of post-harvest consumption of ephydrids and entomobryids suggests that greater provision of these and similar taxa could benefit the health of linyphiid populations post-harvest. Such taxa could be

supported by increased habitat complexity (Michalko *et al.* 2017) and continuous refuges following harvest, particularly grass margins and in-field 'beetle banks' (MacLeod *et al.* 2004; Mansion-Vaquie *et al.* 2017). The relative avoidance of thrips of the family Thripidae post-harvest is likely a consequence of their dominance of the post-harvest invertebrate community, and the need to diversify dietary intake by the spiders. It should be noted that the primers used in this study may exhibit some bias against thrips in PCRs (Figure S3.4), thus this result may alternatively be due to underrepresentation of thrips in the diet; greater sequencing depth or alternative PCR primers could mitigate this in future studies. Such biases are also observed for most metabarcoding primers, including poor amplification of thrips by the commonly-used primers ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale *et al.* 2011; Gomez-Polo *et al.* 2016; Piñol *et al.* 2018; Silva *et al.* 2019).

Across both periods, linyphiids were prominent in the diets of the linyphiids screened. Since sequences conspecific with the predator were removed and contamination by tag-jumping accounted for, these were legitimate detections of intra-guild predation between linyphiid species. The inability to detect cannibalism via metabarcoding could mean that these instances of intra-guild predation are in fact an underestimate. That linyphiids are so prominent in their own diet, particularly post-harvest, and were preferentially consumed across both periods, could indicate high competition for prey resources. Linyphiids will usurp one another for optimal web sites based on prey abundance, sometimes consuming one another in the process (Harwood and Obrycki 2005), which is likely to increase during periods of prey scarcity. If prey choice is indeed influenced by nutritional requirement, linyphiids may also predate one another as perfect vessels for all of their nutritional needs. This intraguild predation is well-documented in linyphiids (Harwood and Obrycki 2005; Davey *et al.* 2013) and is a concern regarding their effectiveness as biological control agents. Intraguild predation may, however, sustain linyphiid populations when suitable prey are absent. Increased habitat complexity could thus reduce intraguild predation through improved prey provisioning (Michalko *et al.* 2017) and improve the biocontrol capacity of linyphiids. Given that this harvest interface period lies beyond typical crop spraying times, the importance of natural enemies for biocontrol is arguably even greater than during active spraying periods. Linyphiids are consuming pests in this period (e.g. aphids, as shown in this study), so reductions in intraguild predation by altered management regimes could further enhance this primary control of pests as they begin overwintering, maximally impacting the early-season return of pests. With these initial results and a proof-of-concept for the analysis of prey choice in field-collected spiders, further assessment of spider dietary dynamics with a larger cohort was carried out in **Chapter 4**, with a nutrient-based analysis of prey choice within that cohort following in **Chapter 5**.

A moment on the lips, a lifetime on the thrips: an analysis of the diet and biocontrol potential of spiders in cereal crops



“When spiders unite, they can tie down a lion.”

- Ethiopian proverb

Chapter 4 : A moment on the lips, a lifetime on the thrips: an analysis of the diet and biocontrol potential of spiders in cereal crops

4.1. Abstract

1. Spiders are among the dominant invertebrate predators in agricultural systems, particularly regarding the predation of insect pests. Their dietary dynamics in the field are, however, poorly understood. This chapter investigates the taxonomic, demographic, temporal and weather factors affecting spider diet with specific focus on their biocontrol potential and intraguild predation.
2. Spiders of five genera (*Bathyphantes*, *Erigone*, *Microlinyphia* and *Tenuiphantes*; Araneae: Linyphiidae; and *Pardosa*; Araneae: Lycosidae) were collected from barley fields in Wales, UK between April and September 2018, and the gut contents of 300 individuals were analysed using metabarcoding and high-throughput sequencing.
3. From the 300 spiders screened, 89 prey operational taxonomic units were identified from 45 families, including a wide range of pests and predators. Spider diets significantly differed between spider genera and life stages, reflected in different propensities for intraguild predation and biocontrol. Adult spiders more frequently predated predators than juveniles, and juveniles more frequently predated pests than adults. Overall, *Tenuiphantes* and *Bathyphantes* exhibited the greatest potential for biocontrol of the pest genera compared.
4. Sustainable production of cereals should aim to optimise conditions throughout the cropping cycle for effective biocontrol, prioritising provision for those spiders which most regularly prey on pest species (i.e. juveniles, *Bathyphantes* and *Tenuiphantes*). This might include spatially segregated intercropping of cereals with legumes to provide refugia and a source of alternative prey throughout the cropping period, whilst also benefitting the soil and agricultural production.

4.2. Introduction

Conservation biocontrol, employing naturally occurring predators as biological control agents of pests, has been discussed for decades (Symondson and Liddell 1993; Gurr *et al.* 2000; Symondson *et al.* 2002; Bale *et al.* 2008), but its relevance has greatly increased. As

insecticide use declines due to developed resistance, increased regulation and detrimental environmental effects, alternative and integrated (i.e. multi-faceted, including the employ of natural enemies) pest management is increasingly pertinent (Fountain *et al.* 2007; MacFadyen *et al.* 2009; Whitehorn *et al.* 2012; Pekár 2013; Loetti and Bellocq 2017). Polyphagous generalist predators, such as spiders and ground beetles (Coleoptera: Carabidae) which are abundant in arable fields, can be effective conservation biocontrol agents for crop pests (Riechert and Lockley 1984; Mark Alderweireldt 1994; Symondson *et al.* 2002; Chapman *et al.* 2013); however, the diets of these biocontrol agents and how their diets dynamically change, both over time and between individuals, has seldom been characterised in the field using broad-scale techniques.

Spiders have several attributes that place them among the most efficient cereal crop predators of pests such as aphids (Sunderland *et al.* 1987). Through a diversity of foraging techniques, including sit-and-wait and active hunting (Turnbull 1973; Riechert and Lockley 1984), spiders influence food webs differentially via variation in strategy-specific prey capture (Michalko and Pekár 2016). Different prey species are active at different times in the diel cycle, resulting in spiders foraging both diurnally and nocturnally (Bollinger *et al.* 2015). Spiders can reach densities of 200-600 m^{-2} in cereal crops (Nyffeler and Sunderland 2003; Shayler 2005) and are even present over winter, being the most active winter predator in many such systems; this facilitates their suppression of pests when populations first emerge or arrive, before their populations establish and grow (Juen *et al.* 2002; Korenko *et al.* 2010; Boreau De Roincé *et al.* 2013). Whereas many generalist predators are disrupted by crop cycling, spiders have similar generation times to typical crop cycles, with peak abundances occurring at these critical early phases of pest population establishment (Riechert and Lockley 1984; Welch *et al.* 2011). When prey reach high densities, spiders will rapidly colonise favourable web locations, sometimes engaging in territorial contests to hold such web sites (Riechert and Lockley 1984; Samu *et al.* 1996; Harwood *et al.* 2001; Bollinger *et al.* 2015). The availability of different prey can also directly affect the structures used for web attachment (e.g. soil-surface-attached webs for springtails and plant-stem-attached webs for falling and crawling aphids; Welch *et al.* 2017). Even once these web sites are abandoned though, their empty webs can capture and subsequently kill pests, indirectly suppressing pest populations (Sunderland 1999; Harwood and Obrycki 2005).

The diet of cereal crop spiders commonly includes springtails, flies and true bugs (Toft 1995; Agustí *et al.* 2003; Piñol *et al.* 2014), including pest species such as aphids (Sunderland *et al.* 1986; Beck and Toft 2000; Mayntz and Toft 2000; Bilde and Toft 2001; Nyffeler and Sunderland 2003), planthoppers (Wang *et al.* 2016; Wang *et al.* 2017), psyllids (Petráková *et al.* 2016), medflies (Monzó *et al.* 2010), lepidopterans (Quan *et al.* 2011; Pérez-Guerrero *et*

al. 2013; Senior *et al.* 2016) and weevils (Vink and Kean 2013). Spiders have been observed preferentially foraging for aphids over alternative prey, such as springtails, possibly due to additional sensory cues from aphids, but also suggesting a nutritional benefit to predating aphids (Welch *et al.* 2016). In fact, Sunderland *et al.* (1986) found that aphids formed 38-63% of the diet of common money spider species. Some aphid species, such as *Sitobion avenae*, are, however, thought to have a toxic effect on spiders, reducing the spider's fecundity, although others, such as *Metopolophium dirhodum,* are thought to have fewer negative effects (Bilde and Toft 2001). Spider predation of aphids is thought to vary between sexes, with female spiders tending to eat more aphids than males, and between taxa, with the subfamily Linyphiinae feeding on more aphids than Erigoninae spiders (Harwood *et al.* 2004); however, itinerant Erigoninae spiders may predate more aphids by actively searching for prey on the ground (Harwood *et al.* 2004; Gavish-Regev *et al.* 2009). It is difficult, however, to predict the efficacy of aphid predators via functional traits, with taxonomy, life stage and morphology serving as poor predictors of predation rates (Bell, Mead, *et al.* 2008).

Optimal predator fitness requires a diverse diet of pest and non-pest prey (Harwood *et al.* 2009). Although alternative prey positively affect spider-mediated biocontrol efficacy by enhancing fitness (Ostman 2004; Roubinet *et al.* 2017), this is taxon-specific and may increase intraguild predation, which detracts from the overall benefit of spider-mediated biocontrol (Traugott and Symondson 2008; Moreno-Ripoll *et al.* 2012; Traugott *et al.* 2012; Davey *et al.* 2013; Moreno-Ripoll *et al.* 2014). To effectively harness their conservation biocontrol potential, the dietary dynamics of spiders in the field must first be understood (Chapman *et al.* 2013).

4.2.1. Objectives and hypotheses

This chapter investigates the diets of common cereal crop spiders (Linyphiidae and Lycosidae) using DNA metabarcoding with the primers developed in **Chapter 3**. The aim of this chapter is to identify differences in dietary intake between spiders and the factors responsible for any differences, ultimately to identify differences in the biocontrol efficiency of different spiders and thus optimal candidates for biocontrol. It was hypothesised that: (i) spider functional traits indicative of ecology, such as genus, sex and life stage, would affect dietary intake as a consequence of differential foraging strategies, life histories and morphologies; (ii) web characteristics (area and height) would correspond with dietary differences, partly explaining any dietary differences observed between spider genera; (iii) time and weather would affect dietary intake as a consequence of the variable availability of prey and modulations to prey and predator behaviour; and (iv) levels of intraguild predation and biocontrol would differ between spiders based on their functional traits. The data generated in this chapter will be

used alongside macronutrient data determined via the protocol developed in **Chapter 2** to analyse nutrient-specific foraging in **Chapter 5**.

4.3. Methodology

4.3.1. Fieldwork

Money spiders (Araneae: Linyphiidae) and wolf spiders (Araneae: Lycosidae) were visually located along transects in two adjacent barley fields at Burdons Farm, Wenvoe in South Wales (51°26'24.8"N, 3°16'17.9"W) and collected from occupied webs and the ground, between April and September 2018. Each belt transect was adjacent to a randomly selected crop tramline and were distributed across the entire field and ran its length. The areas searched were 4 m^2 quadrats at least 10 m apart and all observed linyphiids and lycosids were collected. Spiders were taken from 64 locations along the aforementioned transects. Spiders were placed in 100% ethanol using an aspirator, regularly changing meshing to limit potential contamination like that introduced by alternative techniques such as pitfall or suction sampling (King *et al.* 2012; Athey *et al.* 2017). Linyphiids occupying webs were prioritised for collection, but ground-hunting linyphiid spiders were collected when occupied webs were scarce. For each individual money spider that was taken from its web, the height of the web from the ground and its approximate dimensions were recorded, the latter calculated as approximate web area. Spiders were taken to Cardiff University, transferred to fresh ethanol, adults identified to species-level and juveniles to genus, and stored at -80 °C in 100% ethanol until subsequent DNA extraction.

4.3.2. Extraction and high-throughput sequencing of spider gut DNA

Given their prevalence in field collections, dietary analysis was carried out for the linyphiid genera *Erigone*, *Tenuiphantes*, *Bathyphantes* and *Microlinyphia* (Araneae: Linyphiidae), and the Lycosidae genus *Pardosa* (Figures 4.1-4.2). Spiders were transferred to and washed in fresh 100 % ethanol to reduce external contaminants prior to identification via morphological key (Roberts 1993). Abdomens were removed from spiders and again transferred to and washed in fresh 100% ethanol. DNA was extracted from the abdomens via Qiagen TissueLyser II and DNeasy Blood & Tissue Kit (Qiagen) as per the manufacturer protocol, but with an extended lysis time of 12 hours to account for the complex and branched gut system in spider abdomens (Krehenwinkel *et al.* 2017).

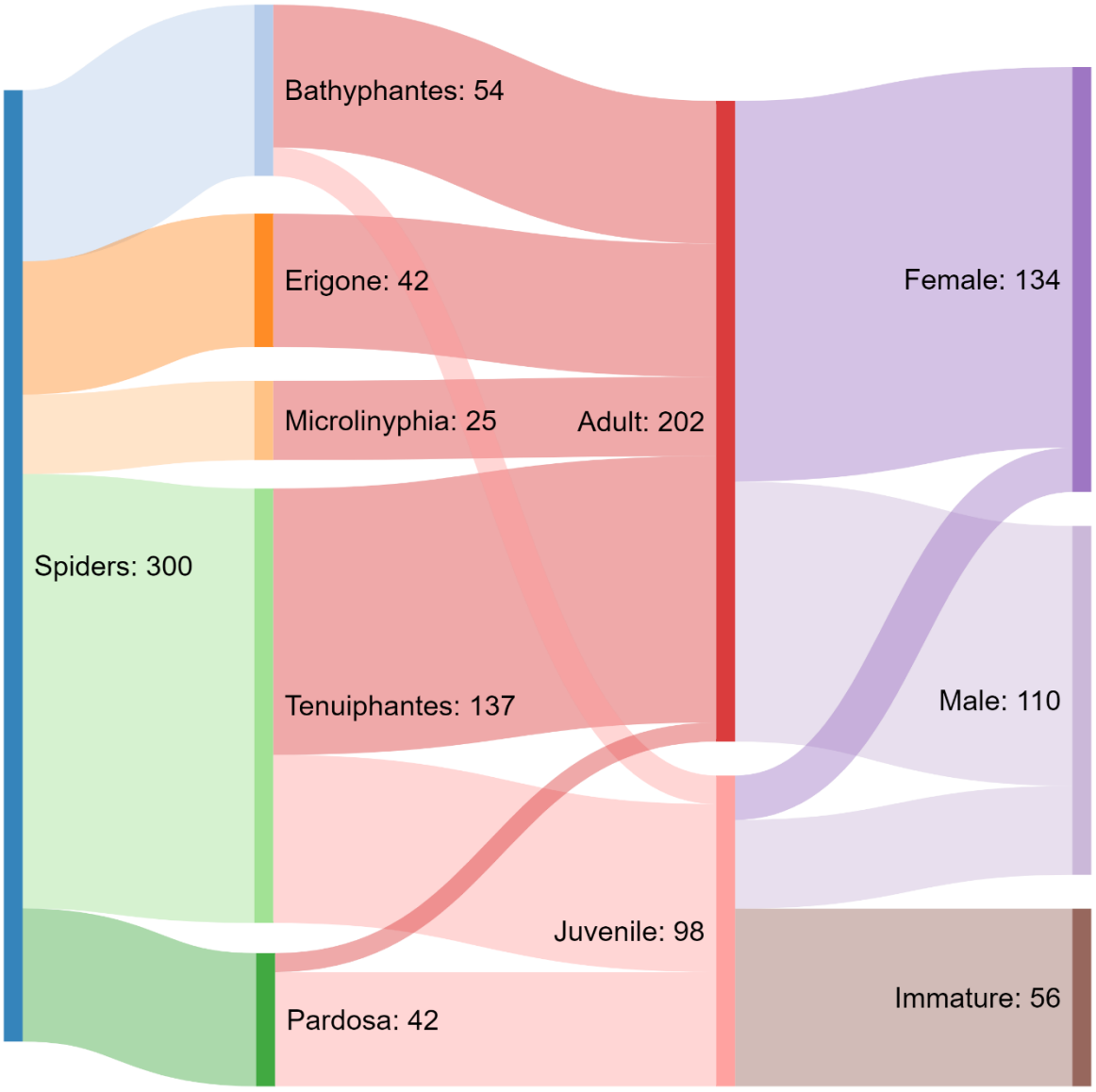


Figure 4.1: The division of spiders analysed via metabarcoding between genera, life stages and sexes. Numbers and labels correspond to the subsection immediately adjacent to it. Diagram created using SankeyMATIC.

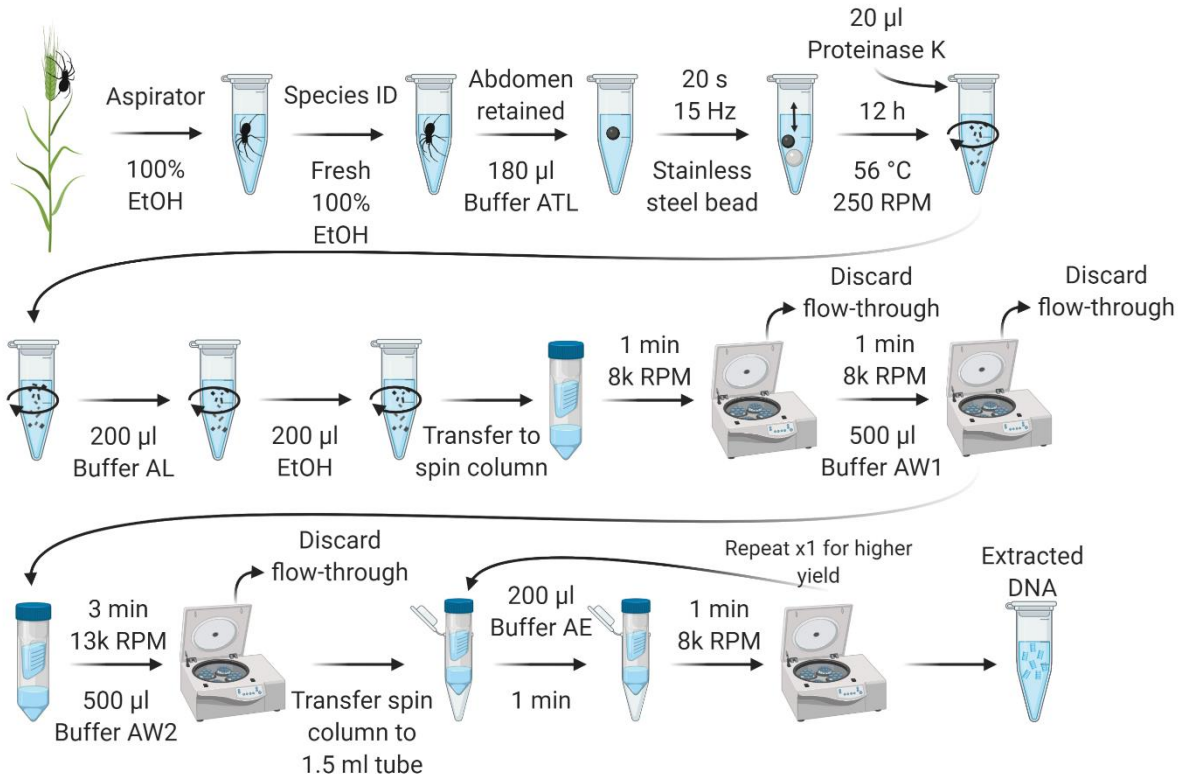


Figure 4.2: DNA extraction protocol. Figure created with BioRender.com.

For amplification of DNA (Figure 4.3), two primer pairs were used. BerenF-LuthienR (Cuff *et al.* 2020) amplified a broad range of invertebrates including spiders, and TelperionF-LaureR, amplified a range of invertebrates but fewer spiders (modified from TelperionF-LaurelinR (Cuff *et al.* 2020) via one base-pair change; 5'-ggrtawacwgttcawccagt-3'). These two primer pairs amplified 314 bp (BerenF-LuthienR) and 302 bp (TelperionF-LaureR) regions of COI. Primers were labelled with unique 10 bp molecular identifier tags (MID-tags) so that each individual had a unique pairing of forward and reverse for identification of each spider post-sequencing. PCR reactions of 25 µl volumes contained 12.5 µl Qiagen PCR Multiplex kit, 0.2 µmol (2.5 µl of 2 µM) of each primer and 5 µl template DNA. Reactions were carried out in the same thermocycler, optimised via temperature gradient, with an initial 15 minutes at 95 °C, 35 cycles of 95 °C for 30 seconds, the primer-specific annealing temperature for 90 seconds and 72 °C for 90 seconds, respectively, followed by a final extension at 72 °C for 10 minutes. BerenF-LuthienR and TelperionF-LaureR used annealing temperatures of 52 °C and 42 °C, respectively.

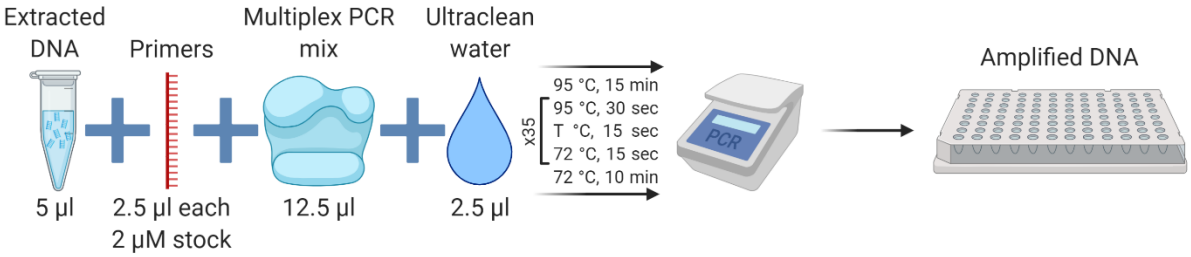


Figure 4.3: PCR protocol. T is the annealing temperature for each primer (BerenF-LuthienR: 52 °C; TelperionF-LaureR: 42 °C). Figure created with BioRender.com.

Within each PCR 96-well plate, 12 negative controls (extraction and PCR), 2 blank controls and 2 positive controls were included (i.e. 80 samples per plate), based on Taberlet *et al.* (2018). Positive controls were mixtures of invertebrate DNA comprised of non-native Asiatic species in four different proportions (Table S4.1) and blanks were empty wells within each plate to identify tag-jumping into unused MID-tag combinations. PCR negative controls were DNase-free water treated identically to DNA samples. A negative control was present for each MID-tag to identify any contamination of primers. All PCR products were visualised in a 2 % agarose gel with SYBR®Safe (Thermo Fisher Scientific, Paisley, UK) and placed in categories based on their relative brightness (Figure 4.4). The concentration of these brightness categories was quantified via Qubit dsDNA High-sensitivity Assay Kits (Thermo Fisher Scientific, Waltham, MA, USA) with at least three representatives of each category per plate. The PCR products were then proportionally pooled according to these concentrations. Each pool was cleaned via SPRIselect beads (Beckman Coulter, Brea, USA), with a left-side size selection using a 1:1 ratio (retaining ~300-1000 bp fragments). The concentration of the pooled DNA was then determined via Qubit dsDNA High-sensitivity Assay Kits and pooled together into one library per primer pair. Library preparation for Illumina sequencing was carried out on the cleaned libraries via NEXTflex Rapid DNA-Seq Kit (Bioo Scientific, Austin, USA) and samples were sequenced on an Illumina MiSeq via a V3 chip with 300-bp paired-end reads (expected capacity ≤25,000,000 reads).

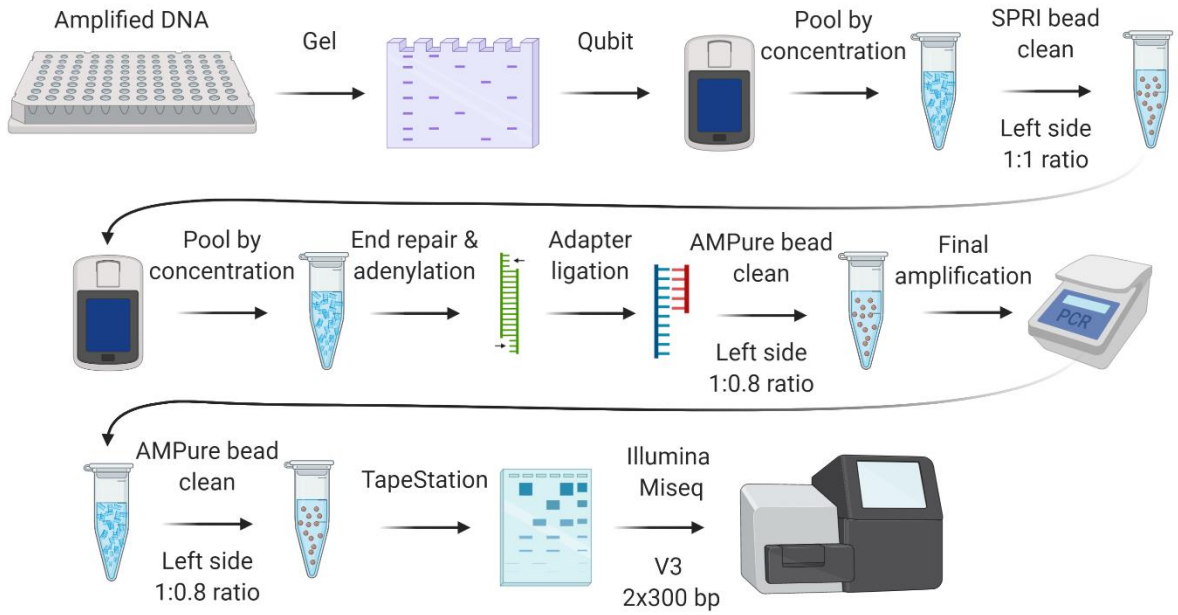


Figure 4.4: Pooling and cleaning of DNA and sequencing library preparation. Figure created with BioRender.com.

4.3.3. Bioinformatic analysis

The Illumina run generated 11,165,405 and 10,959,010 reads for BerenF-LuthienR and TelperionF-LaureR, respectively, which were quality-checked and paired via FastP (Chen *et al.* 2018) to retain only sequences of at least 200 bp with a quality threshold of 33, resulting in 10,561,874 and 9,355,112 paired reads. The paired reads were demultiplexed and assigned to their respective spider sample according to their MID-tags via the “trim.seqs” command in Mothur v1.39.5 (Schloss *et al.* 2009), leaving 7,854,610 and 7,437,929 reads with exact matches to the primer and MID-tags.

Replicates were removed, and denoising and clustering to zero-radius operational taxonomic units (ZOTUs; clustered without % identity to avoid multiple species represented within a single operational taxonomic unit (OTU)) completed via Unoise3 in Usearch11 (Edgar 2010). The resultant sequences were assigned a taxonomic identity from GenBank via BLASTn v2.7.1. (Camacho *et al.* 2009) using a 97% identity threshold (Alberdi *et al.* 2017). The BLAST output was analysed in MEGAN v6.15.2 (Huson *et al.* 2016). Where the top BLAST hit, determined by lowest e-value, was resolved at a higher taxonomic level than species-level, the results were checked; where possibly erroneous entries were preventing species-level assignment (e.g. poorly-resolved identifications on GenBank), finer resolution was assigned based on the next-closest match. Where ZOTUs were assigned the same taxon, these were aggregated.

Data clean-up followed the protocol described by Drake (2020). The maximum value for a ZOTU present in blank or negative controls was identified and subtracted from all read counts for that ZOTU to remove background contaminants. Simultaneously, known lab contaminants (e.g. German cockroach *Blattella germanica*), artefacts and errors of the sequencing process, unexpected reads in positive controls and positive control taxon reads in dietary samples were identified. These were calculated as a percentage of their respective sample's read count and any read counts lower than the highest of these percentages for their respective sample were removed to eliminate additional instances of contamination. These thresholds were defined as 0.38% and 0.39% for BerenF-LuthienR and TelperionF-LaureR, respectively. The data from the two libraries (i.e. from each primer pair) were then aggregated together by sample and aggregated again by taxon. Non-target taxa (e.g. fungi) and instances in which predator DNA was amplified (i.e. ZOTUs with high read counts matching the individual's morphological identity) were removed. All remaining read counts were converted to presence-absence.

4.3.4. Weather data

Weather data were taken from publicly available reports from the Cardiff Airport weather station (6.6 km from the study site) via "Wunderground" (Wunderground 2020) from 1/1/2018 to 17/9/2018 (the last field collection). Specifically, daily high, low, average and historical average temperatures (°C), daily average dew point (°C), maximum daily wind speed (mph), daily sea level pressure (Hg) and day length (min; sunrise to sunset) were recorded. Precipitation data, unavailable via Wunderground, were downloaded via the UK Met Office Hadley Centre Observation Data (UK Met Office 2020) as regional precipitation (mm) for South West England & Wales. These weather data were collected due to the perceived importance of meterological factors in spider behaviour, particularly ballooning (Greenstone 1990; Weyman 1993; Suter 1999; Bell *et al.* 2005; Morley and Robert 2018). For analysis against spider diets, weather data were converted to means for the preceding seven days to correspond to the longevity of DNA in the guts of spiders.

4.3.5. Statistical analysis

All analyses were conducted in R v4.0.0 (R Core Team 2020). Levin's niche breadth (Levins 1969) was calculated for the spider genera, sexes and life stages studied using the 'niche.width' function of the "spaa" package (Zhang 2016) and standardised following equation one from Razgour *et al.* (2011). Pianka's niche overlap (Pianka 1973) was calculated and compared against random simulations for spider genera, sexes and life stages using the

'niche_null_model' function in the 'EcoSimR' package (Gotelli *et al.* 2015) with 9999 repetitions. Prey species that occurred only once across all of the dietary samples were removed before further analyses to prevent outliers skewing the results, which is particularly problematic for non-metric multidimensional scaling. Pairwise co-occurrence analysis was carried out to identify prey species which occurred together more or less than expected by chance. The 'cooccur' function in the 'cooccur' package (Griffith *et al.* 2016) was used to calculate the observed and expected frequencies of co-occurrence between each pair of taxa.

Spider diets were compared between variables using multivariate generalized linear models (MGLMs) via 'manyglm' in the 'mvabund' package (Wang *et al.* 2012) with a binomial error family and Monte Carlo resampling. Initial model independent variables included Julian day, mean week daylength, mean week temperature, mean week precipitation, mean week dew point, mean week wind, mean week air pressure, spider genus, spider family, spider life stage (juvenile or adult, the latter defined by fully developed genitalia), spider sex, spider ectoparasites (presence/absence; when physically present on spiders) and all two-way interactions between these variables. Models were simplified using 'step' from the base R 'stats' package to determine an optimal model based on the lowest AIC value by backward elimination. The optimal model for the analysis of spider diets contained the variables Julian day, mean week daylength, spider genus and spider life stage.

Coarse dietary differences were visualised by non-metric multidimensional scaling (NMDS) via metaMDS in the 'vegan' package (Oksanen *et al.* 2016) with Jaccard distance in two dimensions and 999 tries. For NMDS, outliers (usually samples containing rare taxa) were identified by plotting and subsequently removed to facilitate separation of samples and achieve minimum stress. For visualisation of the effect of continuous variables on diet, surf plots were created from the dietary NMDS using the 'ordisurf' command from the 'vegan' package (Oksanen *et al.* 2016), and re-plotted using 'ggplot' with the colour-blindless-friendly 'viridis' colour palette (Garnier 2018). For plotting categorical variables against the diet NMDS, spider plots were created using 'ordispider' with 'ggplot' and the 'RColorBrewer' 'Accent' colour palette (Neuwirth 2014).

The height and area of webs were compared between spider genera and sexes, and an interaction between the two, with a generalized linear model (GLM) each for height and area using a Gaussian error family and an identify link with the 'glm' function in base R. To improve adherence of the GLMs to assumptions and model fit, web height was square-root transformed and web area log transformed. Web comparisons were visualised using boxplots with jittered points overlaid via the 'ggplot2' 'geom_boxplot' function. Spider diet was compared against web characteristics for spiders for which both data were available using the MGLM process outlined above, but with starting models containing only web height, web area and an

interaction between the two, with the same binomial error family, but with a ‘cloglog’ link function.

Intraguild predation and biocontrol variables were created by counting the number of pest natural enemy taxa (Table S2), and, separately, of agriculturally relevant “pest” taxa (taxa containing species that detract from agricultural productivity; Table S2) in each spider’s diet. These were analysed against spider genus, spider maturity and Julian day (the latter scaled from the full range of days to a continuous range from -3 to 3 using the ‘scale’ function to reduce issues with scaling) via GLMs. “Site” (denoting the 4 m^2 area from which spiders were collected within fields) was initially included as a random effect in generalized linear mixed-models, but no significant effect was observed when comparing this model against a standard GLM via a likelihood ratio test of nested models using the ‘lrtest’ command in the ‘lmtest’ package (Zeileis and Hothorn 2002). Standard GLMs were thus used to avoid issues relating to singularity in the mixed models. The assumptions for the resultant Poisson error family GLMs were tested using the “testResiduals” function of the ‘DHARMa’ package (Hartig 2020). Intraguild predation and biocontrol differences between significant terms were visualised using violin plots with the quartiles, median and 95% upper limit annotated using the ‘geom_violin’ function in ‘ggplot2’.

4.4 Results

4.4.1. Descriptive information and observations

Across the 300 spiders screened, 89 different prey ZOTUs were identified from 45 families. Spiders contained prey from an average of 2.57 ZOTUs and 2.23 families with 81.3 % (244) of spiders containing detectable prey DNA. Coarse dietary differences were observed between genera (Table 4.1), sexes (Table 4.1) and life stages (Tables 4.1 and S4.3-S4.5).

Table 4.1: Spider dietary contents for all spiders (expressed as percentages of the spider population of each column that contained DNA for each prey taxon), each of the five studied genera, and the two sexes and two life stages studied. Dietary composition is given as the percentage of spiders in each category that predated each prey taxon. Darker colours denote larger percentages. Absolute values are given in Tables S4.3-S4.5.

Prey Family	All Spiders	*Bathyphantes*	*Erigone*	*Microlinyphia*	*Pardosa*	*Tenuiphantes*	Female	Male	Adult	Juvenile
Aeolothripidae	1.64	0.00	0.00	0.00	0.00	3.23	3.81	0.00	1.79	1.32
Anthocoridae	0.41	0.00	2.86	0.00	0.00	0.00	0.95	0.00	0.60	0.00
Anthomyiidae	0.41	0.00	0.00	3.85	0.00	0.00	0.95	0.00	0.60	0.00
Aphelinidae	4.92	0.00	5.71	11.54	4.76	4.84	5.71	5.38	4.17	6.58
Aphididae	19.26	26.32	14.29	3.85	0.00	25.00	13.33	29.03	20.83	15.79
Bourletiellidae	15.57	2.63	17.14	3.85	4.76	23.39	9.52	22.58	13.10	21.05
Braconidae	6.15	13.16	8.57	0.00	0.00	5.65	9.52	5.38	8.33	1.32
Cecidomyiidae	9.02	10.53	0.00	11.54	42.86	4.84	5.71	6.45	7.14	13.16
Chironomidae	1.64	2.63	0.00	3.85	0.00	1.61	1.90	1.08	1.79	1.32
Chloropidae	13.93	2.63	8.57	65.38	14.29	8.06	20.00	9.68	16.07	9.21
Chrysopidae	0.82	0.00	0.00	3.85	0.00	0.81	0.00	2.15	1.19	0.00
Cicadellidae	4.92	5.26	0.00	7.69	4.76	5.65	3.81	3.23	2.98	9.21
Damaeidae	0.41	0.00	0.00	0.00	0.00	0.81	0.95	0.00	0.60	0.00
Delphacidae	6.56	7.89	2.86	3.85	9.52	7.26	9.52	3.23	7.74	3.95
Dolichopodidae	0.41	2.63	0.00	0.00	0.00	0.00	0.95	0.00	0.60	0.00
Drosophilidae	1.64	2.63	0.00	3.85	0.00	1.61	3.81	0.00	2.38	0.00
Encyrtidae	0.82	0.00	0.00	3.85	0.00	0.81	1.90	0.00	1.19	0.00
Entomobryidae	2.87	0.00	0.00	0.00	0.00	5.65	1.90	3.23	0.60	7.89
Ephydridae	0.41	0.00	2.86	0.00	0.00	0.00	0.00	1.08	0.60	0.00
Eupodidae	16.80	23.68	0.00	0.00	0.00	25.81	10.48	16.13	9.52	32.89
Figitidae	0.41	0.00	0.00	0.00	0.00	0.81	0.95	0.00	0.60	0.00
Hemerobiidae	0.82	0.00	0.00	3.85	0.00	0.81	0.00	2.15	1.19	0.00
Unknown Hemipteran Family	0.82	0.00	0.00	0.00	0.00	1.61	0.00	2.15	1.19	0.00
Hypogastruridae	1.23	0.00	5.71	0.00	0.00	0.81	1.90	1.08	1.79	0.00
Ichneumonidae	1.64	2.63	0.00	7.69	0.00	0.81	1.90	1.08	2.38	0.00
Isotomidae	4.92	10.53	11.43	0.00	0.00	3.23	6.67	5.38	6.55	1.32
Katiannidae	3.28	13.16	2.86	0.00	0.00	1.61	3.81	3.23	3.57	2.63
Linyphiidae	6.97	2.63	14.29	0.00	0.00	8.87	8.57	5.38	7.74	5.26
Lycosidae	4.10	0.00	0.00	3.85	23.81	3.23	4.76	2.15	2.98	6.58
Mymaridae	0.82	0.00	2.86	0.00	0.00	0.81	1.90	0.00	0.60	1.32
Nabidae	0.41	0.00	0.00	0.00	0.00	0.81	0.95	0.00	0.60	0.00
Noctuidae	0.41	0.00	0.00	0.00	0.00	0.81	0.00	0.00	0.00	1.32
Phalacridae	0.82	5.26	0.00	0.00	0.00	0.00	0.95	1.08	0.60	1.32
Phoridae	3.28	0.00	5.71	11.54	4.76	1.61	5.71	1.08	2.98	3.95

Psychodidae	0.41	2.63	0.00	0.00	0.00	0.00	0.95	0.00	0.60	0.00
Rhinotermitidae	18.44	0.00	0.00	0.00	0.00	36.29	16.19	22.58	17.26	21.05
Sciaridae	10.25	7.89	5.71	3.85	4.76	14.52	13.33	8.60	10.71	9.21
Sminthuridae	7.38	26.32	5.71	0.00	0.00	4.84	12.38	5.38	10.71	0.00
Sphaeroceridae	1.23	5.26	0.00	0.00	0.00	0.81	2.86	0.00	1.79	0.00
Staphylinidae	4.92	2.63	8.57	7.69	4.76	4.03	8.57	2.15	6.55	1.32
Syrphidae	0.82	0.00	2.86	0.00	0.00	0.81	0.95	1.08	1.19	0.00
Thripidae	36.07	18.42	22.86	30.77	4.76	51.61	21.90	47.31	27.38	55.26
Tomoceridae	0.41	0.00	0.00	0.00	0.00	0.81	0.00	1.08	0.00	1.32
Triozidae	0.41	0.00	2.86	0.00	0.00	0.00	0.95	0.00	0.60	0.00
Trombidiidae	3.69	0.00	2.86	3.85	4.76	4.84	2.86	6.45	5.36	0.00

Niche breadth varied between spider genera, sexes and life stages (Figure 4.5). Of the genera studied, *Pardosa* and *Microlinyphia* had the smallest niche breadth, while the dietary niches of *Erigone*, *Bathyphantes* and *Tenuiphantes* were approximately twice as broad. Similarly, the dietary niche of female spiders was approximately twice as broad as that of male spiders, as was true of adult spiders compared against juveniles.

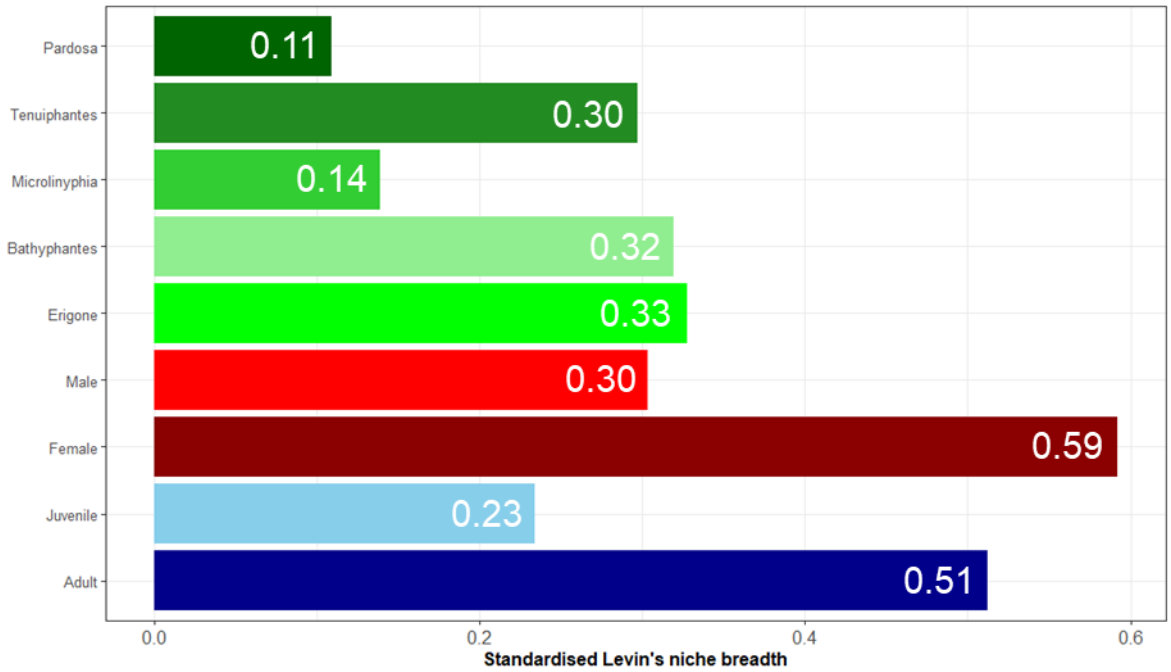


Figure 4.5: Standardised Levin's niche breadth for spider groups. Green, red and blue denote genera, sexes and life stages, respectively, with different shades arbitrarily representing different sub-groups. Numeric values within the bars denote the value of each niche breadth.

Dietary niches overlapped between genera (observed Pianka index = 0.348, simulated Pianka index = 0.138 (upper 95% CI = 0.189), SES = 7.475; Figure S4.1), life stages (observed Pianka index = 0.799, simulated Pianka index = 0.208 (upper 95% CI = 0.364), SES = 7.158; Figure S4.2) and sexes (observed Pianka index = 0.791, simulated Pianka index = 0.207 (upper 95% CI = 0.292), SES = 12.287; Figure S4.3) substantially more than in random simulations.

Across all spider diets, 26 prey co-occurrences were identified significantly more (17) or less (9) than expected by random simulation (Figure 4.6; Table S4.6).

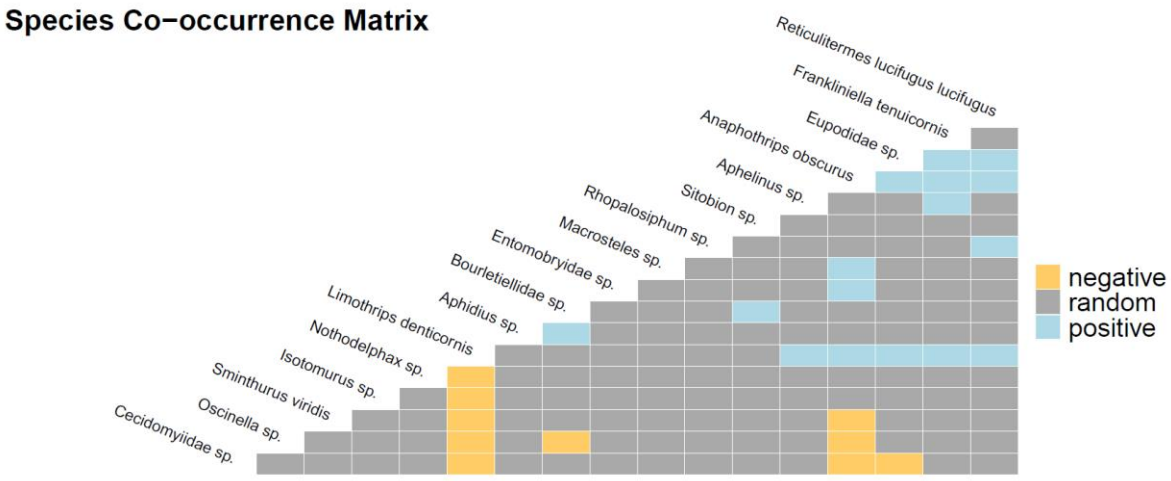


Figure 4.6: Species co-occurrence matrix for those species identified from spider diets. Yellow, grey and blue points denote significantly negative, random and significantly positive co-occurrences, respectively.

4.4.2. Dietary dynamics

In the full analysis, specific spider diets were significantly related to Julian day (MGLM: Dev = 149.9, d.f. = 235, p = 0.001; Figures 4.7 & S4.4, Table 4.2), mean week day length (MGLM: Dev = 350.6, d.f. = 234, p = 0.001; Figures 4.8 & S4.5, Table 4.2), spider genus (MGLM: Dev = 499.2, d.f. = 230, p = 0.001; Figures 4.9 & S4.6, Table 4.2) and spider maturity (Dev = 125.3, d.f. = 229, p = 0.001; Figures 4.10 & S4.7, Table 4.2). Specifically, the predation of four, five, eight and three taxa significantly differed based on Julian day, mean week daylength, genus and maturity, respectively (Table 4.2). As the study period progressed, fewer springtails of *Isotomurus sp. Börner, 1903 (Entomobryomorpha: Isotomidae)*. and *Sminthurus viridis* were predated, but more *Sminthurinus aureus* (Lubbock, 1836; Symphypleona: Katiannidae) springtails and *Oscinella* sp. At peak daylength, more thrips of *Anaphothrips obscurus*, *Frankliniella tenuicornis* and *Limothrips denticornis* (Haliday, 1836; Thysanoptera: Thripidae), and *Isotomurus* sp. springtails were predated, but fewer *Hypogastrura viatica* (Tullberg, 1872; Poduromorpha: Hypogasturidae). Of those prey that were differentially predated by spider

genera, *Tenuiphantes* predated many *Anaphothrips obscurus*, Bourletiellidae, Eupodidae and *Reticulitermes lucifugus* (Rossi, 1792; Blattodea: Rhinotermitidae), *Bathyphantes* similarly predated many Eupodidae but also *Sitobion* sp., *Microlinyphia* predated many *Oscinella* sp., *Pardosa* predated many Cecidomyiidae, and *Erigone* predated a moderate number of Bourletiellidae. Of the prey differentially predated by different life stages, adults predated more Trombidiidae, but juveniles more Eupodidae and *Limothrips denticornis*.

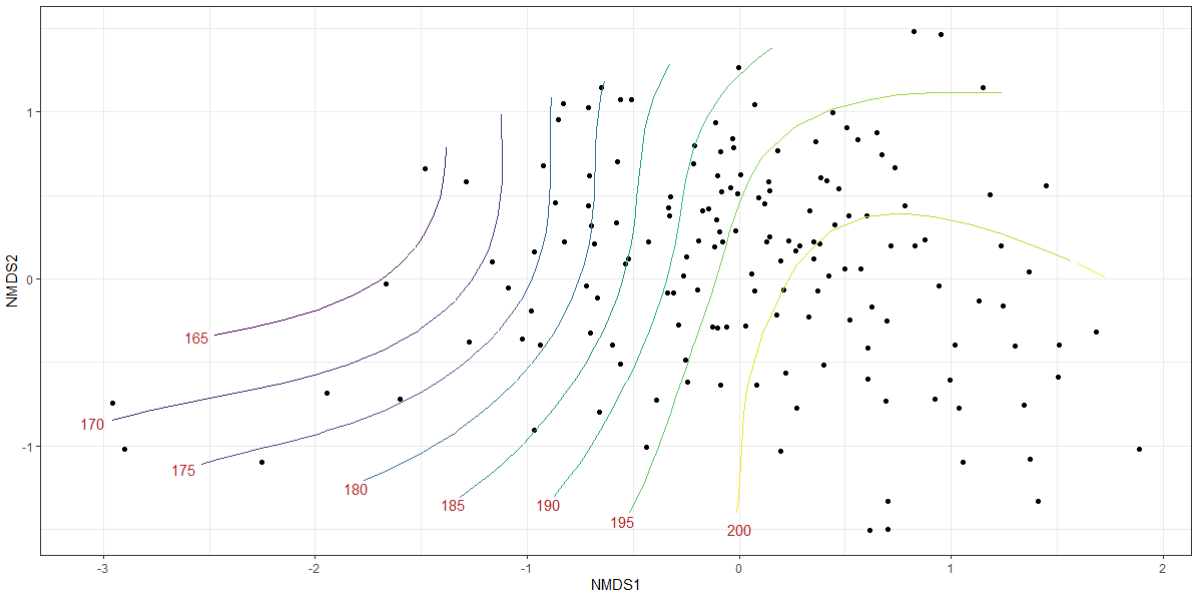


Figure 4.7: Surf plot derived from non-metric multi-dimensional scaling of spider diets with Julian day contours. Axes represent a two-dimensional variation in spider diet. Each point represents the diet of a spider, with distance between them indicating their dissimilarity (i.e. proximate points are similar, distant points are dissimilar). Contours represent Julian days throughout the sampling period (purple and yellow denoting the earliest and latest days, respectively, with a gradual scaling between). Prey species are overlaid in Figure S4.4. Stress = 0.082.

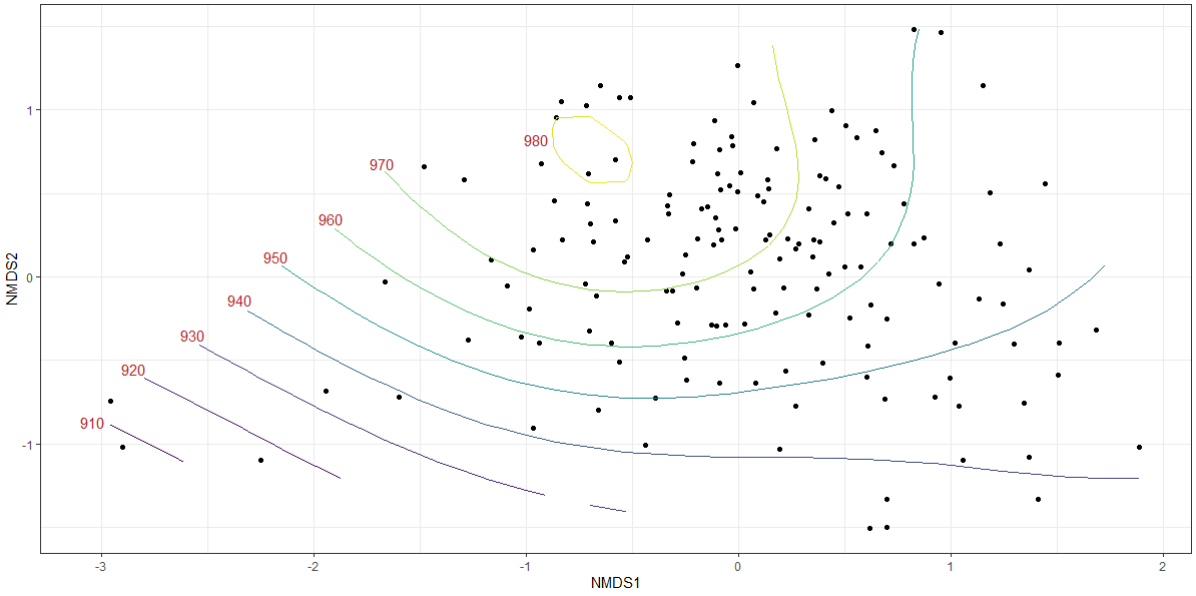


Figure 4.8: Surf plot derived from non-metric multi-dimensional scaling of spider diets, with mean week daylength contours. Axes represent a two-dimensional variation in spider diet. Each point represents the diet of a spider, with distance between them indicating their dissimilarity (i.e. proximate points are similar, distant points are dissimilar). Contours represent mean week daylength (min; sunrise to sunset) throughout the sampling period (purple and yellow denoting the shortest and longest days, respectively, with a gradual scaling between). Prey species are overlaid in Figure S4.5. Stress = 0.082.

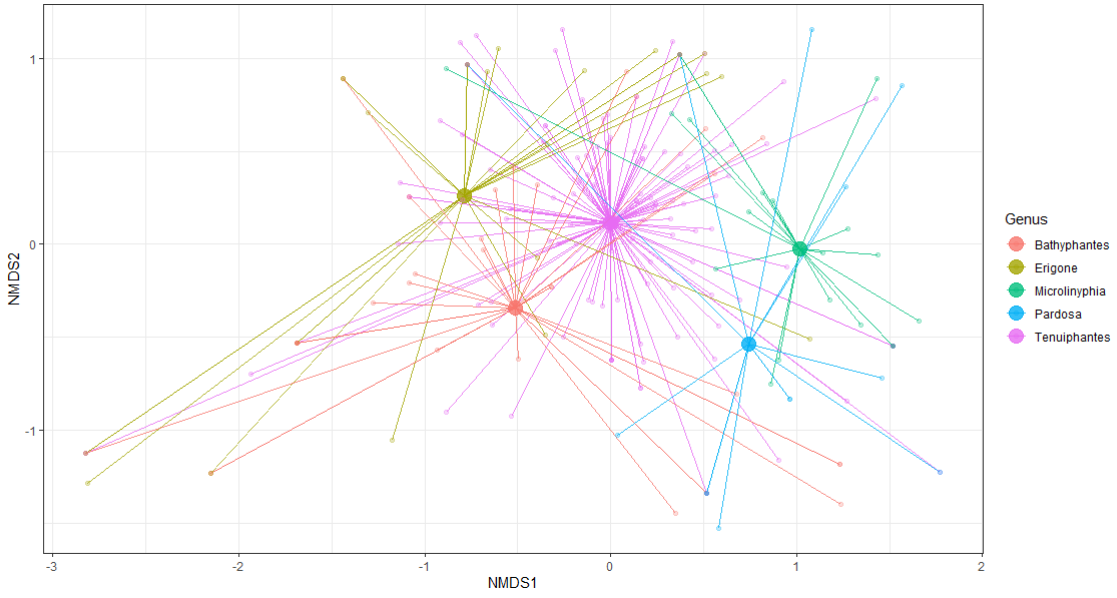


Figure 4.9: Spider plot derived from non-metric multi-dimensional scaling of spider diets. Colours denote spider genera (red, gold, green, blue and purple denoting *Bathyphantes*, *Erigone*, *Microlinyphia*, *Pardosa* and *Tenuiphantes*, respectively). Axes represent a two-dimensional variation in spider diet. Each point represents the diet of a spider, with distance between them indicating their dissimilarity (i.e. proximate points are similar, distant points are dissimilar). Each smaller point represents the diet of a spider, joined by the centroids of diets in each group (larger nodes; mean coordinates in that group). Prey species are overlaid in Figure S4.6. Stress = 0.082.

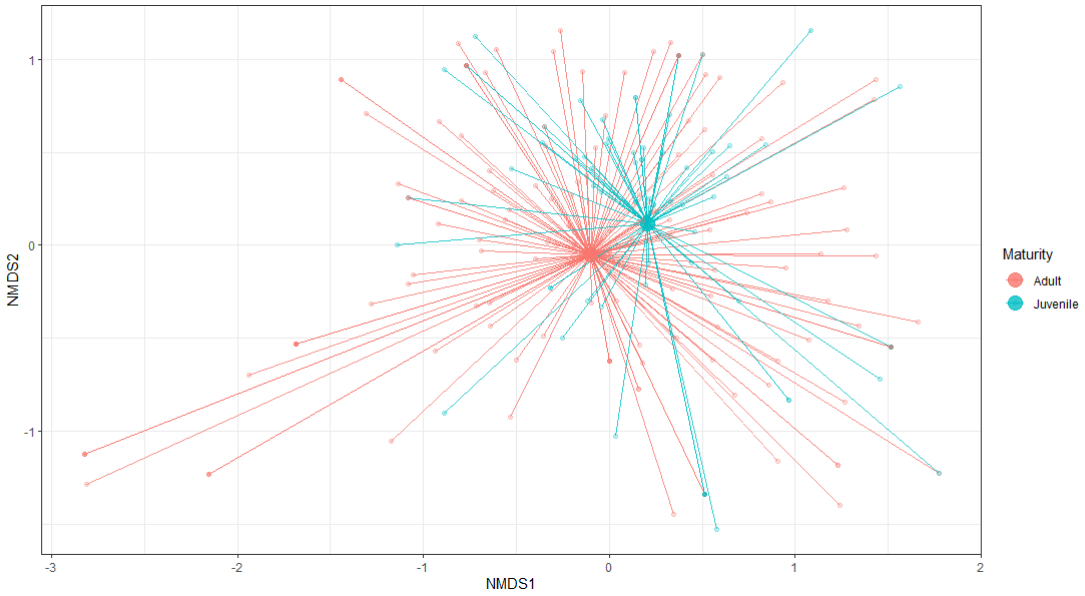


Figure 4.10: Spider plot derived from non-metric multi-dimensional scaling of spider diets. Colours denote spider life stages (red and blue denoting adult and juvenile, respectively). Axes represent a two-dimensional variation in spider diet. Each point represents the diet of a spider, with distance between them indicating their dissimilarity (i.e. proximate points are similar, distant points are dissimilar). Each smaller point represents the diet of a spider, joined by the centroids of diets in each group (larger nodes; mean coordinates in that group). Prey species are overlaid in Figure S4.7. Stress = 0.082.

Table 4.2: Significant univariate MGLM results by taxon for the overall significant variables. Deviance and probability are given for each.

Taxon	Julian day (d.f. = 235)	Mean week daylength (d.f. = 234)	Genus (d.f. = 230)	Maturity (d.f. = 229)
Isotomurus sp.	Dev = 11.989 p = 0.024	Dev = 14.3 p = 0.020	-	-
Oscinella sp.	Dev = 12.4 p = 0.021	-	Dev = 45.0 p = 0.001	-
Sminthurinus aureus	Dev = 14.1 p = 0.013	-	-	-
Sminthurus viridis	Dev = 33.5 p = 0.001	-	-	-
Anaphothrips obscurus	-	Dev = 73.8 p = 0.001	Dev = 41.183 p = 0.001	-
Frankliniella tenuicornis	-	Dev = 18.4 p = 0.002	Dev = 22.7 p = 0.002	-
Hypogastrura viatica	-	Dev = 13.4 p = 0.032	-	-
Limothrips denticornis	-	Dev = 37.9 p = 0.001	-	Dev = 11.8 p = 0.039
Bourletiellidae	-	-	Dev = 21.4 p = 0.002	-
Cecidomyiidae	-	-	Dev = 23.1	-

			p = 0.002	
Eupodidae	-	-	Dev = 36.7 p = 0.001	Dev = 16.7 p = 0.004
Reticulitermes lucifugus	-	-	Dev = 67.0 p = 0.001	-
Sitobion sp.	-	-	Dev = 17.4 p = 0.032	-
Trombidiidae	-	-	-	Dev = 14.7 p = 0.007

4.4.3. Web differences

Active webs were measured in association with 147 spiders within the focal genera of this study during sampling (Table 4.3; Figure S4.8). Whilst recorded and noted below, *Microlinyphia* were too small in sample size (n = 2 webs for this species) to produce robust models and were thus excluded from subsequent analyses. Web height (Figure 4.11, Table 4.4) and area (Figure 4.12, Table 4.5) significantly differed between genera, but not sexes; female spiders had larger webs (GLM: mean diff. = -0.608 ± 0.345, t = -1.763, p = 0.080) but this was not significant. Diet was not, however, significantly related to specific web heights or areas in those spiders for which dietary and web data were both collected.

Table 4.3: Web heights and areas for different spider genera and sexes. Means are given ± SD. *Pardosa* do not build webs and are therefore not featured.

Group	Female web height mm	Male web height mm	Female web area mm²	Male web area mm²
Bathyphantes	41.3 ± 54.5 (n = 19)	13.0 ± 10.4 (n = 5)	3418 ± 2831 (n = 19)	1580 ± 1176 (n = 5)
Erigone	0.7 ± 1.9 (n = 7)	6.7 ± 11.5 (n = 3)	793 ± 322 (n = 7)	783 ± 401 (n = 3)
Microlinyphia	130 (n = 1)	5 (n = 1)	7200 (n = 1)	1000 (n = 1)
Tenuiphantes	24.5 ± 21.2 (n = 25)	29.3 ± 27.8 (n = 23)	3448 ± 2583 (n = 25)	2192 ± 1536 (n = 23)

Table 4.4: Generalized linear model results for the web height of different spider genera, releveled for each genus. Mean differences are given alongside standard errors. 'n.s.' denotes a non-significant difference.

	Erigone	*Bathyphantes*	*Tenuiphantes*
Erigone	-	Mean diff. = 4.905 ± 1.131, t = 4.338, p < 0.001	Mean diff. = 4.130 ± 1.093, t = 3.777, p < 0.001
Bathyphantes		-	n.s.
Tenuiphantes			-

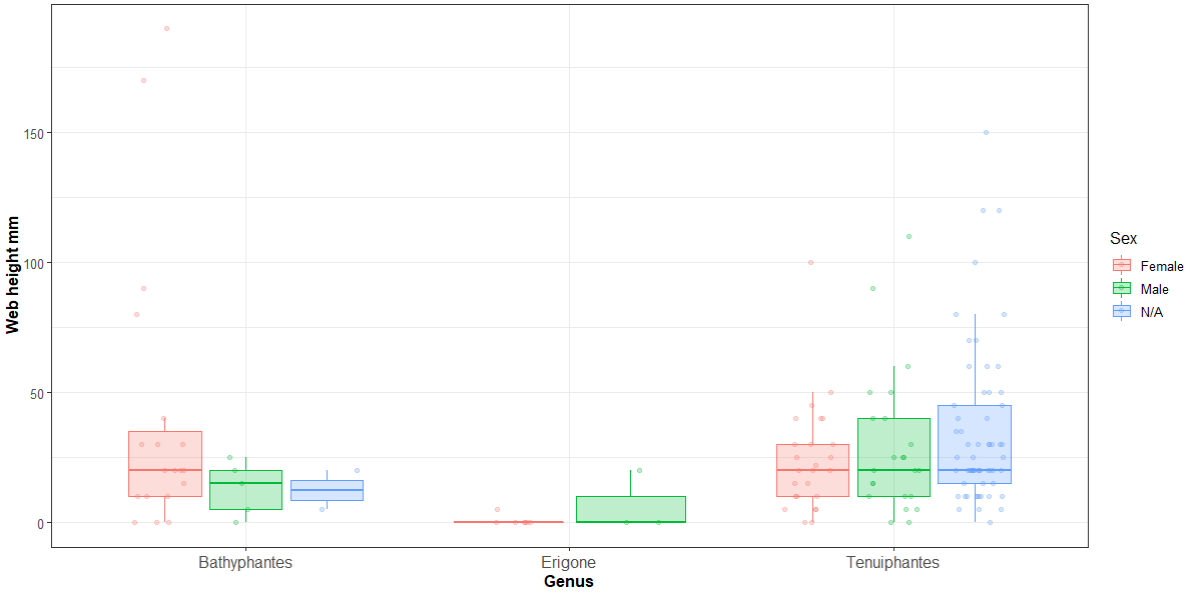


Figure 4.11: Web heights by spider genus and sex with jittered point overlay. Spiders denoted as 'N/A' are those juvenile spiders for which a sex could not be determined.

Table 4.5: Generalized linear model results for the web area of different spider genera, releveled for each genus. Mean differences are given alongside standard errors. 'n.s.' denotes a non-significant difference.

	Erigone	*Bathyphantes*	*Tenuiphantes*
Erigone	-	Mean diff. = 1.196 ± 0.303, t = 3.941, p < 0.001	Mean diff. = 1.240 ± 0.293, t = 4.225, p < 0.001
Bathyphantes		-	n.s.
Tenuiphantes			-

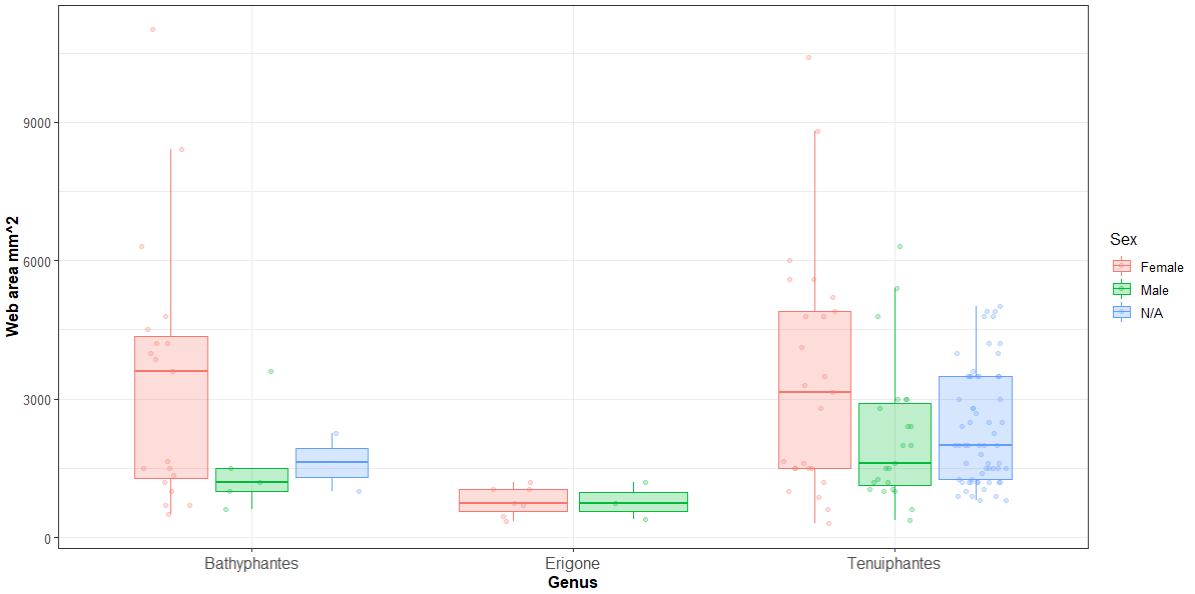


Figure 4.12: Web areas by spider genus and sex with jittered point overlay. Spiders denoted as 'N/A' are those juvenile spiders for which a sex could not be determined.

4.4.4. Intraguild predation and biocontrol

Prey were categorised as pests or fellow predators. Predation of pests (inherently linked with biocontrol) significantly differed between spider life stages, with juveniles predating significantly more pests (Adult-Juvenile GLM: mean diff. = 0.349 ± 0.107, z = 3.271, p = 0.001; Figure 4.13), and differed significantly between genera (Figure 4.14, Table 4.6), but not consistently over time. Similarly, intraguild predation also significantly differed between spider life stages, with adults predating more predators (Adult-Juvenile GLM: mean diff. = -0.866 ± 0.331, z = -2.612, p = 0.009; Figure 4.13), and differed significantly between genera (Figure 4.14, Table 4.7), but not consistently over time.

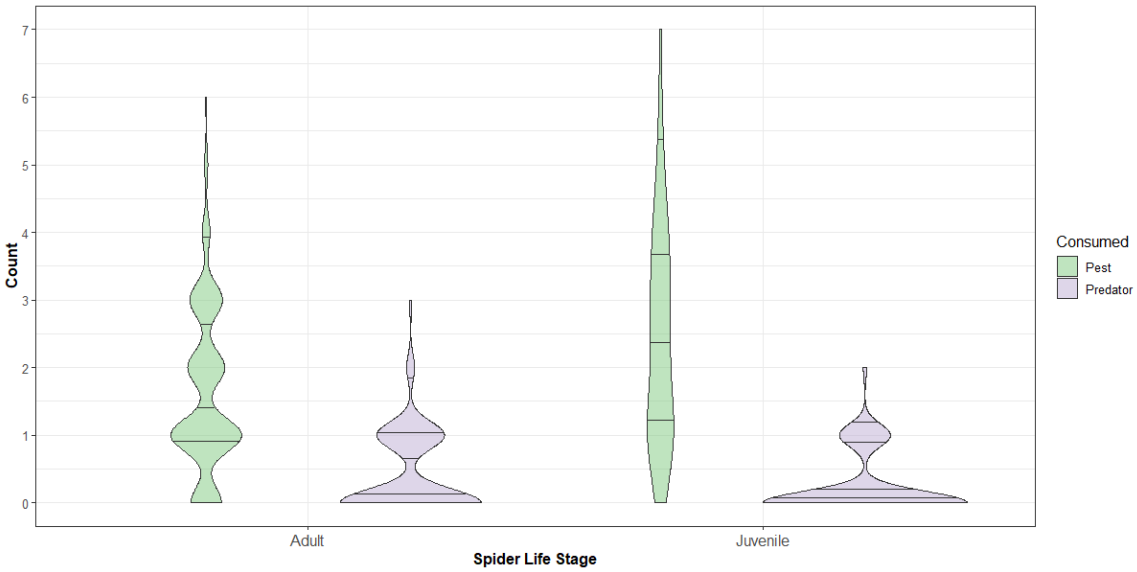


Figure 4.13: Violin plot of pest and predator predation rates (number of different taxa of each consumed by each predator) for adult and juvenile spiders. Horizontal lines within the plotted shapes denote, from bottom to top, the lower quartile, median, upper quartile and upper 95% CI. The width of the plotted shape differs to reflect the relative frequency of predators that predated different numbers of prey taxa.

Table 4.6: Generalized linear model results for predator predation (intraguild predation) of different spider genera, releveled for each genus. Mean differences are given alongside standard errors. 'n.s.' denotes a non-significant difference.

	Erigone	*Bathyphantes*	*Microlinyphia*	*Tenuiphantes*	*Pardosa*
Erigone	-	Mean diff. = -0.823 ± 0.454, z = -1.812, p = 0.070	n.s.	n.s.	n.s.
Bathyphantes		-	n.s.	Mean diff. = 0.912 ± 0.409, z = 2.231, p = 0.026	Mean diff. = 1.351 ± 0.603, z = 2.238, p = 0.025
Microlinyphia			-	n.s.	n.s.
Tenuiphantes				-	n.s.
Pardosa					-

Table 4.7: Generalized linear model results for pest predation (biocontrol) of different spider genera, releveled for each genus. Mean differences are given alongside standard errors. 'n.s.' denotes a non-significant difference.

	Erigone	*Bathyphantes*	*Microlinyphia*	*Tenuiphantes*	*Pardosa*
Erigone	-	Mean diff. = 0.651 ± 0.233, z = 2.798, p = 0.005	Mean diff. = 0.576 ± 0.254, z = 2.264, p = 0.024	Mean diff. = 1.012 ± 0.207, z = 4.878, p < 0.001	n.s.
Bathyphantes		-	n.s.	Mean diff. = 0.361 ± 0.145, z = 2.496, p = 0.013	Mean diff. = -0.924 ± 0.282, z = -3.277, p = 0.001
Microlinyphia			-	Mean diff. = 0.436 ± 0.178, z = 2.456, p = 0.014	Mean diff. = -0.849 ± 0.300, z = -2.829, p = 0.005
Tenuiphantes				-	Mean diff. = -1.285 ± 0.248, z = -5.175, p <0.001
Pardosa					-

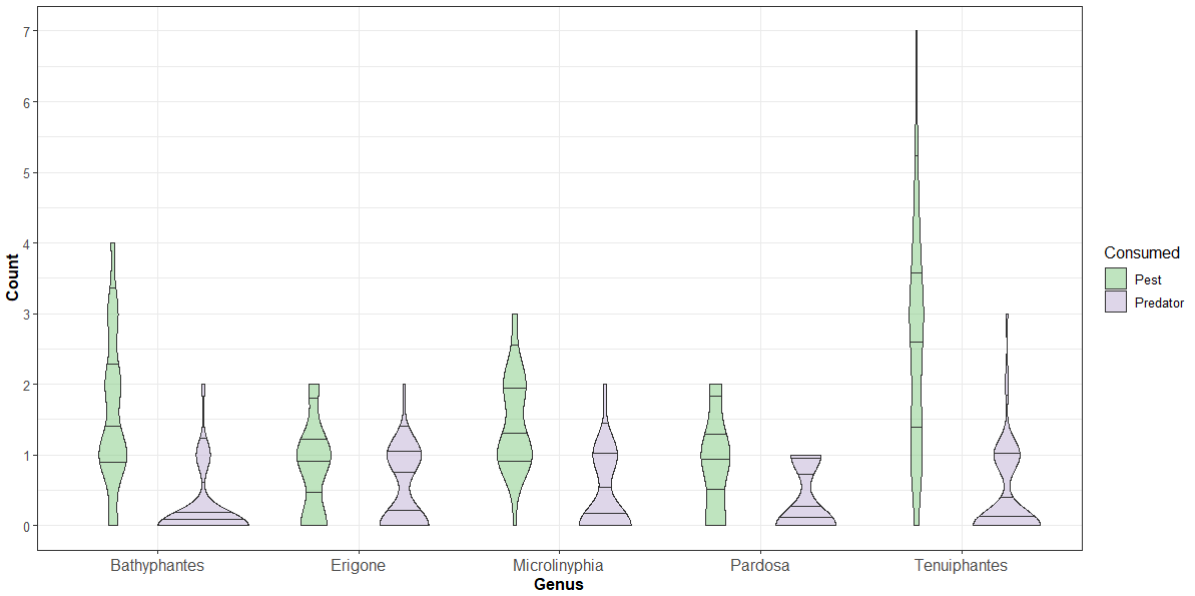


Figure 4.14: Violin plot of pest and predator predation rates (number of different taxa of each consumed by each predator) for different spider genera. Horizontal lines within the plotted shapes denote, from bottom to top, the lower quartile, median, upper quartile and 95% CI. The width of the plotted shape differs to reflect the relative frequency of predators that predated different numbers of prey taxa.

4.5. Discussion

This chapter identifies key differences in dietary intake between spiders with different functional traits, ultimately resulting in their differential benefit to conservation biological control. Specifically, juvenile spiders and those of the genera *Bathyphantes* and *Tenuiphantes* were of optimal benefit to biocontrol (i.e. predated many pests and relatively few predators). The large dietary breadth of spiders in cereal crops was also highlighted. Thrips were identified as the most common prey of these spiders, which is noteworthy given that their predation by spiders has remained undetected in many previous dietary analyses, likely due to the insufficient breadth of amplification by previously used PCR primers. The variation of spider diet over time and with spider genus and life stage, particularly regarding their predation of other predators and crop pests, indicates the dynamic ecology of these predators and their variable benefit to agriculture.

4.5.1. Broad dietary observations and co-occurrences

The spiders screened contained a diverse range of prey across 45 families, with an average of 2.57 ZOTUs and 2.23 families per spider, confirming the polyphagous habit of these spiders. The dietary niche breadth was, however, variable between spiders, with female spiders having the greatest breadth despite often living less itinerantly than males in some of the focal species (Foelix 2011); this may indicate that remaining on the web increases foraging success and that itinerancy is a successful strategy simply for male reproductive output. Adult spiders had a far greater dietary niche breadth than juveniles which may simply be a result of their larger size (thus requiring greater nutritional input and facilitating predation of larger prey) and greater experience with handling prey. Variation in dietary niche breadth between spider genera was less than that between life stages or sexes, but *Pardosa* sp. and *Microlinyphia pusilla* had notably narrower dietary niches despite, at least for *Pardosa*, being a far larger and more active spider group with efficient digestive enzyme production (Samu 1993; Suter and Benson 2014). The far greater-than-random dietary niche overlap between all of these groups does nonetheless suggest that there are several key prey species common to all of the screened spider groups, despite differences in niche breadth.

Several prey species were present in many of the significant co-occurrence relationships, occurring regularly with various other prey in the diets of the spiders. The thrips species of the family Thripidae *Anaphothrips obscurus*, *Limothrips denticornis* and *Frankliniella tenuicornis* were featured prominently in these relationships, with only 2 of the 10 negative co-occurrence relationships not including these thrips whilst, of the 16 positive co-occurrence relationships, 12 included these thrips. That the only other thrips species detected in this dietary analysis,

Aeolothrips intermedius Bagnall, 1934 (Thysanoptera: Aeolothripidae), is absent from any co-occurrence relationships is of note given its distinct ecology (being a predator of other thrips; Elimem and Chermiti 2012), which may result in its ecological separation from the other thrips concerned. The thrips co-occur most frequently with one another and with eupodid mites, but mostly negatively with *Oscinella* sp., *Sminthurus viridis* and cecidomyiid flies, otherwise showing few trends. Co-occurrences may result from behavioural and spatial overlap of prey, but that cannot be inferred from these results. Similarly, spiders may be altering their foraging behaviour or choices after predating one prey to target dissimilar prey, possibly to diversify nutritional intake (Mayntz *et al.* 2005). Given the prevalence of thrips in these co-occurrence relationships, it is important to note that thrips were the most frequently predated prey family across the study, with just the family Thripidae occurring in over 36% of spiders; this may, of course, simply relate to their disproportionate abundance in the field, in which case the likelihood of them co-occurring with other prey would be greater. Despite fundamental inferences emerging, co-occurrence data must be interpreted with caution and these results do not necessarily infer ecological trends (Blanchet *et al.* 2020), although this has been demonstrated in the case of a large carabid beetle and its worm prey (Bell *et al.* 2010).

The widespread predation of thrips is, however, a significant finding itself. The regular co-occurrence of thrips in spider diets with many other prey could suggest that predation of these prey is not nutritionally optimal alone and that other prey are predated alongside them to redress nutritional deficiencies elicited by their paucity of nutritional value; this would, however, need clarifying through analysis of the nutritional quality of the various prey taxa consumed. Of course, greater quantities of thrips would increase net nutrient intake by biomass alone, which is undetectable via the presence/absence data provided by metabarcoding, but this could confer negative fitness consequences as is the case when solely feeding on other fluid-feeding herbivores such as aphids (Bilde and Toft 2001). Given the high local abundance of thrips when present in cereal fields (Morse and Hoddle 2006), they may serve as a convenient source of intermediate nutrition between more nutritionally beneficial prey. Given the status of the three most commonly predated thrips as pests, this would highlight the importance of alternative prey in facilitating predation of pests by generalist predators in cereal crop systems.

Similarly, aphids, another prevalent group mostly comprising pests in this study, were very common in the diet of most spider groups, with 19.26% of all spiders containing detectable aphid DNA. The rate of linyphiid predation of aphids was, however, lower than the 38-63% detected by Sunderland *et al.* (1986), and the lack of any detectable aphid DNA in the lycosids screened falls short of the previously-reported estimate that aphids comprised 20% of lycosid diet (Sunderland *et al.* 1986; Kuusk *et al.* 2008). This disparity may result from the broad-spectrum primers used and the nature of metabarcoding resulting in the outcompeting of any

degraded DNA by fresher prey (and, in the case of the general primers, predator) DNA (Murray *et al.* 2011), thus possibly reducing the apparent individual dietary breadth. This DNA degradation is affected by the feeding mode and digestive physiology of the predator (Greenstone *et al.* 2007) and fluid-feeding predators do, in fact, have much longer gut DNA half-lives, facilitating detection of prey over longer time periods post-feeding than in other predators (Greenstone *et al.* 2007), reducing the extent of the problem of prey DNA degradation in spiders. The PCR primers used in this study, adapted from Cuff *et al.* (2020), successfully improved upon the exclusion of linyphiids by the primers TelperionF-LaurelinR, with LaureR excluding most *Tenuiphantes* and *Bathyphantes* DNA, affording a greater read depth for the linyphiids. This larger read depth afforded to each sample should have successfully captured a greater diversity than in some previous metabarcoding studies of spider diet (Cuff *et al.* 2020). Some non-target DNA amplicon sizes were, however, produced by these spider exclusion primers, possibly as a result of the low melting temperature used; these may have been closely-related nuclear mitochondrial pseudogenes, found in many invertebrates (Moulton *et al.* 2010).

4.5.2. Dietary differences based on spider functional traits

Key dietary differences were identified between spiders based on their functional traits, although these were not always consistent with ecological distinctions between these spiders. The distinction between adult and juvenile spider diets is less extreme than that observed between genera, possibly due to the representation of sub-adult spiders as juvenile; this was undertaken due to the difficulty in distinguishing between juvenile and sub-adult spiders (Roberts 1993). The far greater predation of *Limothrips denticornis* and Eupodidae by juveniles highlights a possible preference for smaller and less mobile prey, with adults targeting more nutritionally valuable but ‘riskier’ prey; this ‘boldness’ is a well-documented consequence of development in many animals (Johnson and Sih 2007; Montiglio and DiRienzo 2016). Adults predated more Trombidiidae, large predatory mites; this may, however, be detection of the recent presence of these mites on either the focal adult or an animal that they recently predated since these mites are known to attach themselves to spiders to feed (Tomić *et al.* 2015). That this would be more prevalent in adults is unsurprising given the less debilitating effect the mite would have on a larger organism both from the nutrients it appropriates but also the physical impairment it elicits through attachment. Adult spiders, through their larger body surface and a greater time *in situ*, would also be more likely to obtain ectoparasites.

Coarse dietary differences can be observed between male and female spiders too, such as the more frequent predation of thrips by male spiders, but this sex difference was not retained during model simplification; such differences between sexes could indicate differences in ecology, such as female spiders being less itinerant (Foelix 2011), or different nutritional needs associated with egg production (Wheeler 1996). Female spiders have previously been recorded predating pests such as aphids at a greater rate than male spiders (Harwood *et al.* 2004); the opposite was identified in this study, with more than twice as many male spiders having detectable aphid DNA in their gut.

The disparity in diet between spider genera is unsurprising given the different life histories and ecological niches of the focal genera. From the analyses, the genera can be roughly split into two groups: first, *Microlinyphia* and *Pardosa*; and second, *Erigone*, *Bathyphantes* and *Tenuiphantes*. The latter group all typically employ sit-and-wait foraging at similar positions in the crop canopy (except *Erigone* which is typically more itinerant; Sunderland *et al.* 1986), whilst *Pardosa* hunt more actively, despite remaining in relatively small areas (Hallander 1967; Shayler 2005). That the diet of *Microlinyphia* is less distinct from that of *Pardosa* highlights that this web-building behaviour is not the principal separating factor since *Microlinyphia* will build and maintain webs akin to those of other linyphiids (Benjamin *et al.* 2002). During the field component of this study, however, female *Microlinyphia* were almost exclusively found at the apex of the crop canopy, sometimes inhabiting large sheet webs, directly contrasting the small close-to-the-ground webs of the males (pers. obs. Jordan Cuff); such separation of the sexes would necessitate a high degree of mobility, possibly resulting in regular itinerant behaviour that might increase the probability of encountering those prey met by highly mobile *Pardosa*. Female *Microlinyphia* were also markedly larger than most of the other linyphiid individuals studied, possibly allowing them to mechanically subdue and predate larger prey otherwise only attainable by *Pardosa* among the focal genera of this study, thus rendering their diets slightly less disparate.

The prey differentially predated between spider genera largely reflected the coarse grouping of genera described above. *Sitobion* spp., *Anaphothrips obscurus* and *Frankliniella tenuicornis* were mostly predated by *Tenuiphantes*, *Bathyphantes*, *Erigone* and *Microlinyphia*, but rarely by *Pardosa*. Conversely, Cecidomyiidae were mostly predated by *Pardosa*, with the linyphiid genera predating few or none. These differences are likely due to the disparity in ecology and size between the large and highly active lycosids of *Pardosa* and the relatively inactive and small linyphiids. Those prey predominantly predated by linyphiids are siphon-feeding insects, unlike the cecidomyiids predated by lycosids, which may be responsible for their physical or behavioural separation, thus their relative exclusivity to these groups.

Several prey were predated exclusively or predominantly by a single genus. *Oscinella* spp. were mostly predated by *Microlinyphia*, with a few instances of *Tenuiphantes* and *Bathyphantes* predation. That only *Pardosa* and *Erigone* did not predate these flies, and given that they bore into shoots, could indicate that they are typically found higher in the crop, most accessible by the female *Microlinyphia* found at the crop apex. Eupodid mites were only predated by *Bathyphantes* and *Tenuiphantes*, suggesting their presence at a specific crop height given the vertical co-occurrence of these two genera. Bourletiellid springtails were, however, almost exclusively predated by *Tenuiphantes* and *Erigone*, which differ in their web positioning and size. It is worth noting though that the year in which these spiders were collected (2018) had a particularly hot and dry summer and, when conditions were arid, *Tenuiphantes* were regularly collected from the edges of cracks in the ground where the soil had dried and contracted (pers. obs. Jordan P. Cuff). During these sub-optimal conditions, *Tenuiphantes* may have sought prey from these ground fissures, thus increasing predation of subterranean prey such as springtails.

Reticulitermes lucifugus was detected in the diet of 36% of *Tenuiphantes* individuals, but no other spiders in this study. *Reticulitermes lucifugus* is not, however, recorded in the United Kingdom. Other *Reticulitermes* spp. are recorded from mainland Europe, including Western France, with records of *Reticulitermes grassei* in Kent and Devon (England), thought to originate from southwest France (Clément *et al.* 2001). Whilst it is possible that a *Reticulitermes* sp. was present, the likelihood is low and further surveys would need to confirm this. Outbreaks of termites have occurred in the UK but are usually sufficiently overt to be noticed and eradicated rapidly (Verkerk and Bravery 2004). The immediate concern was that this was contamination, but the presence of this species in only *Tenuiphantes* and its absence from any controls, especially given the stringent measures taken to ensure clean data pre- and post-sequencing, suggests that this is not the case. It is far more likely that this is not a *Reticulitermes* species and is instead a British insect for which a DNA barcode is yet to be recorded (likely a poorly studied hypogeal species), the COI gene of which very closely resembles *Reticulitermes lucifugus*, which would usually, but does not necessarily, infer the taxonomic proximity of those species. That this prey is found exclusively in the diet of *Tenuiphantes* is noteworthy regardless, as it could indicate a strict preference at some point in the life history of *Tenuiphantes*, at least at this site. This may, as discussed regarding the predation of bourletiellid springtails above, relate to the retreat of *Tenuiphantes* into subterranean cracks during arid conditions.

4.5.3. Webs as a potential determinant of spider diet

A consideration regarding the distinction in diet between spider genera, at least in the case of the linyphiids, is the distinction in web structure and positioning. The webs measured in the field during this study correspond with the literature, with the webs of *Tenuiphantes* and *Bathyphantes* being larger and higher than those of *Erigone* and with females typically possessing slightly larger webs than males (Sunderland *et al.* 1986). Importantly, *Erigone* exhibit greater behavioural plasticity in that they can regularly leave their webs which play a smaller role in prey capture, instead acting more like a base from which the spider forages (Alderweireldt 1994). These differences did not, however, explain dietary variation in those spiders for which both web and dietary data were collected. That structural differences in webs were not associated with dietary differences supports the finding that linyphiids adjust their webs for optimal prey capture, thus overcoming spatial differences in prey availability (Welch *et al.* 2016). These previous findings do, however, suggest that webs are structurally varied to capture the specific prey abundant at each web site most effectively, whilst the results of this study could imply that these structural changes are in fact purposed to increase more general or consistent prey capture. The typical webs of linyphiids are known to capture a broad spectrum of prey, lending to their facilitation of balanced nutritional intake through presentation of a diversity of prey options to the resident spider (Ludwig *et al.* 2018).

Physical separation of webs, both through site selection and structural variation, also promotes niche separation of spiders, thus reducing competitive interactions; web variation may thus be purposed not to modulate prey capture, but to avoid overlap with the webs of other spiders (Opatovsky *et al.* 2016). The webs of linyphiids do not exhibit the adhesive properties of araneid webs (Benjamin *et al.* 2002), instead depending on physical interception of prey and a proactive response from the resident spider, thus it is important to consider that predation is still limited by the mechanical capability and reaction of the resident spider, despite any structural changes to the web. The smaller sample size for which both dietary and web data were available in this study reduces the veracity of any conclusions drawn from these data, but these findings nonetheless provide the first comparison of web features against molecular-derived dietary data.

4.5.6. Temporal and meteorological determinants of spider diet

The senescence of cereal crops render them unsuitable for many of the herbivorous prey taxa identified in this study, possibly partly explaining the temporal variation in spider diets. The prey taxa that were differentially predated across the study period were largely springtails (*Isotomurus* sp., *Sminthurinus aureus* and *Sminthurus viridis*) which would likely be active

throughout the entire study period. That the prevalence of these species in the diet changed over time could instead suggest a response to temperature or other weather-related variables; these were, however, dropped from the models during simplification, suggesting that the progression of time offers a greater explanation. The arid conditions at the peak of summer, in this study year particularly, may have driven many invertebrates into fissures in the ground, as discussed above. This may have altered the interaction between the focal spiders and these springtails. Whilst none of these springtails are predated near day 200 (late July), they do, however, exhibit distinct temporal trends. Whilst *Sminthurus viridis* is predated frequently before this period, they are not predated at all afterwards, whereas *Sminthurinus aureus* is predated more after than before, suggesting a switch in prevalence. *Isotomurus* sp., similar to *Sminthurus viridis*, are less frequently predated following this period. *Oscinella* sp. may have differentially presented in spider diets across the study period due to their phenology, with large peak abundances typically occurring in July (Vickerman 1980), aligning with their peak prevalence in the diet of these spiders. During these peak abundances, it is likely that a greater proportion of spiders will predate *Oscinella* sp. not only based on their increased population densities, but also due to the reduced availability of other prey such as springtails.

Whilst significant association between dietary composition and time could suggest phenological changes in the prey available or corresponding changes to spider behaviour across the study period, the association with daylength would more precisely suggest dietary distinctions coinciding with late June. The association of the three Thripidae species identified in the spiders' diets, *Anaphothrips obscurus*, *Frankliniella tenuicornis* and *Limothrips denticornis*, with daylength may relate to their greater abundance on the crop at this stage of development, prior to senescence but once the shoots have sufficiently grown to support larger populations. As above, the reduced availability of common prey such as springtails may also increase predation of thrips. The association of *Isotomurus* with daylength alongside its temporal association suggests that its predation by spiders reduces toward peak daylength, possibly due to the negative phototaxis exhibited by many springtails (Fox *et al.* 2007). *Hypogastrura viatica*, the only other species associated with daylength, was rarely predated, and only prior to peak daylength. The relative lack of spiders sampled at the peak of daylength was simply due to the far reduced availability of spiders relevant to this study in the field at this time (pers. obs.), likely due to the arid and unfavourable weather conditions forcing them into ground fissures.

4.5.7. Differences in spider biocontrol based on functional traits

The biocontrol dynamics identified in this study provide a valuable insight into the optimal biocontrol agents within the focal spiders. That juveniles predated more pests, and adults more predators, indicates the importance of maintaining young spider populations *in situ* since adult spiders, whilst important for providing the next generation of spiderlings, may be negatively impacting biocontrol overall through their intraguild predation of alternative biocontrol agents. The predation of pests by juveniles is logical given that these will largely be herbivorous and thus less 'risky' prey, whereas "bolder", larger and more experienced adults will have greater success in attacking other predators. It is, however, noteworthy that some common pest prey, such as aphids, contain toxins that detriment development, fecundity and survival (Bilde and Toft 2001). This highlights the importance of available non-toxic herbivorous prey for the provision of developing spiderlings. This greater rate of predation of potentially toxic prey, may, however, suggest that their detriment is effaced in juvenile spiders, possibly by physiological adaptation, or that these prey provide a substantial nutritional benefit to juvenile spiders.

Tenuiphantes, being the most common spider genus in this study and in many British studies of cereal crop linyphiids (Harwood *et al.* 2003; Sheppard *et al.* 2005; Davey *et al.* 2013; Cuff *et al.* 2020), predated the greatest proportion of pests among the focal genera, but this may be due to the greater number of juvenile spiders sampled. That *Tenuiphantes* predated so many pests is nonetheless positive considering their abundance and widespread distribution in cereal fields. *Bathyphantes* similarly predated many pests, but also far fewer predators, indicating the additional benefit of reduced intraguild predation. The other focal genera performed similarly, with *Microlinyphia* predating slightly more pests than *Erigone* and *Pardosa*, indicating their slightly less beneficial contribution as biocontrol agents, but exhibiting some control of pests nonetheless. That no distinction was found across the study period indicates that the role of linyphiids in biocontrol is a consistently important consideration in integrated pest management plans. The particularly beneficial contributions of *Tenuiphantes* and *Bathyphantes* indicates a benefit to ensuring sufficient crop height for these species early in the cropping season considering that they build their webs slightly higher in the crop (Sunderland *et al.* 1986). This could indicate a benefit to winter over spring cropping. It is notable, however, that many spiders present early in the cropping season are thought to be immigrant spiders (Opatovsky *et al.* 2012), having ballooned potentially long distances into the fields, often as juveniles. That juveniles had the greatest rate of biocontrol increases the hypothetical benefit of juvenile immigrant spiders, which are already known to provide a critical role in biocontrol, particularly given the ecologically turbulent process of crop cycling (Opatovsky *et al.* 2012; Cuff *et al.* 2020).

Spiders will often attack prey on encounter regardless of hunger, termed superfluous killing; return to this prey later may relate to the time necessary for external digestion of the prey after the spider has immobilised it, and the abundance of alternative prey (Riechert and Lockley 1984; Sunderland 1999). The spider might not return to predate the prey, resulting in more prey killed than are required by the spider, which is advantageous for biocontrol (Riechert and Lockley 1984; Sunderland 1999). It does, however, conflate the impact of these spiders as biocontrol agents as assessed through dietary analysis since the spiders may be providing a far greater service than that represented by their dietary intake, or lesser if actually exhibiting a greater rate of intraguild predation. Equally, molecular analysis of gut contents via PCR can overlook scavenging, secondary predation, and parasitism which should be considered a possibility for some results (Greenstone *et al.* 2007; Paula *et al.* 2015). Scavenging on dead prey by spiders, possibly balancing out their superfluous killing (von Berg *et al.* 2012), may further disrupt the interpretation of these data given that it would be included as a trophic interaction. Regardless, given the location of prey by vibrational cues by most spiders (Turnbull 1973; Molina *et al.* 2009; Virant-Doberlet *et al.* 2011), scavenging is likely to be less regular than live capture of prey, thus these spiders are clearly providing a critical service in the suppression of pest species.

4.5.8. Limitations

The data presented in this study are constrained by the interpretation of presence or absence of prey, thus precluding any detection of multiple predation events on the same prey species which is likely to have occurred. Much debate surrounds the quantification of metabarcoding outputs due to inherent biological and technical biases (Murray *et al.* 2011; Thomas *et al.* 2016; Deagle *et al.* 2013; Piñol *et al.* 2018). Quantification of PCR-based dietary metabarcoding results is difficult, if not impossible, due to differential digestion rates of prey, primer biases and random sampling during sequencing (Murray *et al.* 2011; Leray and Knowlton 2017). Correction factors can be determined to allow for amplification bias and digestion rate (Thomas *et al.* 2016) and, similarly, semi-quantitative predation rates can be calculated from metabarcoding data (Egeter *et al.* 2015; Welch *et al.* 2014), but these can be laborious to design and may differ greatly between taxa and physiological traits; regardless, the developmental stage of the prey and the DNA density of the predated tissue are difficult to correct for with DNA data (Murray *et al.* 2011). Regardless, this study marks the most comprehensive field-based analysis of linyphiid diet, particularly in agriculture and concerning biocontrol.

4.5.9. Summary

This study successfully identified optimal candidates for biocontrol among the common spiders of cereal crops by determining the diet of a range of spiders with various functional traits. Through these analyses, dietary differences were highlighted between spiders of different genera, sexes, life stages and over time, with important implications for biocontrol. That some spider genera (i.e. *Bathyphantes* and *Tenuiphantes*) and juveniles exhibited greater biocontrol efficacy highlights their importance in the management of generalist predators in agricultural fields. Predation of pests could be further encouraged by provision of suitable conditions in cereal crops, such as sufficient crop height early in the season and adequate alternative prey provision. The latter could be achieved by additional compost applications (Agustí *et al.* 2003; Shayler 2005) or increased habitat diversity, for example by introduction of intercropping, field margins or beetle banks (Sunderland and Samu 2000; Butts *et al.* 2003; MacLeod *et al.* 2004; Mansion-Vaquie *et al.* 2017; Michalko *et al.* 2017). By managing the generalist predator populations of cereal crops to maximise the activity of those spiders with the greatest impact on biocontrol, conservation biocontrol can be increasingly employed for the sustainable control of crop pests in integrated pest management schemes.

Dude, Where's My Carbs? Nutrient-specific Foraging by Spiders in Cereal Crops

“The spider that knows what it will gain sits waiting patiently in its web.”

- Nigerian proverb

Chapter 5 : Dude, where's my carbs? Nutrient-specific foraging by spiders in cereal crops

5.1. Abstract

1. To effectively harness conservation biocontrol, the mechanisms underlying trophic interactions must first be elucidated. Nutrient-specific foraging is the ecological theory that consumers select their resources based on macronutrient content; this has been demonstrated across many *ex situ* lab feeding trials but has not yet been shown in the field.

2. Money spiders (Araneae: Linyphiidae), wolf spiders (Araneae: Lycosidae) and their local prey communities were collected from barley fields in Wenvoe, Wales, UK between April and September 2018. Using DNA metabarcoding, the diets of 300 spiders were screened. Prey communities were identified, and the macronutrient contents of a range of species were determined using the microscale macronutrient protocol MEDI. Using hierarchical clustering, taxa were classified into "tropho-species" (i.e. novel macronutrient-based taxonomic ranks). Prey co-occurrence in the diet of spiders and in-field preferential selection of taxa and tropho-species were assessed via null statistical models.

3. Individual-level (co-occurrence analysis) and population-level (prey choice analysis) results indicated an overall tendency of spiders to balance their nutritional intake. Prey of macronutrient contents deviating from average were typically present together with nutritionally average prey in individual spiders' recent diets. Depending on genus, sex and life stage, spiders obtain similar macronutrient proportions from different prey resources. These data indicate an overall importance of the proportional macronutrient content of prey for foraging choices in spiders. Spiders predated several taxa more or less than expected based on prey density, including several key crop pest taxa.

4. Both individual-level and population-level results provide evidence for nutrient-specific foraging in the field. The tropho-species concept facilitated reclassification of complex invertebrate communities by their macronutrient content, simplifying analysis of nutrient-specific foraging. The predominant importance of the content of all three macronutrients in determining spider prey choice indicates a possibility for influencing prey choice by modulating the availability of these nutrients through changes to prey community structure and habitat heterogeneity.

5.2. Introduction

To harness conservation biocontrol more effectively, the mechanisms underlying prey choice must first be understood. Trophic interactions involve the transfer of mass between organisms, primarily driven by behaviour, physiology and the need to acquire not just energy, but a blend of nutrients (Machovsky-Capuska *et al.* 2016; Machovsky-Capuska *et al.* 2018; Potter *et al.* 2018). The influence of macronutrient intake on animal fitness highlights the nutritional basis inevitably underpinning trophic interactions, with nutritional deficits incurring severe consequences for fitness, survival, body composition, behaviour, immunity, reproductive performance and development, among other effects (Wilder and Rypstra 2008; Woch *et al.* 2009; Barry and Wilder 2013; Neeson *et al.* 2013; Bong *et al.* 2014; Rho and Lee 2015; Bunning *et al.* 2016; Littlefair *et al.* 2016; Srygley 2017). Ecological theory thus suggests that prey choice is driven, or at least affected, by the need to redress or avoid nutritional deficits, termed nutrient-specific foraging (Mayntz *et al.* 2005).

Nutrient-specific foraging is conceptually consistent with optimal foraging theory, particularly in the assumptions that foraging drives fitness, is determined by heritable components and adapts rapidly to a changing environment (Pyke 1984; Simpson *et al.* 2004; Jensen *et al.* 2012). Such nutrient-specific foraging has been demonstrated in controlled *ex situ* feeding trials (Mayntz *et al.* 2005; Kohl *et al.* 2015; Fanson *et al.* 2017). In such studies, many predators have been observed exhibiting nutrient-specific foraging, including vertebrates, both marine and terrestrial, such as cats, dogs, mink, dolphin, carp, sole, trout and bass (Yamamoto *et al.* 2001; Rubio *et al.* 2003; Mayntz *et al.* 2009; Rubio *et al.* 2009; Hewson-Hughes *et al.* 2011; Hewson-Hughes, Hewson-Hughes, Colyer, Miller, Hall, *et al.* 2013; Hewson-Hughes, Hewson-Hughes, Colyer, Miller, McGrane, *et al.* 2013; Jensen *et al.* 2014; Kohl *et al.* 2015; Denuncio *et al.* 2017), and invertebrates such as beetles, flies, ants and spiders (Mayntz *et al.* 2005; Raubenheimer *et al.* 2007; Christensen *et al.* 2010; Jensen *et al.* 2011; Jensen *et al.* 2012; Schmidt *et al.* 2012; Mooney *et al.* 2016; Fanson *et al.* 2017).

Mayntz *et al.* (2005) demonstrated nutrient-specific foraging by *Anchomenus dorsalis* (Pontoppidan, 1763; Coleoptera: Carabidae), *Pardosa prativaga* (Koch, 1870; Araneae: Lycosidae) and *Stegodyphus lineatus* (Latreille, 1817; Araneae: Eresidae); all three predators, when reared on either a lipid-rich and protein-poor or protein-rich and lipid-poor diet, would preferentially select resources abundant in the macronutrient previously lacking in their diet. Spiders have also been observed selecting theoretically nutritionally suboptimal prey despite the availability of alternatives, suggesting a benefit to dietary diversification, thought to be macronutrient-based (Welch *et al.* 2016). The primary macronutrient sought by nutrient-specific foraging spiders may, however, change with life stage and across seasons to reflect

the dynamic macronutrient requirements associated with growth and development, reproduction and overwintering (Bressendorff and Toft 2011). The survival of spiders in a state of starvation for months (Lesne *et al.* 2016) suggests that they are able to exhibit a high degree of selectivity in their foraging in the field. Rendon *et al.* (2019) studied nutrient-specific foraging in field-caught *Tasmanicosa leuckartii* (Thorell, 1870; Araneae: Lycosidae) and found that they predated similar proportions of protein-rich and protein-poor prey, but would forgo protein-rich prey that had more effective defence mechanisms in favour of alternative protein-rich prey.

Despite this body of *ex situ* evidence, nutrient-specific foraging has not yet been evidenced in the field. The selection of resources by a forager is undoubtedly affected not only by nutrients, but also by toxins, the energy required to capture different prey and many other factors (Raubenheimer *et al.* 2009; Simpson and Raubenheimer 2012). These factors together form a complex network of ecological considerations which are further confounded in dynamically unpredictable field conditions, study of which has traditionally been constrained by restrictive dietary analysis and statistical methods. Through modern high-throughput sequencing-based dietary analyses (Pompanon *et al.* 2012) and novel statistical methods such as prey choice null modelling (Vaughan *et al.* 2018), increasingly complex studies of prey choice can be conducted to directly assess fundamental theories in ecology (Majdi *et al.* 2018), such as nutrient-specific foraging.

5.2.1. Objectives and hypotheses

This chapter aims to investigate differences in prey choice between spiders before investigating nutrient-specific foraging in the field for the first time. The diet of cereal crop spiders, determined in **Chapter 4** using DNA metabarcoding with the PCR primers developed in **Chapter 3**, will be analysed in the context of prey choice and, specifically, nutrient-specific foraging. The diets of common money spiders (Linyphiidae) and wolf spiders (Lycosidae) collected from barley fields will be compared to local invertebrate abundances (as in **Chapter 3**) to ascertain prey choice. These data will also be analysed alongside prey macronutrient content data, determined using the protocol (MEDI) developed in **Chapter 2** to identify evidence of nutrient-specific foraging. The comparison of the macronutrient contents of those prey available to the spider against the prey selected provides a basis for the first in-field assessment of nutrient-specific foraging. Field-based evidence of nutrient-specific foraging would strengthen the foundations underlying this long-standing ecological hypothesis, or provide refutation, either way providing a greater depth of understanding regarding the mechanisms underlying prey choice. The following hypotheses will be tested: (i) spiders will exhibit prey choice beyond density-dependent selection, these choices differing between

spiders based on taxonomy, sex and life stage; (ii) spider prey families significantly differ in their macronutrient content with some families being more similar; (iii) individual spiders predate nutritionally complementary prey together; and (iv) spider populations predate prey rich in all three macronutrients through selective foraging.

5.3. Methodology

5.3.1. Fieldwork

The same spider dietary data analysed in **Chapter 4** were used in this chapter. Money spiders (Araneae: Linyphiidae) and wolf spiders (Araneae: Lycosidae) were visually located along transects in two adjacent barley fields at Burdons Farm, Wenvoe in South Wales (51°26'24.8"N, 3°16'17.9"W) and collected from occupied webs and the ground between April and September 2018. Each belt transect was adjacent to a randomly selected crop tramline and were distributed across the entire field and ran its length. The areas searched were 4 m^2 quadrats at least 10 m apart and all observed linyphiids and lycosids were collected. Spiders were taken from 64 locations along the aforementioned transects. Spiders were processed as described in Chapter 4.3.1-4.3.3 (Figures 4.1-4.4). Where money spiders were taken from webs, the approximate dimensions of the web were recorded, and the web replaced with a sticky trap supported with wire at the same height and orientation as the web. Two types of web replacement traps were used: one to simulate the smaller, near-ground webs of *Erigone* spp. and one to simulate the larger, aerial webs of *Tenuiphantes* spp.

Near-ground sticky traps comprised a 15 mm x 50 mm sheet of acetate thinly coated with Oecotak A5 (Oecos, Kimpton, UK), an ecologically neutral adhesive, and attached with Oecotak A5 to a 15 mm x 50 mm black plastic base; these traps afforded a sampling area of 7.5 cm^2, representative of the webs of *Erigone atra* and *E. dentipalpis* (Figure 5.1). 'Aerial' sticky traps comprised a 140 mm x 55 mm sheet of acetate coated on both sides with Oecotak A5 adhesive, held adjacent to one another with vertical wire; these traps afforded a sampling area of 77 cm^2, representative of the webs of *Tenuiphantes tenuis* and *Bathyphantes gracilis* (Figure 5.1). Each sticky trap was left in place for 72 hours.

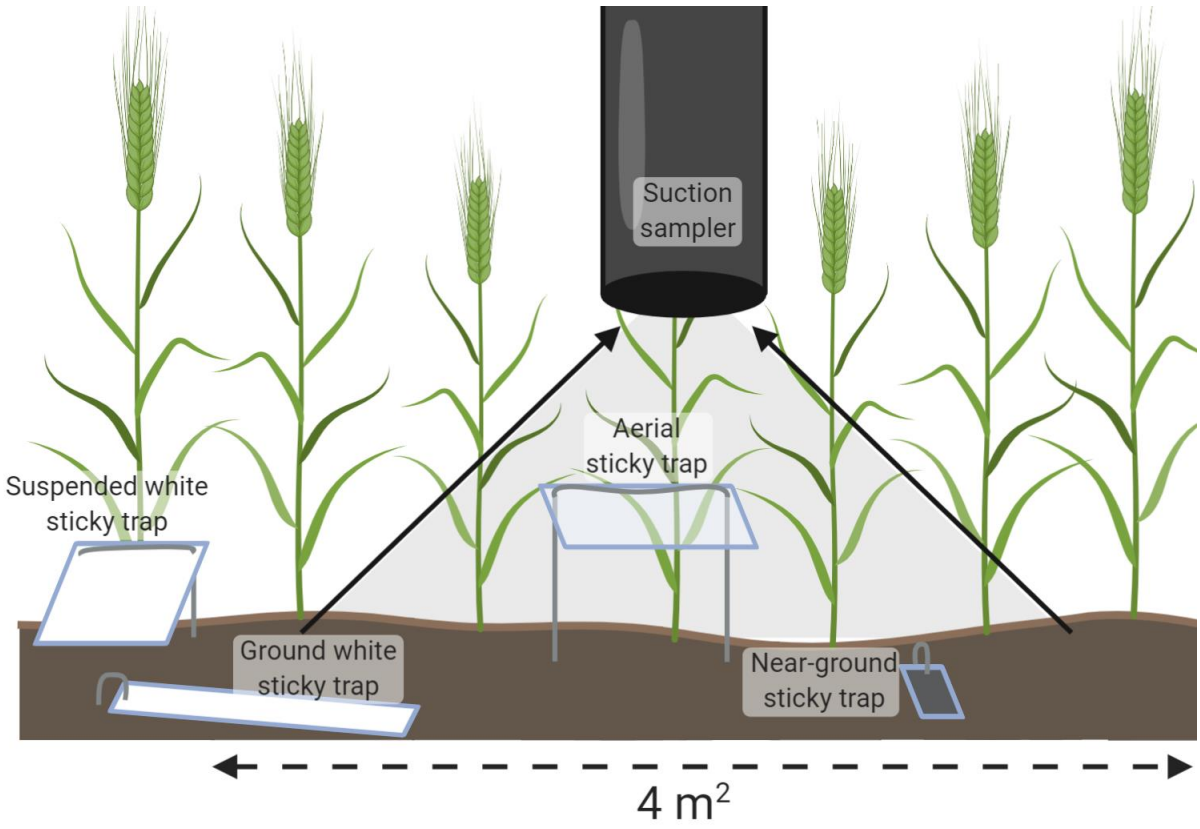


Figure 5.1: The four trap types and suction sampling used to survey the invertebrates present at each web site.

For each area of 4 m^2 in which spiders were found, one white dry sticky trap 100 mm x 125 mm (Oecos) was suspended with wire approximately 25 mm above the ground to catch falling and flying invertebrates (suspended white sticky trap), and a thin strip white sticky trap 250 mm x 10 mm was pinned to the ground to catch ground-running invertebrates (ground white sticky trap; Figure 5.1); both were left to sample prey *in situ* for 72 hours. Ten heads of the crop were removed to estimate the number of crop head-dwelling invertebrates (predominantly aphids) present in each 1 m^2 of crop. Following collection of sticky traps, 4 m^2 of ground and crop stems was suction sampled for approximately 30 seconds, with the collected material emptied into a bag and any organisms immediately killed with ethyl-acetate. Suction sampling used a 'G-vac' modified garden leaf-blower (Figure 5.1), known to have a greater air velocity and capture more taxa relevant to this study, such as spiders, hymenopterans and thrips, when compared to commonly-used Vortis™ samplers (Zentane *et al.* 2016). All material was later frozen for storage before sorting in the lab.

All invertebrates were identified to family level due to the restriction of many of the metabarcoding-derived dietary data at this level, and the difficulty associated with finer taxonomic resolution of many damaged specimens. Exceptions included springtails of the

superfamily Sminthuroidea (Sminthuridae and Bourletiellidae, which were often indistinguishable following suction sampling and preservation due to the fine features necessary to distinguish them) which were left at super-family, mites (many of which were immature or in poor condition) which were identified to order level and wasps of the superfamily Ichneumonoidea (which were identified no further due to obscurity of wing venation due to damage); in these cases, these taxonomic assignments were treated as family-level assignments for later tropho-species aggregation. Identifications were carried out under an Olympus SZX7 stereomicroscope using morphological keys: Araneae (Roberts 1993), Diptera (Ball 2008), Coleoptera (Duff 2012), Hymenoptera (Goulet and Huber 1993), Hemiptera (Unwin 2001), Collembola (Dallimore and Shaw 2013) and Chilopoda (Barber 2008).

The fields used were divided into sixteen equally-sized squares and, in the centre of each square, stick, net and bottle (SNB) traps, designed by Woolley *et al*. (2007), were set to intercept ballooning spiders to give an approximation of the dispersal activity of spiders throughout the study period. The SNB traps were emptied weekly throughout the study period and any spiders within placed in ethanol for identification.

5.3.2. Macronutrient determination

Specimens were taken for macronutrient analysis from the same suction samples collected for invertebrate community identification. Representatives were taken from each family found in the community samples for which specimens were intact, in visually good condition and relatively clean of soil and other contaminants. If specimens were from a relatively uncommon family but unclean, soil and other surface contaminants were physically removed, and the specimen then momentarily dipped in water to remove remaining surface contaminants without greatly dislodging surface lipids. Macronutrient contents were determined following the MEDI protocol (Chapter 2.3) with minor alterations to account for the small size of most of the invertebrates processed. During extraction, half volumes (i.e. 500 µl) of solvents were used. For the lipid assays, 15 µl of sulfuric acid was added for a 15 min incubation, followed by only 200 µl of vanillin reagent to increase the concentration and development of analyte for more accurate readings from smaller invertebrates. Lipid and protein standard series were diluted to 50% of the concentration specified in Chapter 2 (i.e. 0-1 mg ml^{-1}). Carbohydrate assays used 140 µl of reagent with 30 min incubations at 92 °C followed by a further 30 min at room temperature. Carbohydrate standard series were diluted to 1% of the concentrations specified in Chapter 2 (i.e. 0-0.02 mg ml^{-1}) to ensure signals overcame the higher limit of detection relative to typical invertebrate carbohydrate content.

5.3.3. Ex situ *prey choice assays*

Spiders were collected from barley crops at Burdons Farm, Wenvoe, in 2019. Spiders were weighed on Mettler HK 60 scales sensitive to ± 0.0005 g and individually placed in 50 × 15 mm vented Petri dishes with a charcoal/plaster of Paris base kept moist to maintain humidity. Spiders were kept in a controlled-temperature room maintained at 18 °C with a photoperiod of 16 L:8 D. Spiders were initially starved for two weeks and subsequently provided with one each of *Drosophila suzukii* (Matsumura, 1931; Diptera: Drosophilidae), *Metopolophium dirhodum* and *Isotoma anglicana*, all ascertained to be viable prey of linyphiids prior to these experiments. All invertebrate prey were reared in a controlled-temperature room maintained at 18 °C with a photoperiod of 16 L:8 D. Aphids were reared on barley with three pots of ~10 plants rotationally replaced each week. Springtails were reared on a base of 90% plaster of Paris and 10% activated charcoal kept moist and supplemented with fresh baker's yeast each week. Fruit flies were reared on standard maize-sugar-yeast larval media. Spiders were then starved again for two weeks, after which they were re-weighed.

Spiders were randomly allocated to one of three feeding regimes: (i) fed one *D. suzukii* per week for four weeks; (ii) fed two *M. dirhodum* per week for four weeks; or (iii) fed two *I. anglicana* per week for four weeks (Figure 5.2). Only one *D. suzukii* was presented to account for their larger size (and thus disproportionate provision of nutrients). On week five, all spiders were presented with a choice of one each of the three prey. Spiders were monitored for five minutes immediately after prey were introduced, and after 1, 12 and 24 hours, and the predation of prey recorded. All prey were removed after 24 h and spiders were weighed.

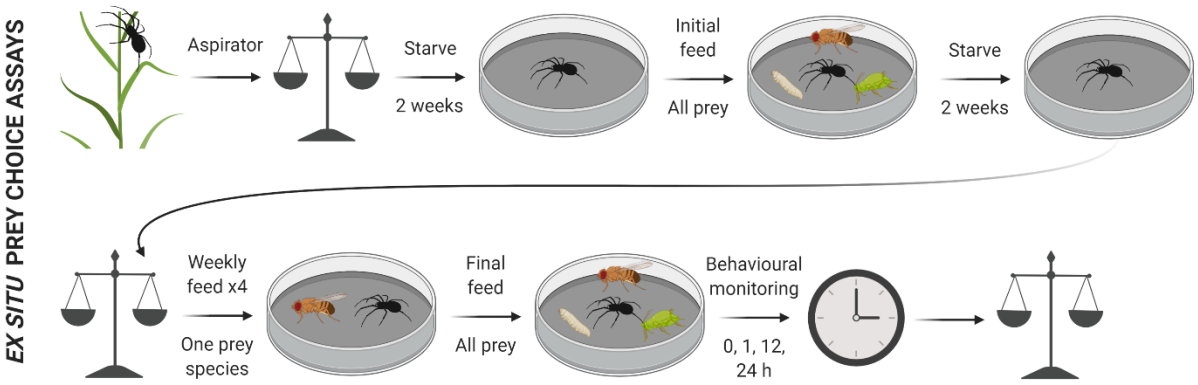


Figure 5.2: *Ex situ* feeding trial workflow.

5.3.4. Statistical analysis

All analyses were conducted in R v.4.0.3 (R Core Team 2020). Macronutrient contents (as proportions of total macronutrient mass) were first compared between families, orders and classes in a multivariate linear model using the 'manylm' function of the 'mvabund' package (Wang *et al.* 2012). Significant differences were visually represented through ternary plots using 'ggtern' (Hamilton and Ferry 2018) and 'ggplot2' (Wickham 2016).

To group taxa into tropho-species, mean macronutrient values for each taxon were first determined to prevent splitting of taxa across clusters; these were represented at the family, order and class levels to allow tropho-species assignment for families for which macronutrient content was not determined, but was at a higher level. Macronutrient values were scaled by subtracting the mean of each column from each contained value and dividing it by the column standard deviation using the 'scale' function. A Euclidean distance matrix was calculated using the 'dist' function. Hierarchical clustering of scaled macronutrient distance matrix used the 'hclust' function. Optimal clustering solutions were determined by comparison of Dunn's index between methods and *k* values; this was calculated using the 'dunn' function in the "clValid" package (Brock *et al.* 2008) for each cluster *k* value above five until the Dunn index decreased, the first instance of the value preceding the decrease deemed the maximum value, thus optimal solution. Clustering solutions based on 'average', 'complete', 'single', 'median', 'centroid' and 'mcquitty' linkages were compared, and the "complete" method selected for subsequent analysis as it resulted in the smallest number of clusters (20; thus the most efficient simplification of the taxa analysed). Three uncommon families (present in small numbers in one community sample each, but no dietary detections) were removed from further tropho-species analyses due to the lack of class-level macronutrient data (Arionidae, Lithobiidae and Polydesmidae).

To name the tropho-species, a second clustering stage was used in which the tropho-species were grouped according to their mean macronutrient content for each of the three nutrients separately. 'Single' linkage clustering was found to be the optimal method for this step and created ten, seven and six groups for carbohydrate, lipid and protein, respectively. These clusters were labelled from one to the total number of clusters for each macronutrient to represent low-to-high content of that nutrient relative to other tropho-species. Names used the structure 'C*x*L*y*P*z*' to denote the relative content of each tropho-species (*x*, *y* and *z* replaced with the cluster number for carbohydrate, lipid and protein, respectively).

Clusters were henceforth termed 'tropho-species', with all taxa within a single cluster representing a single aggregated tropho-species. Heatmap dendrograms were produced using the 'heatmap.2' function in the 'gplots' package (Warnes *et al.* 2020), with cluster colours assigned with the 'Accent' palette of 'RColorBrewer' (Neuwirth 2014) and relative macronutrient content colour scaling produced using the 'viridis' package (Garnier 2018). Ternary plots were produced to visualise the macronutrient content of taxa within each cluster, and differences in mean macronutrient contents between tropho-species.

Tropho-species were assigned to each taxon present in dietary and prey community samples. Where family-level macronutrient data were not obtained (usually low abundance and poor condition invertebrates or families identified in the diet that were not subsequently observed in community samples), order-level tropho-species assignment was used, or class where order-level data were not available. Pairwise co-occurrence analysis was carried out as specified in Chapter 4.3.5. using the 'cooccur' function in the 'cooccur' package (Griffith *et al.* 2016), but with taxonomic identities substituted with tropho-species. The previously generated co-occurrence data were also imported from Chapter 4.4.1. for post-hoc alignment of tropho-species identities with a taxonomically derived co-occurrence matrix.

In situ spider prey choice was analysed using network-based null models in the 'econullnetr' package (Vaughan *et al.* 2018) with the 'generate_null_net' command, visually represented with the 'plot_preferences' command, as in Chapter 3.3. Suction sample data were used to represent prey availability. Prey choice analyses were carried out first with prey taxonomic identities, and secondly and separately with prey tropho-species identities. Prior to the taxonomic prey choice analysis, an hemipteran identified no further than order level through dietary analysis was removed due to the inability to pair it to any present prey taxa with certainty.

Ex situ prey choice assay data were analysed via multivariate analysis of variance (MANOVA) using the 'manova' function. The dietary regime was analysed against the change in body mass across the study period, the time point in the final feed by which each spider had first

fed and the separate time points at which each spider had consumed each of the three prey (non-consumption represented as 48 hours). Data collected from spiders that died during the experiments were excluded from the former analyses but retained for a generalized linear model (GLM) with Poisson errors that analysed the number of weeks until death against the initial mass, the dietary regime, the sex and maturity of the spider, and all pairwise interactions between these variables for all spiders that died during study. Model assumptions were checked for the Poisson GLM via the 'testResiduals' function in the 'DHARMa' package (Hartig 2020).

5.4. Results

5.4.1. Field surveys of potential prey

Of the survey methods trialled, only suction sampling was taken forward for prey choice analysis given the under-representation of many common spider prey groups (e.g. springtails, rove beetles) by other methods following initial identification. The SNB traps rarely captured any spiders, resulting in insufficient data for any subsequent analyses.

5.4.2. Taxonomic macronutrient comparison and tropho-species determination

Macronutrient content was determined from 201 individual invertebrates across 64 families, 11 orders and 3 classes. Macronutrient content significantly differed between families (MLM: $F_{137} = 8.673$, $p = 0.002$; Figure S5.1), orders (MLM: $F_{190} = 8.470$, $p = 0.002$; Figure S5.2) and classes (MLM: $F_{198} = 18.840$, $p = 0.002$; Figure S5.3). The families Lycosidae, Chloropidae, Scathophagidae, Sepsidae and Linyphiidae had the lowest carbohydrate content, whilst Syrphidae, Anthomyiidae, Acanthosomatidae, Aphididae and Proctotrupidae had the highest. Eupelmidae, Megaspilidae, Ceraphronidae, Linyphiidae and Ichneumonidae had the lowest lipid content, whilst Phalacridae, Stenopsocidae, Sphaeroceridae, Chrysididae and Scathophagidae and had the highest. Phalacridae, Aphididae, Stenopsocidae, Sphaeroceridae and Syrphidae had the lowest protein content, and Linyphiidae, Ichneumonidae, Eupelmidae, Carabidae and Platygastridae the highest. Several taxa had relatively average proportions of all three macronutrients, including Anthocoridae, Diapriidae, Phoridae, Sminthuroidea, Torymidae, Cicadellidae, Coccinellidae, Delphacidae, Microphysidae, Thripidae and Aeolothripidae; such proportions are herein referred to as nutritionally balanced, whereas those organisms with relative extremes of proportional content are considered nutritionally imbalanced (i.e. nutrient-rich for that specific nutrient). These macronutrient data facilitated the clustering of taxa into 20 independent groups based on their

macronutrient contents (i.e. taxa with similar nutrient contents were aggregated), herein termed “tropho-species” (Figures 5.3-5.4 & S5.4-5.5). The “complete” method was deemed optimal, with other solutions producing too few (4) or many more (29-43) clusters.

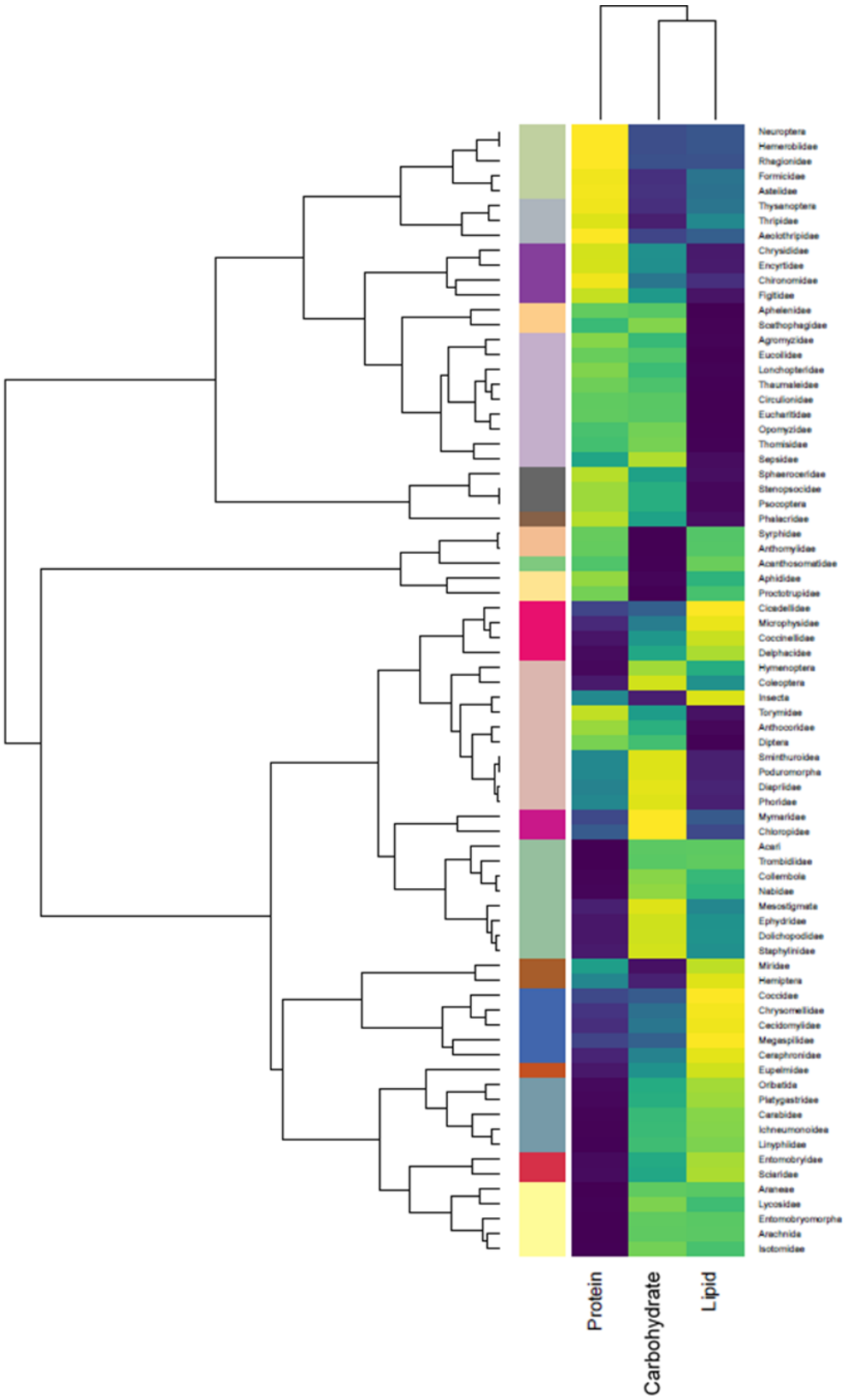


Figure 5.3: Dendrogram and heatmap displaying the 20 clusters (i.e. tropho-species, novel taxonomic ranks assigned based on macronutrient content) denoted by the leftmost coloured blocks. The height of the dendrogram branches indicates the relative dissimilarity between the resultant divergent clusters. Proportional content of each macronutrient (yellow to purple denoting low to high proportional content) and the taxa aggregated in each cluster are given. Many tropho-species contain at least one macronutrient in very high or low proportion, with few exhibiting "balanced" macronutrient content. Alternative dendrogram given in Figure S5.4.

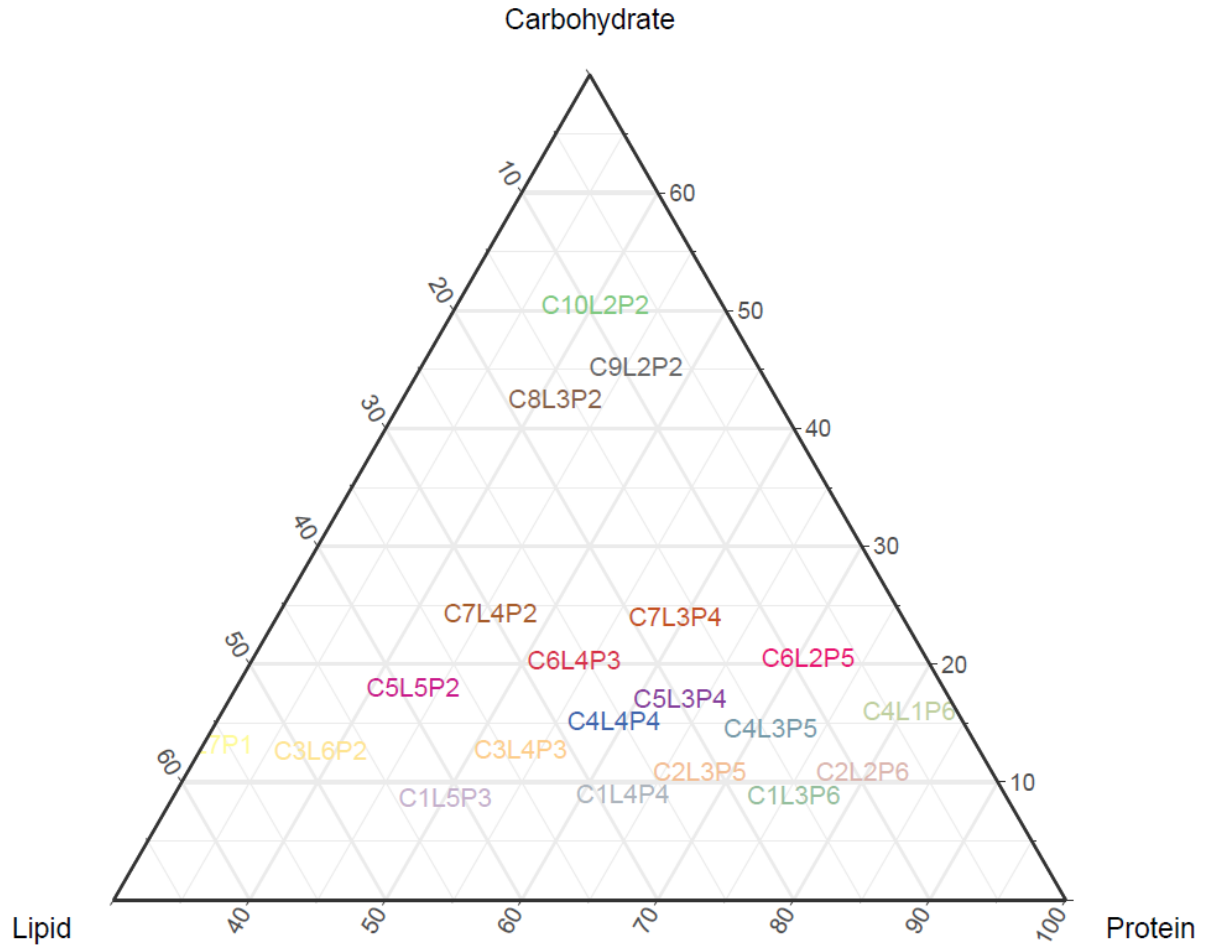


Figure 5.4: Ternary plot of macronutrient content of the tropho-species (represented by each tropho-species label, also coloured differently). Proximity to each labelled point of the triangle corresponds to the proportion of that macronutrient in the body of the invertebrate, with scaled lines clockwise of their respective label in the same orientation as their axis labels. All taxa comprising each tropho-species are plotted separately in Figure S5.5.

Clustering of the average content of each individual macronutrient across tropho-species optimally formed ten, seven and six clusters for carbohydrate, lipid and protein, respectively. Tropho-species were then named based on these groupings to create informative titles: 'C*x*L*y*P*z*', whereby the relative carbohydrate (*x*), lipid (*y*) and protein (*z*) content are given, with higher numbers denoting higher content, up to a maximum of the total number of clusters (e.g. C10L7P6 would contain the highest relative grouping for each macronutrient; Figure 5.4, Table 5.1). Tropho-species are herein referenced by these names, taken to confer relative macronutrient content to the reader.

Table 5.1: Tropho-species mean macronutrient contents and the taxa that comprise each group. Tropho-species names use the structure "CxLyPz", referring to the carbohydrate (x), lipid (y) and protein (z) cluster number, with higher numbers denoting higher content, up to a maximum of the total number of clusters (carbohydrate = 10, lipid = 7, protein = 6). The three macronutrient columns for each tropho-species are coloured according to their relative concentration, with yellow, green and purple denoting low to medium to high content, respectively, with scaled colouring between them.

Tropho-species	Mean carbohydrate % macronutrient mass	Mean lipid % macronutrient mass	Mean protein % macronutrient mass	Taxonomic composition
C1L3P6	7.48	16.27	76.25	Arachnida, Araneae, Entomobryomorpha, Isotomidae, Lycosidae
C1L4P4	7.58	28.78	63.64	Chloropidae, Mymaridae
C1L5P3	7.31	41.97	50.72	Aphelenidae, Scathophagidae
C2L2P6	9.42	10.23	80.35	Carabidae, Ichneumonoidea, Linyphiidae, Oribatida, Platygastridae
C2L3P5	9.50	22.16	68.33	Acari, Collembola, Dolichopodidae, Ephydridae, Mesostigmata, Nabidae, Staphylinidae, Trombidiformes
C3L4P3	11.37	34.39	54.24	Agromyzidae, Circulionidae, Eucharitidae, Eucoilidae, Lonchopteridae, Opomyzidae, Sepsidae, Thaumaleidae, Thomisidae
C3L6P2	11.30	49.13	39.57	Psocoptera, Sphaeroceridae, Stenopsocidae
C3L7P1	11.82	57.26	30.92	Phalacridae
C4L1P6	14.59	4.22	81.19	Eupelmidae
C4L3P5	13.13	15.15	71.72	Entomobryidae, Sciaridae
C4L4P4	13.79	26.33	59.88	Anthocoridae, Coleoptera, Diapriidae, Diptera, Hymenoptera, Insecta, Phoridae, Poduromorpha, Sminthuroidea, Torymidae
C5L3P4	15.63	20.54	63.83	Cicadellidae, Coccinellidae, Delphacidae, Microphysidae
C5L5P2	16.61	39.69	43.71	Chironomidae, Chrysididae, Encyrtidae, Figitidae
C6L2P5	19.13	9.38	71.49	Cecidomyiidae, Ceraphronidae, Chrysomellidae, Coccidae, Megaspilidae
C6L4P3	18.89	26.70	54.41	Aeolothripidae, Thripidae, Thysanoptera

C7L3P4	22.59	17.41	60.00	Hemiptera, Miridae
C7L4P2	22.96	30.84	46.20	Asteiidae, Formicidae, Hemerobiidae, Neuroptera, Rhagionidae
C8L3P2	41.09	17.00	41.91	Aphididae, Proctotrupidae
C9L2P2	43.83	9.69	46.49	Acanthosomatidae
C10L2P2	49.12	10.05	40.83	Anthomyiidae, Syrphidae

5.4.3. Tropho-species co-occurrence analysis

The taxonomically derived co-occurrence data (co-occurrence of taxa in the diets of spiders) highlight significant positive and negative co-occurrences of a broad range of tropho-species (Table 5.2).

Table 5.2: Significant taxa co-occurrences in the diet of spiders taken from the field with the corresponding tropho-species for each taxon listed (originally presented in Table S4.6). The direction of the relationship determined by co-occurrence analysis is given by '+' in red and '-' in blue, denoting positive and negative co-occurrences (i.e. co-occur more or less frequently than expected), respectively. The three macronutrient columns for each tropho-species are coloured according to their relative concentration, with yellow, green and purple denoting low to medium to high content, respectively, with scaled colouring between them.

Taxon 1	Tropho-species 1	Carbohydrate	Lipid	Protein	Taxon 2	Tropho-species 2	Carbohydrate	Lipid	Protein	Relationship
Anaphothrips obscurus	C6L4P3				Cecidomyiidae sp.	C6L2P5				-
Anaphothrips obscurus	C6L4P3				Entomobryidae sp.	C4L3P5				+
Anaphothrips obscurus	C6L4P3				Eupodidae sp.	C2L3P5				+
Anaphothrips obscurus	C6L4P3				*Frankliniella tenuicornis*	C6L4P3				+
Anaphothrips obscurus	C6L4P3				*Limothrips denticornis*	C6L4P3				+
Anaphothrips obscurus	C6L4P3				*Macrosteles* sp.	C5L3P4				+
Anaphothrips obscurus	C6L4P3				*Oscinella* sp.	C1L4P4				-
Anaphothrips obscurus	C6L4P3				*Reticulitermes lucifugus*	C4L4P4				+

Anaphothrips obscurus	C6L4P3				*Sminthurus viridis*	C4L4P4				-
Aphelinus sp.	C1L5P3				*Frankliniella tenuicornis*	C6L4P3				+
Aphelinus sp.	C1L5P3				*Limothrips denticornis*	C6L4P3				+
Aphidius sp.	C2L2P6				Bourletiellidae sp.	C4L4P4				+
Bourletiellidae sp.	C4L4P4				*Oscinella* sp.	C1L4P4				-
Bourletiellidae sp.	C4L4P4				*Sitobion* sp.	C8L3P2				+
Cecidomyiidae sp.	C6L2P5				Eupodidae sp.	C2L3P5				-
Cecidomyiidae sp.	C6L2P5				*Limothrips denticornis*	C6L4P3				-
Eupodidae sp.	C2L3P5				*Frankliniella tenuicornis*	C6L4P3				+
Eupodidae sp.	C2L3P5				*Limothrips denticornis*	C6L4P3				+
Eupodidae sp.	C2L3P5				*Reticulitermes lucifugus*	C4L4P4				+
Frankliniella tenuicornis	C6L4P3				*Limothrips denticornis*	C6L4P3				+
Isotomurus sp.	C1L3P6				*Limothrips denticornis*	C6L4P3				-
Limothrips denticornis	C6L4P3				*Nothodelphax* sp.	C5L3P4				-
Limothrips denticornis	C6L4P3				*Oscinella* sp.	C1L4P4				-
Limothrips denticornis	C6L4P3				*Reticulitermes lucifugus*	C4L4P4				+
Limothrips denticornis	C6L4P3				*Sminthurus viridis*	C4L4P4				-
Reticulitermes lucifugus	C4L4P4				*Rhopalosiphum* sp.	C8L3P2				+

Of 190 possible tropho-species pair combinations, 122 pairs (64.21 %) were removed from the analysis due to an expected co-occurrence of less than 1, leaving 68 pairs for analysis. The tropho-species-derived co-occurrence analysis identified 11 significant co-occurrence relationships out of these 68 possible pairwise combinations (Figure 5.5; Tables 5.3 & S5.1). These comprised three instances of tropho-species co-occurring significantly more than expected and eight instances of tropho-species co-occurring less than expected.

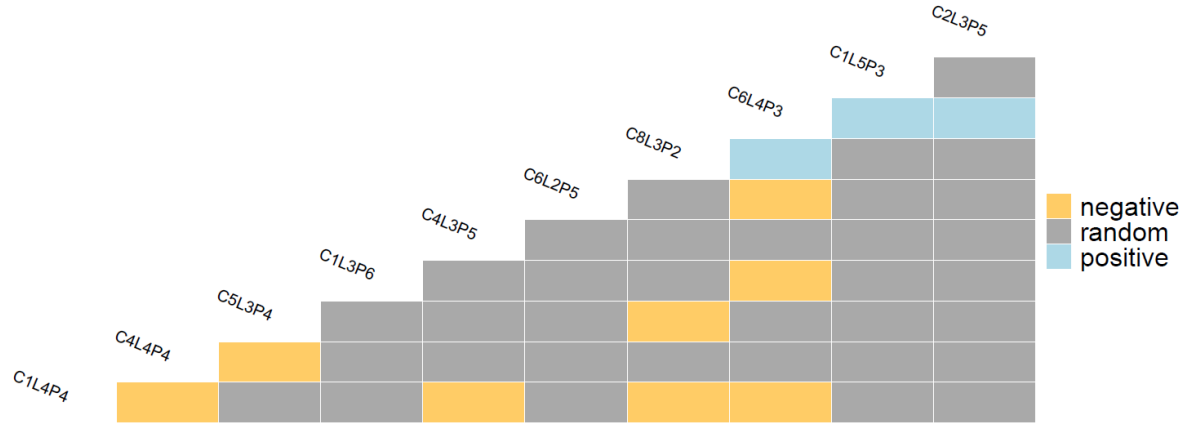


Figure 5.5: Tropho-species co-occurrence matrix for those tropho-species identified from spider diets in the field. Yellow, grey and blue points denote significantly negative, random and significantly positive co-occurrences, respectively.

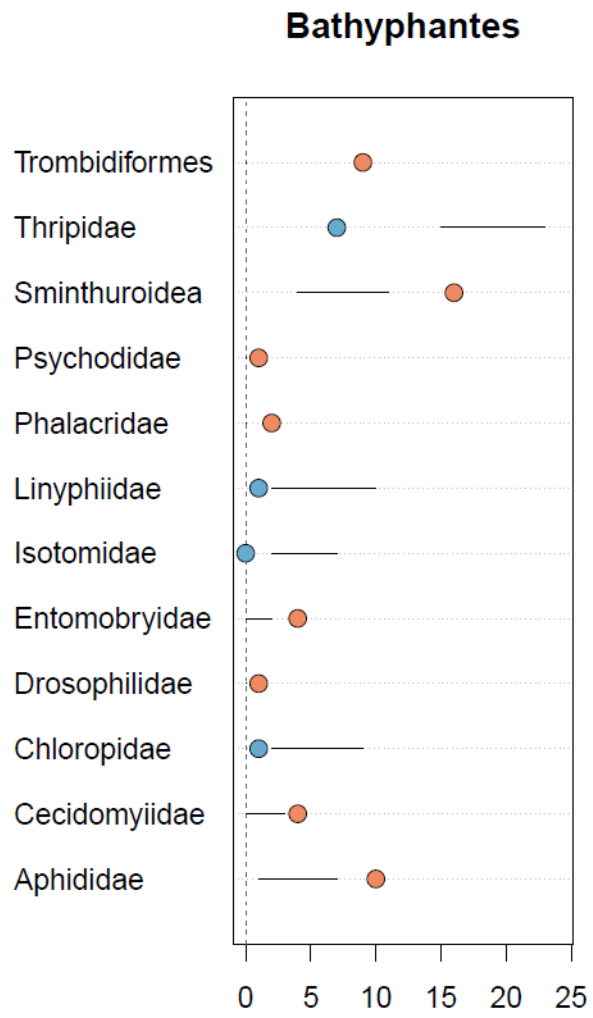
Table 5.3: Significant tropho-species co-occurrences (i.e. determined by tropho-species co-occurring or not) in the diet of spiders taken from the field. The relationship determined by co-occurrence analysis is given by '+' in red and '-' in blue, denoting positive and negative co-occurrences (i.e. co-occur more or less frequently than expected), respectively. The three macronutrient columns for each tropho-species are coloured according to their relative concentration, with yellow, green and purple denoting low to medium to high content, respectively, with scaled colouring between them.

Tropho-species 1	Carbohydrate	Lipid	Protein	Tropho-species 2	Carbohydrate	Lipid	Protein	Relationship
C1L3P6				C6L4P3				-
C1L4P4				C4L3P5				-
C1L4P4				C4L4P4				-
C1L4P4				C6L4P3				-
C1L4P4				C8L3P2				-
C1L5P3				C6L4P3				+
C2L3P5				C6L4P3				+
C4L4P4				C5L3P4				-
C5L3P4				C8L3P2				-
C6L2P5				C6L4P3				-
C6L4P3				C8L3P2				+

5.4.4. In situ *prey choice analysis*

All groups of spiders exhibited taxonomically biased prey choice beyond density-dependent selection across the 81 taxa identified from community and dietary samples (Figures 5.6-5.8 & S5.6-S5.8; Tables 5.4-5.6). Different spider genera exhibited different prey preferences (Figures 5.6 & S5.6; Table 5.4). *Bathyphantes* predated eight taxa significantly more than expected and four taxa significantly less than expected. *Erigone* predated eight taxa

significantly more than expected and two taxa significantly less than expected. *Microlinyphia* predated 9 taxa significantly more than expected and one taxon significantly less than expected. *Tenuiphantes* predated 13 taxa significantly more than expected and 12 taxa significantly less than expected. *Pardosa* predated three taxa significantly more than expected and one taxon significantly less than expected.


Bathyphantes
Trombidiformes
Thripidae
Sminthuroidea
Psychodidae
Phalacridae
Linyphiidae
Isotomidae
Entomobryidae
Drosophilidae
Chloropidae
Cecidomyiidae
Aphididae
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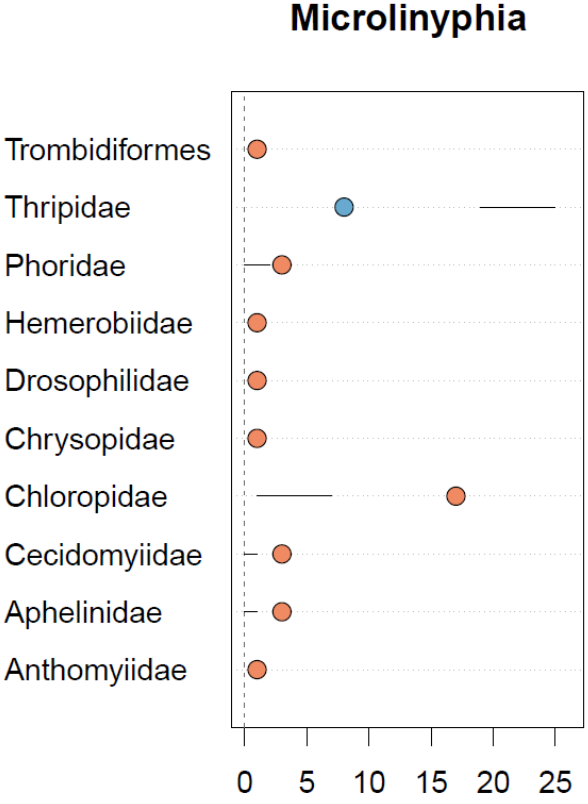
Microlinyphia
Trombidiformes
Thripidae
Phoridae
Hemerobiidae
Drosophilidae
Chrysopidae
Chloropidae
Cecidomyiidae
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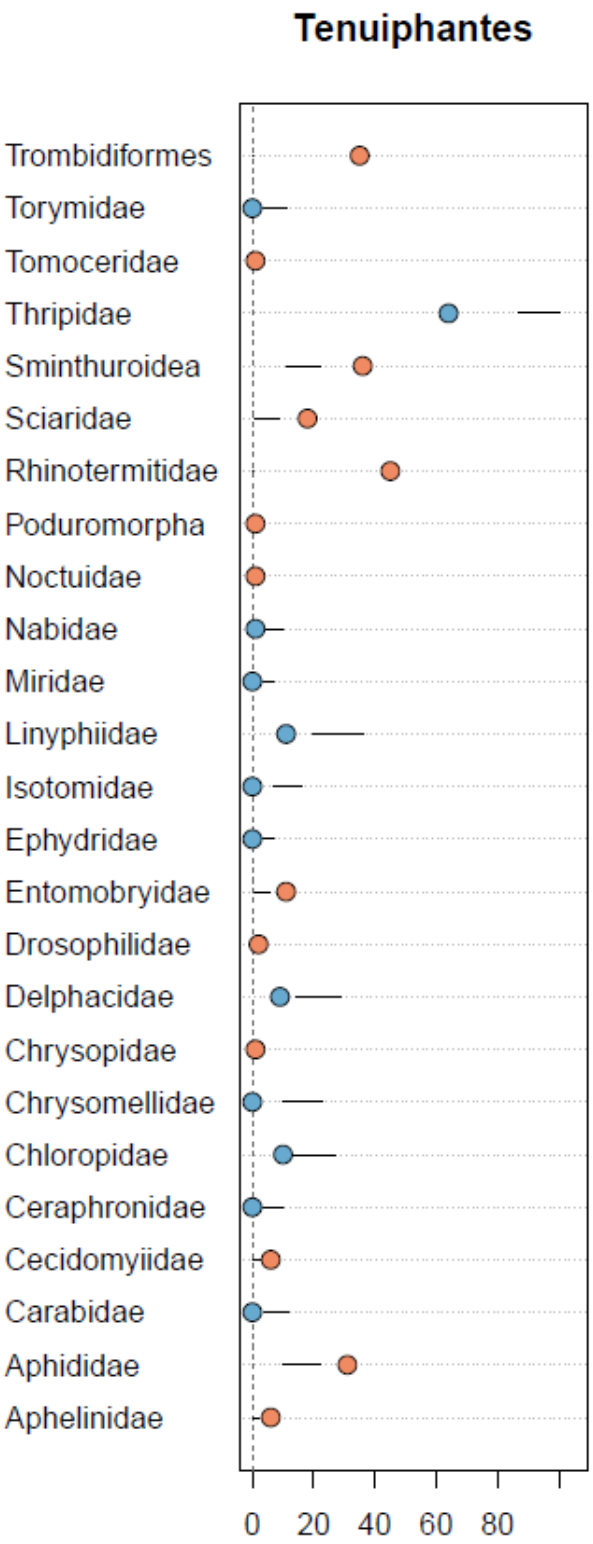
Tenuiphantes
Trombidiformes
Torymidae
Tomoceridae
Thripidae
Sminthuroidea
Sciaridae
Rhinotermitidae
Poduromorpha
Noctuidae
Nabidae
Miridae
Linyphiidae
Isotomidae
Ephydridae
Entomobryidae
Drosophilidae
Delphacidae
Chrysopidae
Chrysomellidae
Chloropidae
Ceraphronidae
Cecidomyiidae
Carabidae
Aphididae
Aphelinidae
0
20
40
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80

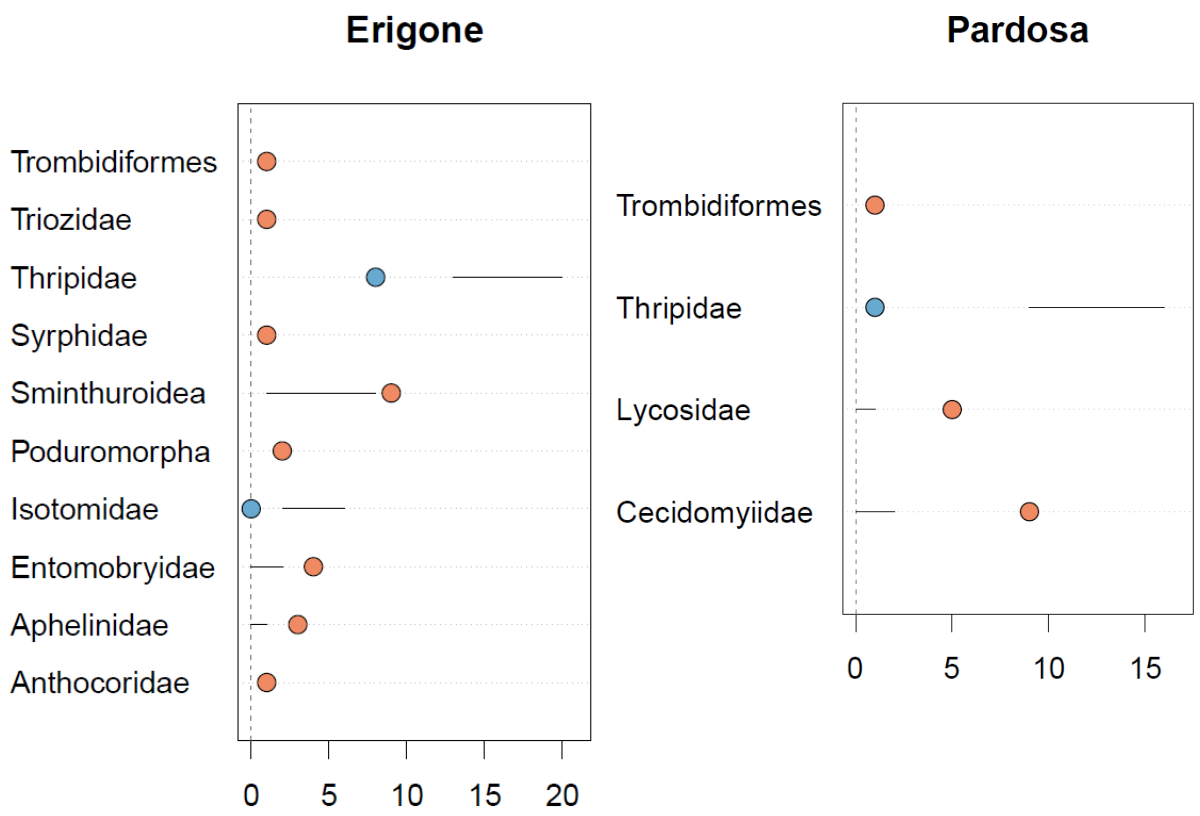

Figure 5.6: Significant deviations from expected frequencies of trophic interactions for spider genera predating prey taxa in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), red = higher than expected (consumed more frequently than predicted from relative abundance). The full results are given in Figure S5.6.

Table 5.4: Taxa that were predated significantly more (+ red) or less (- blue) than expected from prey choice null models for each of the five spider genera studied in the field.

Bathyphantes		*Erigone*		*Microlinyphia*		*Tenuiphantes*		*Pardosa*	
+	-	+	-	+	-	+	-	+	-
Trombidiformes	Thripidae	Trombidiformes	Thripidae	Trombidiformes	Thripidae	Trombidiformes	Torymidae	Trombidiformes	Thripidae
Sminthuroidea	Linyphiidae	Triozidae	Isotomidae	Phoridae		Tomoceridae	Thripidae	Lycosidae	
Psychodidae	Isotomidae	Syrphidae		Hemerobiidae		Smithuroidea	Nabidae	Cecidomyiidae	
Phalacridae	Chloropidae	Sminthuroidea		Drosophilidae		Sciaridae	Miridae		
Entomobryidae		Poduromorpha		Chrysopidae		Rhinotermitidae	Linyphiidae		
Drosophilidae		Entomobryidae		Chloropidae		Poduromorpha	Isotomidae		
Cecidomyiidae		Aphenilidae		Cecidomyiidae		Noctuidae	Ephydridae		
Aphididae		Anthrocoridae		Aphelinidae		Entomobryidae	Delphacidae		
				Anthomyiidae		Drosophilidae	Chrysomellidae		
						Chrysopidae	Chloropidae		
						Cecidomyiidae	Ceraphronidae		
						Aphididae	Carabidae		
						Aphelinidae			

Different spider life stages exhibited different taxonomic biases in their prey choice (Figures 5.7 & S5.7; Table 5.5). Adult spiders predated 19 taxa significantly more than expected and 9 taxa significantly less than expected. Juvenile spiders predated 11 taxa significantly more than expected and 7 taxa significantly less than expected.

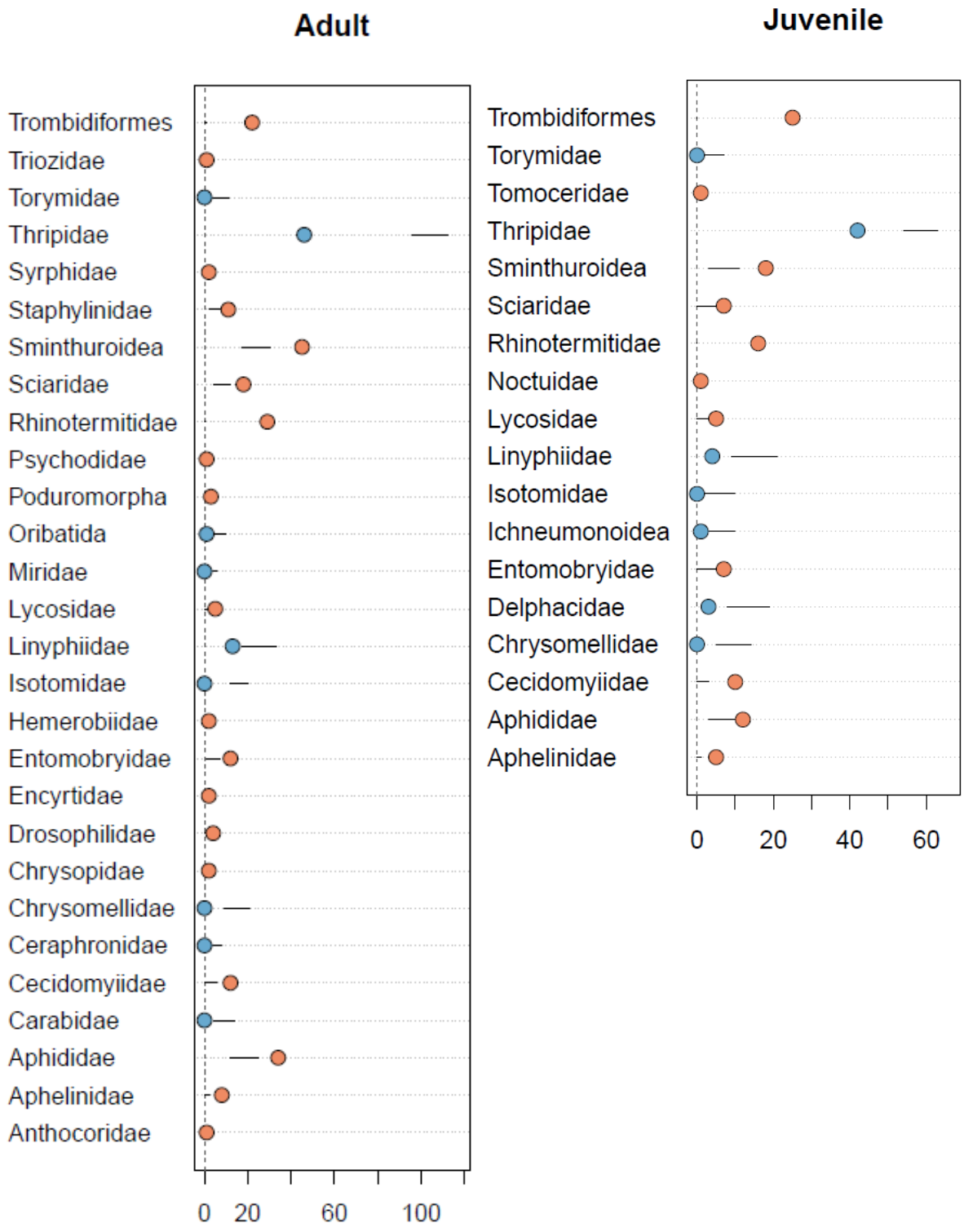

Figure 5.7: Significant deviations from expected frequencies of trophic interactions for spider life stages predating prey taxa in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), red = higher than expected (consumed more frequently than predicted from relative abundance). The full results are given in Figure S5.7.

Table 5.5: Taxa that were predated significantly more (+ red) or less (- blue) than expected from prey choice null models for each of the two spider life stages studied in the field.

Adult		Juvenile	
+	-	+	-
Trombidiformes	Torymidae	Trombidiformes	Torymidae
Triozidae	Thripidae	Tomoceridae	Thripidae
Syrphidae	Oribatida	Sminthuroidea	Linyphiidae
Staphylinidae	Miridae	Sciaridae	Isotomidae
Sminthuroidea	Linyphiidae	Rhinotermitidae	Ichneumonoidea
Sciaridae	Isotomidae	Noctuidae	Delphacidae
Rhinotermitidae	Chrysomellidae	Lycosidae	Chrysomellidae
Psychodidae	Ceraphronidae	Entomobryidae	
Poduromorpha	Carabidae	Cecidomyiidae	
Lycosidae		Aphididae	
Hemerobiidae		Aphelinidae	
Entomobryidae			
Encyrtidae			
Drosophilidae			
Chrysopidae			
Cecidomyiidae			
Aphididae			
Aphelinidae			
Anthocoridae			

Different spider sexes exhibited different taxonomic biases in their prey choice (Figures 5.8 & S5.8; Table 5.6). Female spiders predated 18 taxa significantly more than expected and 7 taxa significantly less than expected. Male spiders predated 13 taxa significantly more than expected and 8 taxa significantly less than expected.

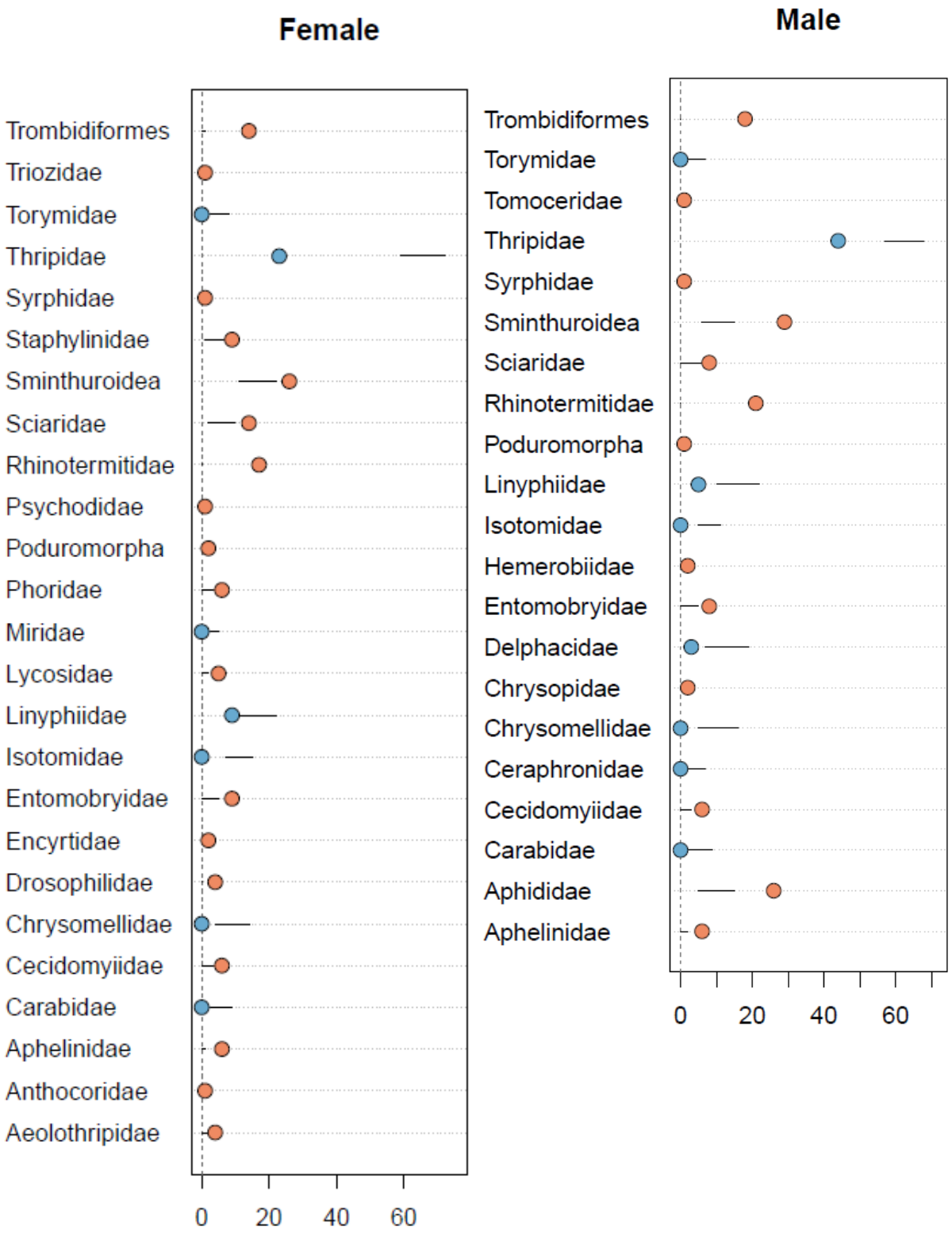

Figure 5.8: Significant deviations from expected frequencies of trophic interactions for spider sexes predating prey taxa in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), red = higher than expected (consumed more frequently than predicted from relative abundance). The full results are given in Figure S5.8.

Table 5.6: Taxa that were predated significantly more (+ red) or less (- blue) than expected from prey choice null models for each of the two spider sexes studied in the field.

Female		Male	
+	-	+	-
Trombidiformes	Torymidae	Trombidiformes	Torymidae
Triozidae	Thripidae	Tomoceridae	Thripidae
Syrphidae	Miridae	Syrphidae	Linyphiidae
Staphylinidae	Linyphiidae	Sminthuroidea	Isotomidae
Sminthuroidea	Isotomidae	Sciaridae	Delphacidae
Sciaridae	Chrysomellidae	Rhinotermitidae	Chrysomellidae
Rhinotermitidae	Carabidae	Poduromorpha	Ceraphronidae
Psychodidae		Hemerobiidae	Carabidae
Poduromorpha		Entomobryidae	
Phoridae		Chrysopidae	
Lycosidae		Cecidomyiidae	
Entomobryidae		Aphididae	
Encyrtidae		Aphelinidae	
Drosophilidae			
Cecidomyiidae			
Aphelinidae			
Anthocoridae			
Aeolothripdiae			

5.4.5. Nutrient-specific foraging analysis via tropho-species prey choice models

All groups of spiders exhibited tropho-species preferences beyond density-dependent selection (Figures 5.9-5.11 & S5.9-S5.11; Tables 5.7-5.9). Different spider genera exhibited different tropho-species biases in their prey choice (Figures 5.9 & S5.9; Table 5.7). *Bathyphantes* predated five tropho-species significantly more than expected and three tropho-species significantly less than expected. *Erigone* predated four tropho-species significantly more than expected and three tropho-species significantly less than expected. *Microlinyphia* predated three tropho-species significantly more than expected and two tropho-species significantly less than expected. *Tenuiphantes* predated five tropho-species significantly more than expected and seven tropho-species significantly less than expected. *Pardosa* predated two tropho-species significantly more than expected, and two tropho-species significantly less than expected.

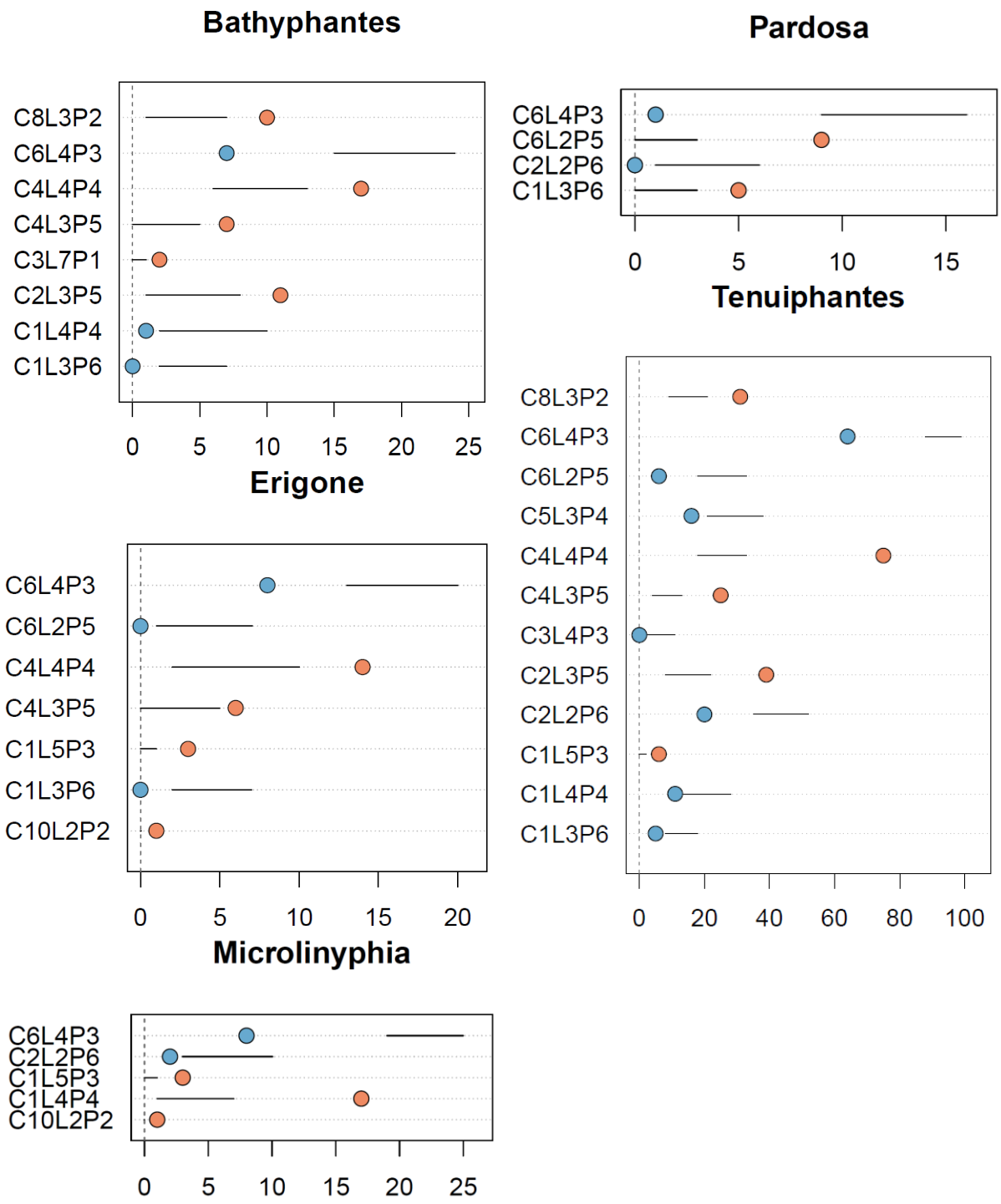

Figure 5.9: Significant deviations from expected frequencies of trophic interactions for spider genera predating prey tropho-species in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), red = higher than expected (consumed more frequently than predicted from relative abundance). The full results are given in Figure S5.9.

Table 5.7: Tropho-species that were predated significantly more (+ red) or less (- blue) than expected from prey choice null models for each of the five spider genera studied in the field.

Bathyphantes		*Erigone*		*Microlinyphia*		*Tenuiphantes*		*Pardosa*	
+	-	+	-	+	-	+	-	+	-
C8L3P2	C6L4P3	C4L4P4	C6L4P3	C1L5P3	C6L4P3	C8L3P2	C6L4P3	C6L2P5	C6L4P3
C4L4P4	C1L4P4	C4L3P5	C6L2P5	C1L4P4	C2L2P6	C4L4P4	C6L2P5	C1L3P6	C2L2P6
C4L3P5	C1L3P6	C1L5P3	C1L3P6	C10L2P2		C4L3P5	C5L3P4		
C3L7P1		C10L2P2				C2L3P5	C3L4P3		
C2L3P5						C1L5P3	C2L2P6		
							C1L4P4		
							C1L3P6		

Different spider life stages exhibited different tropho-species biases in their prey choice (Figures 5.10 & S5.10; Table 5.8). Adults spiders predated six tropho-species significantly more than expected and six tropho-species significantly less than expected. Juvenile spiders predated four tropho-species significantly more than expected and three tropho-species significantly less than expected.

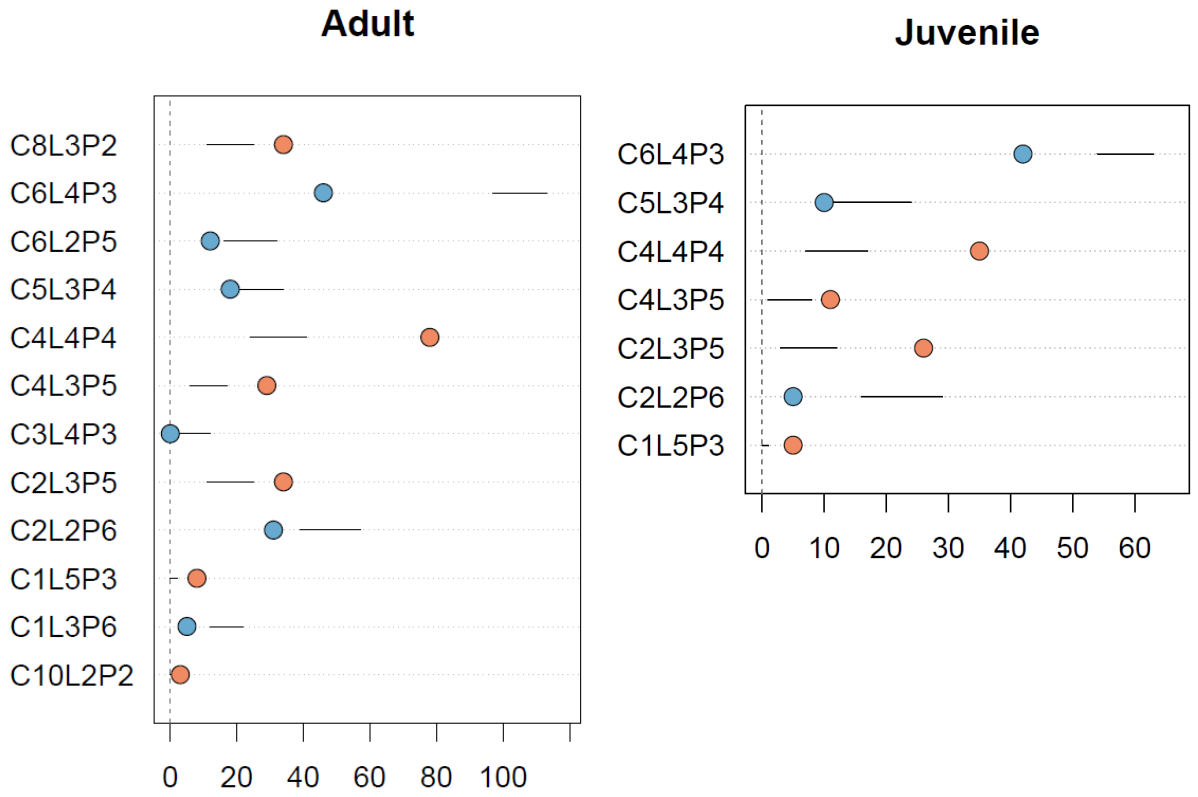

Figure 5.10: Significant deviations from expected frequencies of trophic interactions for spider life stages predating prey tropho-species in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), red = higher than expected (consumed more frequently than predicted from relative abundance). The full results are given in Figure S5.10.

Table 5.8: Tropho-species that were predated significantly more (+ red) or less (- blue) than expected from prey choice null models for each of the two spider life stages studied in the field.

Adult		Juvenile	
+	-	+	-
C8L3P2	C6L4P3	C4L4P4	C6L4P3
C4L4P4	C6L2P5	C4L3P5	C5L3P4
C4L3P5	C5L3P4	C2L3P5	C2L2P6
C2L3P5	C3L4P3	C1L5P3	
C1L5P3	C2L2P6		
C10L2P2	C1L3P6		

Different spider sexes exhibited different tropho-species biases in their prey choice (Figures 5.11 & S5.11; Table 5.9). Female spiders predated six tropho-species significantly more than expected and five tropho-species significantly less than expected. Male spiders predated five tropho-species significantly more than expected and six tropho-species significantly less than expected.

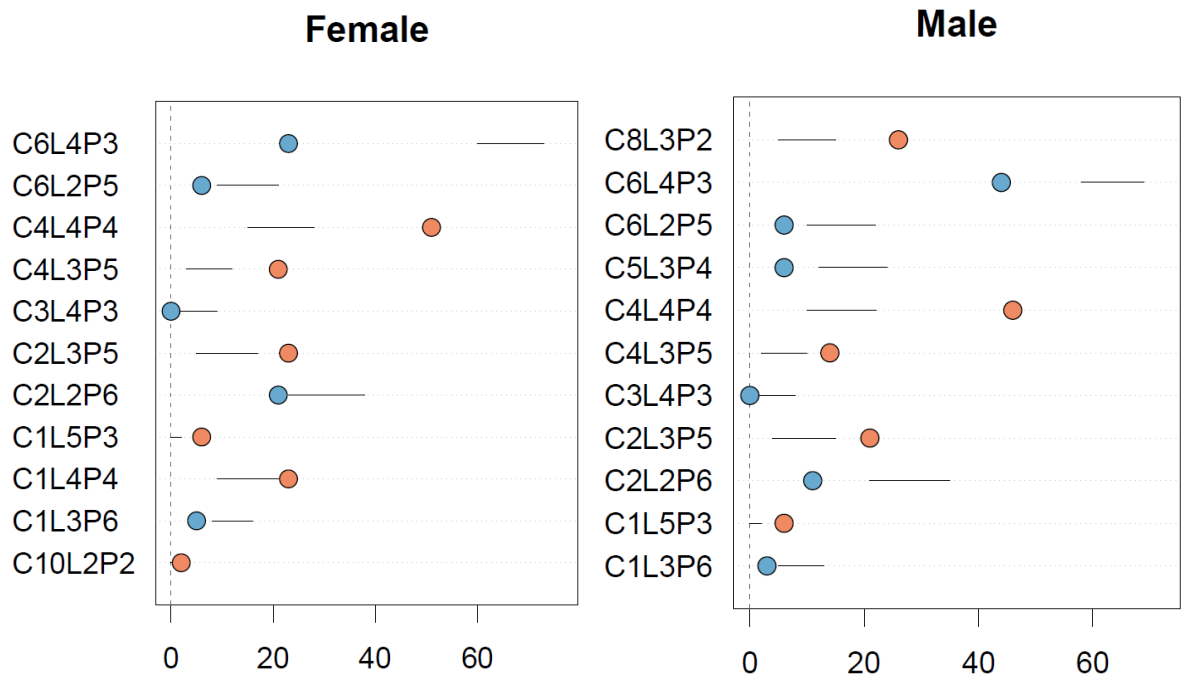

Figure 5.11: Significant deviations from expected frequencies of trophic interactions for spider sexes predating prey tropho-species in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), red = higher than expected (consumed more frequently than predicted from relative abundance). The full results are given in Figure S5.11.

Table 5.9: Tropho-species that were predated significantly more (+ red) or less (- blue) than expected from prey choice null models for each of the two spider sexes studied in the field.

Female		Male	
+	-	+	-
C4L4P4	C6L4P3	C8L3P2	C6L4P3
C4L3P5	C6L2P5	C4L4P4	C6L2P5
C2L3P5	C3L4P3	C4L3P5	C5L3P4
C1L5P3	C2L2P6	C2L3P5	C3L4P3
C1L4P4	C1L3P6	C1L5P3	C2L2P6
C10L2P2			C1L3P6

5.4.6. Ex situ *prey choice assays*

Of the 54 spiders that survived the initial starvation period (20 aphid diet, 21 fly diet, 13 springtail diet), 33 survived the full feeding trial (14 aphid diet, 13 fly diet, 6 springtail diet). The time at which spiders consumed their first prey in the final feeding point, the time at which spiders had eaten each prey and the change in spider body mass were not significantly associated with dietary regime. Equally, mortality was not significantly associated with the initial body mass of the spider, dietary regime, or the sex or maturity of the spider.

5.5. Discussion

This chapter provides the first evidence toward nutrient-specific foraging in the field by highlighting the predation of nutritionally complementary prey by individual spiders, and the preferential predation of prey rich in all three macronutrients by spider populations. Spider prey families significantly differed in their macronutrient contents, facilitating the novel hierarchical clustering approach to macronutrient-based prey choice analysis. Spiders exhibited distinct non-density-dependent prey preferences, with these preferences differing between genera, sexes and life stages.

5.5.1. Spider prey choice analyses

Spider prey choice was found to deviate from density-dependent selection, with these deviations differing between genera, sexes and life stages, indicating differential prey preferences. Several consistent prey choice patterns arose between spider groups, notably the significantly higher-than-expected predation of Trombidiformes by all groups, and the significantly lower-than-expected predation of Thripidae. The latter is likely due to the overwhelming abundance of thrips in prey communities, especially when considering that, despite this lower-than-expected predation, they still comprised the most commonly predated prey group (Chapter 4.4). It is noteworthy that G-vac suction samplers are pre-disposed to collect a greater abundance of thrips than other comparative suction sampling methods (Zentane *et al.* 2016), possibly resulting in their over-representation. Regardless, this finding suggests that spiders may switch to other prey in order to diversify their diet rather than solely predating the most abundant and accessible prey group. The presence of defensive compounds and other toxins in some insect herbivores (Bilde and Toft 2001; Zvereva and Kozlov 2016) could promote this, although nutritional diversification is an equally feasible hypothesis.

Many other prey were predated significantly more than expected across an incomplete range of the spider groups, including Sminthuroidea, Entomobryidae, Cecidomyiidae and Aphididae. Since the former two of these, as well as the aforementioned Trombidiformes, are often associated with subterranean or sheltered microhabitats, their greater-than-expected predation may in fact relate to their poor representation by suction sampling, which will undoubtedly neglect such discrete taxa. To comprehensively sample the prey community spanning the dietary spectrum of a generalist predator is difficult, particularly given the varied ecological niches and behaviours that those prey will naturally represent. This study attempted several survey techniques, but preliminary comparisons made clear that prey such as these soil-dwelling springtails and mites were even more under-represented by other techniques such as sticky trapping. These findings highlight that the choice of appropriate prey sampling techniques is unarguably critical in such studies of prey choice.

That some linyphiids predated one another significantly less than expected may be an artefact of the lack of detection of cannibalism via metabarcoding; all detections of linyphiids in the diet of other linyphiids are instances of inter-specific predation. Cannibalism in linyphiids and lycosids is well-documented, particularly in juveniles (Hallander 1970; Turnbull 1973; Lesne *et al.* 2016) and as an outcome of courtship (Wilder and Rypstra 2010); if it was detectable in this study, linyphiid cannibalism could bring predation of con-familials up to a level proportional with their abundance (thus the frequency expected by the prey choice models), or even highlight greater-than-expected predation of linyphiids. Importantly, *Pardosa* (the only non-linyphiid studied) predated linyphiids in line with the null models, suggesting that undetected cannibalism could be resulting in underestimation of linyphiid predation; indeed, even *Erigone* and *Microlinyphia* predated other linyphiids in line with the null models, highlighting a taxonomic, but not life stage or sex related, bias in intra-familial predation.

Coarse differences in prey choice can be observed between spider genera; most immediately, *Pardosa* exhibit far fewer deviations from expected dietary proportions than the other genera. Given the ground-active habit of *Pardosa* (Kuusk *et al.* 2008), suction sampling could simply align more closely with their prey interception behaviour. It is, however, likely that the larger metabolic requirement of the larger-bodied and more active *Pardosa* (Kleiber 1932; Hemmingsen 1960; Andersson 1970; Greenstone and Bennett 1980; Kotiaho *et al.* 1998), and their restricted access to flying and climbing prey given their absence of webs and reduced climbing, may reduce their selectiveness when searching for prey. The greater-than-expected predation of other lycosids by *Pardosa* could suggest lethal competition for prey resources or sites, or consumption of one another to obtain the perfect ratio of nutrients to assimilate for their own tissues (Fox 1975).

Both *Erigone* and *Tenuiphantes* predated all springtail taxa other than isotomids significantly more than expected; for *Erigone* this is likely a consequence of their lower webs (Chapter 4.4; Sunderland *et al.* 1986), whilst for *Tenuiphantes* it could suggest the weather-dependent plasticity of web placement discussed in Chapter 4.5. *Tenuiphantes* was periodically found much closer to the ground, particularly during arid periods, which may relate to the availability of nutrients since web plasticity has been observed in response to nutritional changes (Blamires *et al.* 2016). The greater-than-expected predation of aphids by both *Bathyphantes* and *Tenuiphantes* aligns with their high potential for biocontrol, as discussed in Chapter 4.5; the height and area of their webs may be ideally situated to catch aphids falling from the crop canopy, termed 'aphid rain'. The perceived poor nutritional quality of aphids for spiders does, however, contrast with their disproportionately preferential predation (Toft 1995; Bilde and Toft 2001; Toft 2005), possibly indicating their ease-of-capture or a complementary nutrient profile compared to other consumed prey. As also discussed in Chapter 4.5, Rhinotermitidae, which were predated significantly more than expected by *Tenuiphantes*, have not been recorded from the field site and were not observed during invertebrate surveys, provoking questions surrounding the hypothetical presence of a cryptic population which would require specialist surveys, verification and action.

Adult and juvenile spiders exhibited overlapping but distinct prey choice, with adults predating significantly more active predators such as staphylinids, hemerobiids and chrysopids than expected, whereas juveniles predated significantly more tomocerids and noctuids. Juveniles also predated significantly fewer ichneumonids and delphacids than expected, whereas adults predated fewer oribatids, ceraphronids and carabids. The preference of adult spiders for more predators and fewer smaller mites, whilst juveniles predated more springtails and fewer parasitoids and more mobile prey such as hoppers ratifies the discussion in Chapter 4.5 around the increased propensity for intraguild predation in adult spiders, but predation of relatively innocuous prey, often pests, by smaller and less experienced juveniles.

Similarly, spider sexes exhibit overlapping but distinct prey preferences, with female spiders predating significantly more predatory taxa including triozids, staphylinids, lycosids, and aeolothrips than expected, and males significantly more lacewings (both hemerobiids and chrysopids) and aphids. That female spiders exhibit a greater preference for other ground-hunting predators may reflect their larger mean body sizes (Lång 2001; Walker and Rypstra 2003) and thus a greater mechanical advantage in subduing such prey. Overall differences between the sexes could, at least in cases such as *Erigone*, relate to a greater itinerance of males (Foelix 2011) thus enhancing their encounter rate with a broader range of prey. The greater male preference for aphids is, however, contrary to previous literature which has indicated a female-biased predation of aphids (Harwood *et al.* 2004). These prey choice

results identify taxonomically biased prey preference and, given the distinction in macronutrient content identified between taxa, this could be at least partially explained by nutrient-specific foraging.

5.5.2. Prey macronutrient comparison and tropho-species determination

Prey macronutrient content significantly differed between spider prey families, facilitating the hierarchical clustering-based aggregation of prey into “tropho-species” for simplified analyses of nutrient-based prey choice. As was the case in the preliminary data presented in Chapter 2.4, invertebrate bodies typically contained a greater proportion of protein (mean 61.7% ± 16.2) than carbohydrate (mean 14.8% ± 10.7) or lipid (mean 23.5% ± 14.1). Protein and lipid content exhibited substantial variation, but less variation was observed in carbohydrate content; this may suggest an evolutionarily stable assimilation of this macronutrient within invertebrate bodies, perhaps because high concentrations of sugars can be detrimental and toxic to both the accumulating invertebrate and any predator that feeds upon it (Schlotterer *et al.* 2009; Alcántar-Fernández *et al.* 2019). The distinct macronutrient contents between taxa facilitated the identification of 20 separate clusters through hierarchical clustering. These clusters, termed tropho-species, were polyphyletic in terms of ‘Linnean’ taxonomy, indicating that whilst traditional taxonomy does partly explain macronutrient content, inter-cladal convergence (e.g. Linyphiidae and Ichneumonidae being more similar in macronutrient content than other families in the same orders) justifies a macronutrient-based aggregation of prey for a focused and simplified analysis of nutrient-based prey choice. The naming convention (‘C*x*L*y*P*z*’) facilitates a rapid understanding of the approximate proportions of macronutrient in each tropho-species. To facilitate discussion, carbohydrate, lipid and protein values of 1-3, 1-2 and 1-2, respectively, are herein defined as nutrient-poor for that respective nutrient, and values of 8-10, 5-6 and 4-5, respectively are defined as nutrient-rich.

Other simplifications and models of macronutrient content in analyses of nutritional ecology have been conducted, such as a geometric framework of nutrient space (termed nutritional geometry) which requires pre-determination of optimal intake, analysed against the behavioural and physiological constraints imposed on the consumer (Simpson and Raubenheimer 1993; Simpson and Raubenheimer 1995). This framework similarly suggests aggregation of nutritional information, in this case into two axes (Simpson and Raubenheimer 1995); however, this would be impossible to apply to the analyses used in this study. Preliminary attempts were made in this study to create a single quantitative “macronutrient index” through ordination, but the variation in lipid and protein content was perpendicular to that of carbohydrates when ordinated, creating two complementary axes; this is presumably

in part due to the representation of macronutrient contents as proportions. Given the hierarchical nature of macronutrient differences (despite some convergences) displayed in this study, a categorical clustering of taxa in this manner is justifiable and accurate.

Frameworks such as the geometric nutrient framework, designed primarily around lab-based experiments, also require a fairly extensive prior knowledge of the study system, and depend on measurements of absolute quantities of nutrients, rather than proportions (Raubenheimer 2011). In the complexity and unpredictability of field-based studies, this is impractical and could encourage inaccuracies and biases if incorrect assumptions are made about the study system. The tropho-species concept, however, is a post-hoc data-driven approach which only requires information regarding the abundance of prey, their predation by predators and the prey macronutrient contents.

5.5.3. Co-occurrence of nutritionally distinct spider prey

Co-occurrence analyses confirmed that nutritionally complementary prey were consumed together by individual spiders. Two types of co-occurrence analysis were employed in this study: one focused on the taxonomic family of each prey originally analysed in Chapter 4, herein termed “taxonomic co-occurrence”, for which tropho-species assignments were denoted in this chapter, and the other focused on co-occurrence of tropho-species irrespective of “Linnean” taxonomy, herein termed “tropho-species co-occurrence. By viewing the previously generated taxonomic co-occurrence data in the context of the tropho-species assignment of each taxon, co-occurrence relationships comprising two taxa of the same tropho-species can be detected, as is the case for tropho-species C6L4P3 in three instances. The high prevalence of C6L4P3 taxa across co-occurrence relationships (with both taxa of the same and different tropho-species) is unsurprising given the common consumption of thrips (which belong to C6L4P3) by many spiders (Chapter 4.4). The greater-than-random co-occurrence of C6L4P3 taxa with fellow C6L4P3 taxa could, however, highlight the consistent consumption of a particular tropho-species by many spiders in order to obtain that specific nutritional profile, in this case relative nutritional balance (i.e. average proportions of all three macronutrients). This may, of course, instead relate to ecological factors, such as the ease-of-capture of relatively defenceless thrips. This focused predation of nutritionally balanced prey may maintain a balanced intake of all three macronutrients. It has previously been hypothesised that carnivores do not forage for specific nutrients since their prey will ultimately have nutrient profiles similar to their own, forcing them to forage quantitatively rather than qualitatively (Stephens and Krebs 1986; Kohl *et al.* 2015); however, this thesis, by highlighting

that invertebrate bodies do in fact significantly differ in their nutrient content, even between closely-related taxa, contradicts this, as do most existing studies of nutrient-specific foraging.

Many taxonomic co-occurrence relationships highlighted consistent tropho-species trends. In some cases, nutritionally balanced taxa were predated together more than expected, but these tropho-species were also sometimes predated together less than expected. Such inconsistencies undoubtedly relate to the distinct ecologies of the species predated, with different species representing their tropho-species in different co-occurrence relationships; for example, one co-occurrence involving *Anaphothrips obscurus* and C4L4P4 is positive, and another negative.

Most significant co-occurrences between taxa included thrips, which belong to the nutritionally balanced tropho-species C6L4P3. Aside from the previously discussed co-occurrences with taxa of the same tropho-species, many of these co-occurrences were with taxa with substantial nutritional imbalances. Similarly, other nutritionally balanced tropho-species such as C4L4P4 occurred alongside imbalanced taxa more than expected. This pattern is, however, disrupted by the co-occurrence of these nutritionally balanced taxa with some nutritionally imbalanced taxa less than expected. In no cases, however, did nutritionally imbalanced taxa co-occur more than expected; indeed, the only significant co-occurrence relationship involving two nutritionally imbalanced taxa was negative and between two protein-rich tropho-species, suggesting an avoidance of over-predation of protein-rich prey. Regardless of the inconsistencies, which may result from distinct prey behaviours and ecologies, this lack of co-occurrence of nutritionally imbalanced prey suggests an importance of nutritionally balanced prey in sustaining those predators that have imbalanced their nutrient intake through selective foraging.

The tropho-species co-occurrence results are indicative of broader nutritional patterns by aggregating nutritionally similar prey together. Those pairings which co-occurred significantly more than random ratified the inference from the taxonomic co-occurrence results that nutritionally balanced prey are often consumed alongside nutritionally imbalanced prey, possibly to redress deficits incurred by predation of the imbalanced prey. The co-occurrences less regular than expected are typically between nutritionally balanced tropho-species, sometimes including tropho-species with imbalanced carbohydrate content. That carbohydrate-rich tropho-species are not predated alongside nutritionally balanced prey, as is the case for lipid-rich prey, suggests that high carbohydrate intake is not redressed by these spiders. Similarly, high protein tropho-species, except in one case, negatively co-occurred with nutritionally balanced and carbohydrate-poor tropho-species. Many of the positive co-occurrences observed between taxa are obscured when aggregating tropho-species taxa together, possibly due to the loss of ecological separation of behaviourally dissimilar taxa; for

example, predominantly ground-restricted linyphiids were separated from largely airborne ichneumonids, which was not the case for the tropho-species co-occurrence analysis. By aggregating all intra-tropho-species taxa, inter-tropho-species co-occurrences were also obscured. Most of the observed co-occurrences were, however, consistent, notably including that the only significant co-occurrence of nutritionally imbalanced tropho-species was the negative co-occurrence of two protein-rich groups.

Co-occurrence analyses are well-poised to identify short-term tropho-species preferences by identifying prey that were consumed together in relatively short time periods (DNA detectability half-lives in spider guts vary from 10 h (Kuusk *et al.* 2008) to several days (Kobayashi *et al.* 2011; Macıas-Hernández *et al.* 2018) or weeks (Greenstone *et al.* 2014; Pompozzi *et al.* 2019) (Greenstone *et al.* 2014)), thus identifying responses to short-term nutritional requirements. Short-term nutritional preferences are likely to be ecologically significant as indications of nutritional deficits. During periods such as that following diapause, for example, predators have been observed initially seeking increased lipid intake, with a steady return over ten days to more standard protein and lipid intake proportions (Raubenheimer *et al.* 2007). The elucidation of consistent predation of nutritionally balanced prey together with imbalanced prey is thus possibly evidential of nutrient-specific foraging, indicative of a short-term redressal of concentrated macronutrient intake. The limitations of this approach are, however, noteworthy, and ecological interpretations of co-occurrence data must be considered cautiously (Blanchet *et al.* 2020). Whilst nutrient-specific foraging could explain the co-occurrence of taxa and tropho-species, other feasible explanations include coincidental seasonal abundance, spatial or ecological overlap, and even secondary predation. That tropho-species range from containing one taxon to being taxonomically diverse, determined entirely independently of their abundance in the prey community or diet, also provides a problem in the potentially vast over-representation of some tropho-species, and the ecological overlap between others. Whilst these considerations hamper the reliability of co-occurrence analysis as standalone evidence in favour of nutrient-specific foraging, it does not entirely detract from their contribution to a multi-faceted evidence base including alternative forms of analysis.

5.5.4. Nutrient-based prey choice in spider populations

Tropho-species prey choice models highlighted the selective foraging by spiders for each macronutrient from different prey, with the source of these nutrients differing between spiders based on taxonomy, sex and life stage. Overall, greater inter-group distinctions are immediately identifiable in the tropho-species prey choice models than in the taxonomic prey choice models, but key areas of overlap still exist. Several tropho-species were almost

universally predated at a significantly different rate to that expected from the null models. Nutritionally balanced C4L4P4 was predated significantly more than expected by all life stages, sexes and genera except for *Microlinyphia* and *Pardosa*, as was carbohydrate-poor C1L5P3; however, the only preference consistent across all spider groups was the predation of C6L4P3 significantly less than expected. This avoidance of C6L4P3 may simply be a result of the overwhelmingly large abundance of this tropho-species in prey community samples (mostly as thrips, which were the most widely predated family overall; Chapter 4.4). Tropho-species C2L2P6 was also significantly avoided by all but *Erigone* and *Bathyphantes*; interestingly, this is the tropho-species containing linyphiids, which would theoretically contain the ideal macronutrient contents to sustain their confamilials and even *Pardosa*. Importantly, the lack of conspecific predation data available via metabarcoding may have resulted in the underrepresentation of these interactions. Equally, risk cannot be ignored in such circumstances; spiders may avoid such risky conflicts except when territoriality and reproduction are concerned, given the uncertainty of outcome in such a balanced encounter. The broad commonality in preference by spiders suggests a universal requirement for all three macronutrients.

Spider genera exhibited large distinctions in their tropho-species preferences, but with several commonalities. The only stark inter-generic contrasts could be explained by differences in the foraging ecology of the predator, with most differences existing between the linyphiids and *Pardosa*, which have a particularly distinct ecology from the linyphiids (e.g. foraging strategy, activity levels, lack of web building), or *Microlinyphia* which exhibit a far greater web plasticity than their confamilials (Chapter 4.4). That *Microlinyphia* and *Pardosa* are more similar to one another than *Microlinyphia* to the other linyphiids is, however, curious. Given the extreme difference in position in the crop between males and female of *Microlinyphia* found in the field, this could suggest a more mobile ecology (first discussed in Chapter 4.5) closer to that of *Pardosa* than the other largely web-restricted linyphiids. Many of the tropho-species preferences of the different genera highlight preferences for extremes of at least one macronutrient (i.e. predation of tropho-species from both extremes of a macronutrient). Distinctions in the macronutrient preferences of each genus may depend on the individual ecologies and morphologies of that taxon, but most preferences ultimately balance one another out, resulting in a net balance of nutritional intake.

Adults and juveniles had a large degree of overlap in their prey preferences, with adults exhibiting significant preference and avoidance of everything which juveniles did, but with several key addendums. Adults exhibited additional preference for two carbohydrate-rich tropho-species whilst juveniles expressed no preference for carbohydrate-rich prey at all. Similarly, adults also exhibit avoidance of a carbohydrate-poor tropho-species and,

separately, a protein-rich tropho-species. This could indicate that adults have a greater requirement for carbohydrate, possibly due to the energetic requirements involved in reproduction. The avoidance of protein-rich prey could suggest a reduced requirement for protein in adult spiders given their later stage of development, adults instead favouring carbohydrates as an efficient energy resource for reproduction and foraging. This is consistent with the finding of Al Shareefi and Cotter (2019) that juvenile carnivores exhibit nutritional preferences more similar to those of herbivores in that protein-rich resources are preferred, indicative of a developmental requirement for protein.

Both female and male spiders exhibit preferences for carbohydrate-rich prey, but from different tropho-species (females preferring C10L2P2 and males C8L3P2), indicating a sex-based differentiation in carbohydrate resource. Females do, however, perhaps redress their carbohydrate rich dietary intake through preference for carbohydrate-poor C1L4P4, further exemplifying a potential sex-based niche separation. The distinction in preferences between female and male spiders could be an artefact of greater itinerance in males (Foelix 2011), distinctions in web morphology (Sunderland *et al.* 1986), behavioural differences particularly concerning reproduction, or the greater size of females and thus their greater capacity for subduing larger prey.

5.5.5. A synthesis of individual- and population-level nutrient-specific foraging

The null model data represent a pseudo-population-level analysis of nutrient-specific foraging (i.e. individuals are merged into groups, thus no longer representing variation on an individual scale), complementing the co-occurrence data, which better represent commonalities in individual dietary intake. Together, these analyses suggest that many spiders individually forage for a balanced nutrient intake (most exhibiting a focused predation of nutritionally balanced prey when also predating nutrient-rich prey) whereas, at the population level, spiders will flexibly forage for all three macronutrients from different sources depending on life stage, sex and genus. Importantly, nutrient-specific foraging would suggest that predators forage dynamically to redress recent nutritional imbalances, thus imposing a sequential series of choices. Whilst this cannot be observed from these data, the co-occurrence and prey choice analyses together indicate a short-term predation of nutritionally complimentary prey, and the long-term foraging for nutritional diversification, which is indicative of exactly this phenomenon. These results nonetheless suggest an overall importance of all three macronutrients in the selection of prey by spiders, with different macronutrients impacting foraging preferences in different life stages, sexes and genera. Nevertheless, the combined

co-occurrence and prey choice model results are evidence of nutrient-specific foraging occurring in the field.

Many studies have cited a lipid bias in predators (Margalida 2008; Wilder *et al.* 2013; Wilder *et al.* 2016; Al Shareefi and Cotter 2019), or have demonstrated that the balance of protein and lipid is particularly important in prey choice and nutrient assimilation (Prabhu and Taylor 2008; Mayntz *et al.* 2009; Schmidt *et al.* 2012; Vaudo *et al.* 2016; Denuncio *et al.* 2017; Toft *et al.* 2019; Gomez Diaz *et al.* 2020). In fact, extreme protein richness is thought to have negative consequences for predators (Anderson *et al.* 2020), although some will over-feed on protein-rich prey to obtain sufficient lipid provision (Jensen *et al.* 2011). This lipid bias is thought to differ between carnivores, and herbivores and omnivores, with the latter two preferentially targeting protein-rich resources, and the former more concertedly seeking lipids, possibly due to a lipid limitation in higher trophic levels (Goeriz Pearson *et al.* 2011; Wilder *et al.* 2013; Al Shareefi and Cotter 2019; Christensen *et al.* 2020) or to buffer against future starvation since lipids act as long-term energy stores (Jensen *et al.* 2010). For this reason, many studies of nutritional ecology in carnivores neglect carbohydrates, favouring measurement of just lipids and protein (e.g. Mayntz *et al.* 2005; Jensen *et al.* 2012; discussed by Wilder *et al.* 2016; Christensen *et al.* 2020). Nutrient preferences in carnivores are, however, thought to change between life stages; for example, juveniles of one carnivorous beetle species, *Nicrophorus vespilloides*, exhibit a greater propensity toward protein-rich resources and encounter negative repercussions when fed protein-poor diets (Al Shareefi and Cotter 2019). This developmental difference in nutrient preference may be a distinction most prevalent in holometabolous insects though, which exhibit unique nutritional needs throughout the stages of metamorphosis (Al Shareefi and Cotter 2019). This study, whilst ratifying some of these existing ideas, also demonstrates in-field preferences for carbohydrate in spiders, contingent upon life stage, sex and genus, highlighting the importance of studying all three macronutrients even when the focal predator is carnivorous, especially when studying different sub-groups of predator.

Whilst carbohydrate preferences were identified in this chapter, protein and lipids arguably featured more heavily in many of the distinct preferences of different spider groups. The perceived importance of lipid and protein in prey choice by spiders likely relates to their larger variation in prey bodies, whereas carbohydrate appears considerably less variable between most tropho-species. That a more carbohydrate-centric foraging preference has been suggested of omnivores and herbivores (Al Shareefi and Cotter 2019; Christensen *et al.* 2020) could support this if carbohydrate concentrations were found to exhibit greater variation in plants. This pattern may, however, vary in other predator taxa and in other study systems. In carbohydrate-poor study systems (e.g. deserts, caves, other systems with scarce

photosynthetic flora) this macronutrient may be a greater limiting factor of survival and thus a greater determinant of trophic interactions through its bottom-up penetrance of the trophic network via plants and, sequentially, herbivores (Peterson *et al.* 2016). Conversely, trophic networks containing many carbohydrate-rich prey, such as pollinators which interact regularly with carbohydrate-rich nectar resources, could also hold carbohydrate as a greater determinant of interaction. This might result from a possible avoidance of large quantities of carbohydrate-rich prey, given the potential toxicity of disproportionately high carbohydrate concentrations (Schlotterer *et al.* 2009; Alcántar-Fernández *et al.* 2019). Omnivores and herbivores, for example, are thought to have a more carbohydrate-focused foraging preference, harvestmen being one example within arachnids, despite being widely considered carnivores (Christensen *et al.* 2020).

5.5.6. Limitations

The data analysed are, as in Chapters 3 and 4, constrained by the technical limitations of metabarcoding; these data are difficult, if not impossible, to interpret on an individual scale as quantitative results (Murray *et al.* 2011; Thomas *et al.* 2016; Deagle *et al.* 2013; Piñol *et al.* 2018), do not ascertain the life stage or sex of the prey consumed (which will affect macronutrient content, particularly in holometabolous insects; Nestel *et al.* 2016) and do not disentangle ecologically-distinct trophic processes such as scavenging, secondary predation and symbiosis (Greenstone *et al.* 2007; Paula *et al.* 2015), all of which have important implications for nutrient-specific foraging. Attempts are often made to calculate semi-quantitative values from metabarcoding data using read counts (Deagle *et al.* 2013; Thomas *et al.* 2016; Deagle *et al.* 2019), but the many technical biases inherent to metabarcoding, particularly at the PCR stage (Murray *et al.* 2011; Leray and Knowlton 2017; Piñol *et al.* 2018), alongside differential tissue DNA density (Veltri *et al.* 1990), render the accurate estimation of consumed biomass from read counts contentious at best, but more likely impossible. Importantly, these data are at least quantitative at the population level, presenting a frequency of predation events within the population.

The equation of biomass to the number of individuals predated in a fluid-feeding predator such as a spider, which will often leave prey partially consumed, or even superfluously killed (Riechert and Lockley 1984; Sunderland 1999), would also present a further complication to the quantification of these data. This has direct implications for nutrient-specific foraging, which can occur by the consumption of different quantities of different prey or the extraction of specific nutrients from prey (Kohl *et al.* 2015; Pekár *et al.* 2010). Vertebrate predators, for example, have been observed consuming specific organs such as the liver, thought to relate

to tissue-specific macronutrient content (Kohl *et al.* 2015). Even invertebrate predators have been observed feeding on specific body parts with different macronutrient contents (Pekár *et al.* 2010), although this could instead relate to avoidance of toxic compounds or undesirable nutrients present in the neglected body parts; for example, fire ants *Solenopsis invicta* avoid lipid-rich cricket ovaries when fed supplementary carbohydrate (Wilder and Eubanks 2010). The ecology of the predator may similarly factor into this tissue-specific feeding, with Mayntz *et al.* (2005) observing low mobility predators exhibiting this behaviour, whilst active hunters instead redressed nutritional deficiencies through different prey. Regardless, such circumstances are impossible to identify from these metabarcoding data, leaving only broader-scale evidence for the analysis of nutrient-specific foraging in this case.

Regardless of generality, many universal PCR primers will not detect target taxa if their DNA is present in low concentrations, such as if they were consumed some time ago, or in small quantities (Murray *et al.* 2011). The detectability of prey in the guts of predators using their DNA is thus skewed by degradation of this DNA, effectively creating a temporal gradient, thus favouring detection of recently consumed prey DNA. Semi-quantitative predation rates can be calculated from metabarcoding data based on this, using pre-determined DNA digestion rates (Egeter *et al.* 2015; Welch *et al.* 2014), but the highly variable, although typically slower, metabolism of fluid-feeding predators such as spiders (Greenstone *et al.* 2007) would render these results highly inaccurate, particularly in extremely variable field conditions. The data presented do, however, allow fundamental inferences to be drawn regarding the choice of prey from those available to each predator, and the co-occurrence of prey within the diets of individual spiders.

The invertebrate surveying was taxonomically limited due to the poor condition of many specimens and the obscurity of the morphological features necessary for confident identification (notably some springtails, Ichneumonoidea and mites), which could obscure some finer-scale differences in macronutrient content. Even those taxa identified from the community samples did not necessarily have their macronutrient contents determined since many scarcer taxa did not have any individual representatives in suitable condition for macronutrient determination (e.g. large morphological features missing, visual degradation of tissues or unremovable contaminants). The results generated do, however, represent most of those taxa identified from the community samples and, by convergence, those present in the diet of these spiders. The macronutrient contents are consistent with expectations from the literature (Finke 2005; Bryer *et al.* 2015), highlighting the success of this further micro-scaling of the MEDI protocol.

The lack of consideration of micronutrients, despite their biological importance (Jing *et al.* 2014), could obscure some of the nutritional motives underlying certain trophic relationships.

Specific amino acids, for example, are sought by spiders through prey choice (Greenstone 1979). The difficulty associated with detecting and quantifying micronutrients in such small invertebrates is, however, noteworthy, and the coarser analysis of the implications of macronutrients on prey choice was the primary goal of this study. This does not preclude future studies accounting for micronutrients in expansions of this work. Indeed, such values could be incorporated into tropho-species clustering, even alongside morphological or ecological traits. By expanding on the tropho-species concept, increasingly complex prey choice models can be constructed which account for increasingly complex inter-taxonomic variation to address fundamental ecological questions.

Attempts were made via *ex situ* prey choice experiments to replicate the *in situ* results, but to no avail. Whilst these experiments could not confirm the evidence toward nutrient-specific foraging, they were importantly hampered by several key flaws, most notably an oversimplification of the prey community available to the spiders. Undoubtedly, ecology and behaviour will factor into prey choice, with predators having evolved to obtain an optimal nutritional intake from the prey available in their ecosystem by exploiting the behaviour and ecology of those prey; an unfamiliar nutritional landscape, such as in artificial microcosm experiments, will be evolutionarily mismatched to the predator's innate nutrient-specific foraging behaviours (Simpson and Raubenheimer 2005; Al Shareefi and Cotter 2019).

These *ex situ* experiments provided spiders with three prey of distinct ecological and behavioural niches: one larger flying prey (*Drosophila*), one prey with effective escape behaviours (*Isotoma*) and one relatively stationary prey with defensive kicking behaviour (*Metopolophium*; Villagra *et al.* 2002). That these prey avoid predation by different means inevitably differentially modulates the ability of the predator to subdue them, immediately biasing the results in a choice chamber experiment. Similarly, given the sit-and-wait behaviour of linyphiids, the substantially different activity density of the prey, particularly between *Metopolophium* and *Drosophila*, will undoubtedly affect their rate of capture. To correct for this, optimal *ex situ* choice experiments could present standardised signals associated with prey familiar to the predator without presenting the prey itself. For insects, semiochemical cues form the basis of many successful choice assays (Dicke 1988; Delury *et al.* 1999; Bhasin *et al.* 2000), and even spiders have been successfully assayed using such techniques (Allan *et al.* 1996; Jackson *et al.* 2002), but the greater dependence by spiders on vibrational cues for prey detection and location (Turnbull 1973; Molina *et al.* 2009; Virant-Doberlet *et al.* 2011) could warrant the use of vibrometers in such experiments in the future. If spiders were first familiarised with the vibrational cues of their prey, and the nutrient-content each comprises, effective "contactless" assays could be conducted, removing several key sources of bias that affected this and other *ex situ* studies of spider prey choice.

5.5.7. Further implications of nutrient-specific foraging

The evidence toward nutrient-specific foraging occurring in the field identified through this thesis supports this long-standing ecological theory. The exhibition of complementary macronutrient preferences across all spiders, but slight variations between genera, life stages and sexes, indicates that nutrient-specific foraging is not unique to one taxon, nor to specific developmental or physiological states, inferring greater generality and robustness of these findings. Similarly, the preference by different spider groups for different tropho-species with similar nutritional profiles indicates that nutrient-specific foraging can be enacted across the whole population by redressing nutritional needs via prey appropriate to each specific spider's ecology. Nutrient-specific foraging thus likely occurs across all individuals year-round, even when prey diversity and abundance is lower; this is ratified by the shifting prey preferences following harvest identified in Chapter 3 and confirmed by studies such as that of Whitney *et al.* (2018).

Body size may be a significant factor in the difference in nutrient-specific foraging behaviours between juveniles and adults since it is known to affect prey choice (Eitzinger *et al.* 2018), This is, however, somewhat self-fulfilling since body size is potentially determined, at least in adult insects, by juvenile (i.e. larval) nutrition, with adult diet predominantly instead affecting body composition in holometabolous insects (Poças *et al.* 2019); thus, well-supplemented larvae may continue to be better nutritionally provisioned after maturity. A similar phenomenon is observed in spiders, which develop larger cephalothoraxes when fed mixed diets (Bilde and Toft 2001). This indicates that balanced juvenile nutrition may be pivotal to the reproductive success and proliferation of animals, or at least holometabolous insects. Based on this, the predominant predation of herbivores such as aphids, which are known to contain toxins and comprise a low nutritional value (Bilde and Toft 2001; White *et al.* 2017), by the juvenile spiders in this thesis could have negative consequences for overall spider population health, in both juveniles and adults. This high rate of consumption is, however, justified by the observed preference for aphids in prey choice models which implies some nullification of the negative consequences of aphid predation in juveniles, perhaps due to aphids better matching their nutritional requirements. Since development of defences such as toxins can be driven by intensive predation of a given species (Sugiura 2020), dietary diversification by nutrient-specific foraging could benefit predator populations not only by broadening their dietary niche (thus decreasing dependence on smaller subsets of the local community), but also by reducing selective pressure on those prey, decreasing the likelihood of the development of defences against specific predators.

The differential digestibility of prey with different macronutrient contents is an important factor in nutrient-specific foraging (Gomez Diaz *et al.* 2020); if prey of a given macronutrient content are less digestible, does that non-linearly affect their attractiveness? If this is the case, observations of nutrient-specific foraging could be skewed by predators avoiding or preferring prey independent of their nutritional content, despite effectively choosing based on nutritional value otherwise. This distinction between nutritional content and value extends also to prey containing toxins, such as the aphids discussed above. In these cases, unless alternative prey with similar nutritional profiles are available, a more passive nutrient-independent selection of prey could be expected to occur (Rendon *et al.* 2019), more concertedly led by factors such as predator hunger state (Lang and Gsodl 2001), or prey abundance, dispersal (Pastorok 1981), camouflage (Endler 1978), defences (Provost *et al.* 2006), escape capability (Lang and Gsodl 2001; Provost *et al.* 2006) or size (Bence and Murdoch 1986; Downes 2002; Turesson *et al.* 2002); importantly, these factors could profoundly affect foraging even when nutrient-specific foraging is a predator's primary determinant of choice. This non-nutrient-based choice will inevitably create noisy signals in analyses of nutrient-specific foraging but could ultimately be accounted for through expansion of the tropho-species concept to incorporate these other determinants of choice into the clustering process. That nutrient-specific foraging is detected despite these alternative factors indicates its importance in shaping trophic interactions.

5.5.8. Summary

Spiders exhibited non-density-dependent prey choice and their prey significantly differed in macronutrient content. Evidence for nutrient-specific foraging was elucidated in the field using metabarcoding-based dietary analysis with hierarchical aggregations of prey taxa by their nutritional values, termed tropho-species, determined via a microscale macronutrient protocol. This evidence for nutrient-specific foraging is centred around the balanced intake of all three macronutrients by spiders, with slight variations in tropho-species preferences between spider groups. Spiders predate prey of imbalanced macronutrient content alongside nutritionally balanced prey, possibly to maintain a net nutrient balance, but the overall spider population predates prey rich in all three macronutrients, although the prey rich with each macronutrient differ between spider genera, life stages and sexes.

With evidence for nutrient-specific foraging identified, the fundamental remaining question is how this can be applied for the benefit of agriculture and ecology more broadly. Prey preferences can be modulated by altering the abundance of available prey. This can be achieved not only by inundative inoculation of field sites with a given prey taxon or tropho-species, but also (and arguably more sustainably) by manipulating field conditions and habitat

structure to optimally benefit the desired taxon or tropho-species (Agustí *et al.* 2003; Bell, Traugott, *et al.* 2008; Michalko *et al.* 2017). Additional compost applications, for example, increase the long-term density of alternative collembolan prey for spiders, which has been shown to increase predation of aphids (Agustí *et al.* 2003; Shayler 2005). Given the co-occurrence of nutritionally balanced tropho-species in the diets of spiders, and an overall preference for them across most groups, increasing the abundance of these tropho-species could increase predation pressure on those taxa with more variable nutrient content, and *vice versa*. Regardless, this initial evidence for nutrient-specific foraging in the field may facilitate a greater contextual understanding of trophic interactions across the entire breadth of trophic ecology. By providing evidence toward nutrient-specific foraging, this chapter has addressed the fundamental aim of this thesis.

A Synthesis of Investigations into Nutrient-specific Foraging and the Role of Spiders as Biocontrol Agents

“Somehow the killing of a giant spider, all alone by himself in the dark without the help of a wizard or the dwarves or anyone else, made a great difference to Mr. Baggins. He felt a different person, and much fiercer and bolder in spite of an empty stomach as he wiped his sword on the grass and put it back into its sheath.”

- J.R.R. Tolkien – *The Hobbit*

Chapter 6 : A synthesis of investigations into nutrient-specific foraging and the role of spiders as biocontrol agents

6.1. Overview

This thesis, through the synergistic application of novel biochemical protocols, molecular workflows and prey choice analysis, has identified in-field evidence of nutrient-specific foraging, thus satisfying the primary aim established in Chapter 1.6.2. This aim was linked across four data chapters to address each of the six hypotheses:

Chapter 2:

i. *Macronutrients can be extracted and quantified from a single small invertebrate.*

Through MEDI, macronutrients were successfully extracted from a single invertebrate specimen and measured in parallel. In Chapter 2, the precision of this protocol was sufficient to detect and differentiate between taxa as small as springtails and aphids, but this was extended to incorporate even smaller wasps, flies and soil fauna in Chapter 5 through minor methodological alterations. The demonstrable efficacy of MEDI was fundamental to the completion of this thesis, but also bears relevance to the broader field of nutritional ecology.

Chapter 3:

ii. *PCR primers can be designed for the broad metabarcoding of spider prey whilst reducing amplification of undegraded spider DNA.*

The PCR primers designed in Chapter 3 successfully detected a broad dietary range in the guts of spiders, and the two-pronged approach of using both general and predator-exclusion primers together efficiently accounted for the loss of depth and breadth associated with each method individually. Through further refinement in Chapter 4, the spider-exclusion primers displayed a greater selectivity for spider prey by avoiding amplification of a broader range of spiders, providing a robust dataset for the analyses in subsequent chapters.

Chapter 4:

iii. *Spider diet will vary between genera and with time.*

The identification of temporal and taxonomic distinctions in the diets of the spiders studied in Chapter 4 confirmed the importance of these factors in determining not only dietary variation, but also potential biocontrol efficacy. This was ratified by separate analyses of the predation

of pests and predators. In Chapter 5, taxonomic distinctions in prey choice were identified, inferring that the dietary differences reported in Chapter 4 do in fact reflect differing prey preferences between taxa.

Chapter 5:

iv. Spider prey choice is not based solely on the relative density of their prey.

Whilst preliminarily presented in Chapter 3, Chapter 5 evidenced non-density-dependent prey choice on a large scale, with different preferences shown by spiders of different genera, life stages and sexes. These prey preferences were shown in the context of both taxonomic prey preferences and macronutrient-based tropho-species preferences.

v. Taxonomically close invertebrates will have similar macronutrient contents, whilst distant taxa will be dissimilar, allowing taxonomy-based generalisation of macronutrient contents.

In Chapter 5, taxonomic distinctions in macronutrient content were confirmed, and taxa subsequently clustered based on their nutrient contents. These clusters, termed tropho-species, did aggregate some closely related species, but also included several clusters that were polyphyletic regarding their 'Linnean' taxonomy, indicating a convergent evolution of macronutrient content. A taxonomic trend for macronutrient content is thus apparent, but a cluster-based approach, such as that of the tropho-species concept, facilitates further simplification of nutrient content for ecological analyses.

vi. Nutrient-specific foraging occurs in the field.

Nutrient-specific foraging was investigated in Chapter 5 through both taxonomy- and tropho-species-based approaches. The co-occurrence of prey rich in certain nutrients with nutritionally balanced prey is indicative of the balancing of nutrient intake by selective foraging. Prey choice analysis highlighted the predation of a broad range of prey differentially rich in all three macronutrients, but the prey which provided these macronutrients taxonomically differed depending on the foraging spider's genus, life stage and sex. These data are consistent with a propensity for nutrient-specific foraging in spiders in the field.

From these key findings, many implications arise, spanning the methodological implications of the developed protocols and workflows, the ecological implications of the key experimental findings, and the management implications of these ecological outcomes for agriculture and broader ecology.

6.2. Methodological implications

The initial focus of this thesis inevitably regarded the development of protocols, PCR primers and workflows to be ultimately applied to the investigation of nutrient-specific foraging in the field. In producing these methodological advancements, this thesis has already contributed a tangible benefit to the scientific community, with these techniques already adopted for a broad range of research topics.

6.2.1. Implications for macronutrient determination

Since the determination of macronutrients has often depended on analogues (Finke 2005; Pekár and Mayntz 2014; Bryer *et al.* 2015), correction factors (Janssen *et al.* 2017) and bulk sampling (Finke 2013; Bryer *et al.* 2015), MEDI presents a timely advance in the field of nutritional analysis. Since its development, MEDI has been used in different research groups for macronutrient-based investigations into stream population dynamics, parasitism, taxonomy and life history, with broader applications planned and many more discussed. The accessibility (regarding both ease-of-use and cost) of MEDI could allow its widespread adoption as a much-needed standard protocol in nutritional analysis, unlocking the potential for new applications in trophic ecology.

The adaptability of the protocol, exemplified in Chapter 5 through its further micro-scaling, is of particular utility. Micronutrients, as discussed in both Chapters 2 and 5, are of biological importance (Jing *et al.* 2014) and have been implicated in foraging choices (Greenstone 1979). Their incorporation into MEDI should be considered if relevant applications arise in the future, although compromise over which to include will be necessary unless solvent volumes are increased, given a paucity of material remaining after following the current protocol. Similarly, salt content can be a determinant of foraging behaviour (Simpson *et al.* 2006), with sodium more generally having been referred to as the "seventh macronutrient" (following elemental carbon, hydrogen, oxygen, nitrogen, phosphorous and potassium; Kaspari 2020). Incorporating this wider set of potential prey choice determinants into MEDI should be considered for future studies.

Equally, simplifications of MEDI are worth considering in some circumstances. Given the supposedly dominant importance of lipids and proteins in many trophic interactions, particularly those involving carnivores (Prabhu and Taylor 2008; Mayntz *et al.* 2009; Schmidt *et al.* 2012; Vaudo *et al.* 2016; Denuncio *et al.* 2017; Toft *et al.* 2019; Gomez Diaz *et al.* 2020), the protocol can be simplified to focus solely on these two macronutrients, forgoing

carbohydrate determination as many other studies have (e.g. Mayntz *et al.* 2005; Jensen *et al.* 2012; Rendon *et al.* 2019). Carbohydrates are, however, important in many trophic interactions, even in predators, as evidenced in Chapter 5.4. A three-dimensional (i.e. carbohydrate, lipid and protein) approach to macronutrient content should thus be applied where possible, especially in instances where there is no prior knowledge of the predator's nutritional preferences (Wilder *et al.* 2016; Christensen *et al.* 2020). Chapter 5 of this thesis did, in fact, simplify MEDI itself by neglecting exoskeletal measurement despite its inclusion for some specimens in Chapter 2. Exoskeletal mass is undoubtedly an important feature of trophic interactions as a determinant of body mass to macronutrient mass ratio and as an alternative source of nutrition for some animals. The extremely small mass of chitin present in most of the focal organisms of this thesis due to their small overall body mass, which already provided a challenge for measurement, precluded its measurement on this occasion. Where possible through sufficiently sensitive scales, such measurements are, however, recommended for future studies.

6.2.2. Implications for dietary and prey choice analysis

The PCR primers developed in Chapter 3 (and refined in Chapter 4) successfully facilitated a two-pronged approach to the dietary analysis of linyphiids. By using complementary general and predator-exclusion primers, depth and breadth of amplification were separately achieved, detecting a greater diversity of prey than would have been possible with just one of the two primer pairs. The spider-exclusion primers, TelperionF-LaurelinR (adapted to TelperionF-LaureR in Chapter 4), are the first PCR primers designed for the analysis of linyphiid diet, but their specificity indicates their applicability to a range of predators even including examples beyond spiders, such as ants. Alongside NoSpi2, which was designed to be paired with reverse primer BR2 for wolf spider dietary analysis (Lafage *et al.* 2019), these two primers provide complementary coverage of many spider taxa, providing trophic ecologists the toolkit required to analyse the diet of a large range of spiders.

The general primers BerenF-LuthienR amplified a very broad range of potential prey despite their design focusing on insects, arachnids and soil fauna, ultimately amplifying almost everything they were tested against, including marine vertebrates. For this reason, other research groups have begun to use these primers for the analysis of diet in other animals including bats, wasps and endemic island reptiles. BerenF-LuthienR have reportedly shown great promise with decades-old museum specimens (pers. comms. Beth Clare), highlighting their efficient amplification of highly degraded DNA. Other highly efficient general primers

have, however, emerged throughout the course of this project, including BF3-BR2 (Elbrecht and Leese 2017; Braukmann *et al.* 2019), which may be more applicable to many studies, particularly those concerning freshwater systems. At its inception, each metabarcoding-based dietary study should assess the PCR primers available and thoroughly test, both *in silico* and *in vitro*, the efficacy of those primers in amplifying the target taxa for that study, designing novel primers only as a last resort given the wealth of options now available.

The analysis of prey choice using prey availability and dietary data, whilst a great advancement on studying diet alone, and the first of its kind for spiders in the field presented in Chapter 3 (the second in Chapter 5), inevitably neglects the complexities of species interactions. Simple spatial co-occurrence of predator and prey does not infer the plausibility of their interaction (Blanchet *et al.* 2020), with many factors such as diel synchronicity (Welch and Harwood 2014), size (Eitzinger *et al.* 2018), and motility (Tercel *et al.* 2018) differing profoundly between taxa, thus affecting the likelihood of both encounter and successful capture of prey. Numerical weightings can be applied to the prey choice null models used in this study to account for some of these potential sources of bias (Vaughan *et al.* 2018), but careful pre-sampling consideration should be spent on how best to collect the relevant data and how their effect on prey choice might be best represented numerically. The most important consideration in modelling prey choice based on prey abundance data is, however, the survey method used to ascertain those prey abundance data. In this thesis, vacuum sampling was identified as the most appropriate survey method for representing the often soil-based dietary components of cereal crop spiders, but this should be assessed on a case-by-case basis for each study that these methods are applied to, and additional methods trialled or perhaps even combined where appropriate.

The tropho-species concept formulated in Chapter 5 successfully and simplistically integrated nutritional data into the cooccurrence models of ‘cooccur’ (Griffith *et al.* 2016) and the prey choice null models of econullnetr (Vaughan *et al.* 2018), facilitating analyses of nutrient-specific foraging in the field. As discussed in Chapter 5, the inclusion of additional factors in the tropho-species clustering process is recommended for future studies that assess other aspects of prey choice, either in addition to macronutrient content or entirely apart from it. The tropho-species concept could, for example, incorporate body size information, or focus entirely on the prevalence of toxins in different prey groups. Through the analyses described in Chapter 5, increasingly complex and ambitious studies of prey choice could be carried out, pushing forward the frontiers of trophic ecology.

6.3. Ecological implications

The fundamental aim of this thesis – investigating nutrient-specific foraging – produced results of ecological significance, both directly and indirectly arising from this central hypothesis.

6.3.1. Spider generalism and omnivory

Spiders are consistently referred to as generalists throughout this thesis, but this term is considered contentious by some ecologists (Loxdale and Harvey 2016). Although monophagous spiders are known (Petráková *et al.* 2015), even by conservative measures, most spiders arguably fit the description of a "broad generalist", feeding on a large taxonomic range of prey including other arachnids, insects, springtails and many other groups. Their trophic niche is, however, undoubtedly restricted based on prey size, and spatial and diel co-occurrences, inferring some degree of "specialism"; however, if this were to define a forager as "specialist", are all foragers not specialists? This then denigrates the term "generalist" to redundancy. The lexical value of designations to coarsely describe the trophic breadth of a forager is profound in terms of semantic efficiency, immediacy and accessibility, although these should always be framed in the context of feasible interactions, in which these spiders can surely be considered generalist predators.

The status of generalists and specialists does, however, infer some important nutritional considerations. Specialists more efficiently forage for their prey, affording them the capacity to predate proportionally larger prey (Garcia *et al.* 2018). They may also be better able to extract optimal nutrition from their prey than generalists given their physiological and behavioural adaptation to those prey; this is evidenced by the reduced need for compensatory feeding when a specialist's host is nutritionally suboptimal (Despland and Noseworthy 2006). Generalists are instead thought to predate a greater quantity of prey to redress nutritional needs (Pompozzi *et al.* 2019; Rendon *et al.* 2019), as demonstrated in the results of this thesis. The extent of generalism may, however, be obscured by the lack of information regarding another potential dietary resource: plants.

The carnivorous nature of spiders and other arachnids is increasingly debated, some studies suggesting that many are in fact omnivores, and even examples of herbivory discovered (Nyffeler *et al.* 2015; Benhadi-Marín *et al.* 2019; Christensen *et al.* 2020). Otherwise perceivably carnivorous spiders have been observed exploiting resources such as extra-floral nectaries, aphid honeydew and other plant-based carbohydrate-rich reservoirs (Peterson *et al.* 2010; Nahas and Gonzaga 2017; Benhadi-Marín *et al.* 2019). This has broad ecological implications, particularly regarding foraging choices. In this study, carbohydrate-rich prey were

often preferentially predated, particularly by adult spiders, but this may have been further supplemented by plant-derived resources undetected by the PCR primers employed, possibly as a relatively risk-free resource for juvenile spiders which preferentially predated fewer carbohydrate-rich prey. Equally, web-building spiders may ingest sugars caught on their silken webs when interacting with them. That Chen *et al.* (2010) identified fructose in 18.7% of spiders tested suggests that, whether accidental ingestion, secondary predation or intentional feeding, spiders are acquiring plant-based carbohydrate resources, and with unforeseen regularity.

Indeed, “carnivores” have been observed redressing nutritional deficits incurred by suboptimal prey through omnivory (Ugine *et al.* 2019). Foraging outcomes following extra-floral nectary exploitation do, however, vary depending on the nutritional composition of the nectary resource (Wilder and Eubanks 2010; Wilder *et al.* 2011), indicating a dynamic role of these resources in prey choice. The extent of plant resource exploitation was not determined in this thesis though. Future studies of spider dietary dynamics should consider investigating this, but with caution regarding possible secondary predation by inclusion of plant DNA in the guts of consumed herbivores (Sheppard *et al.* 2005). The determination of macronutrient content in these resources would, however, prove difficult, particularly for resources such as aphid honeydew which, whilst containing high concentrations of sugar (Craig 1960), will surely be highly variable and degrade rapidly once expelled. This omnivory in spiders does ultimately provoke questions similar to those regarding generalism: if a given “carnivore” exploits plant-based resources, at what point do we begin to consider them an omnivore? The scaling from herbivore to carnivore is evidently non-binary, but categorisation is important when designing dietary studies (e.g. selecting appropriate PCR primers) and deciding which macronutrients to measure for nutritional ecological studies given the coarse distinction in preferences observed between carnivores and herbivores (Mayntz *et al.* 2009; Wilder *et al.* 2013; Wilder *et al.* 2016). Regardless of potentially undetected omnivory, this study identified nutritional preferences, and thus nutrient-specific foraging, based on animal prey alone, suggesting that predators redress nutritional deficits through predation despite possible further supplementation via plant-based resources.

6.3.2. Nutrition in invasion and migration ecology

Nutrient-specific foraging has important implications for animals entering novel habitats such as ballooning spiders, but also invasive species (Shik and Dussutour 2020). The evolutionary mismatch between the introduced species and its novel situation (i.e. the unfamiliar nutritional

landscape) would theoretically impose fitness consequences on that species (Simpson and Raubenheimer 2005; Al Shareefi and Cotter 2019); its lack of experience with the native resources and their provision, alongside potential ecological mismatches affecting the accessibility of these resources, would require a great degree of adaptability to optimally forage, or at least a flexible physiology. Many invasive species nevertheless exhibit great biological success in novel environments. The extension of this to ballooning spiders, which can travel hundreds of kilometres, even across oceans, to stochastically arrive at nutritionally novel environments (Bell *et al.* 2005; Greenstone 1990; Weyman 1993), also suggests their adaptability to the novel nutritional landscape of unfamiliar landing sites. The nutritional requirement of invasive species, and their realised exploitation of resources of differing nutritional value, is thus of profound academic interest in the discussion surrounding nutrient-specific foraging.

6.4. Management implications

The model system used in this thesis, generalist predators of pests in barley fields, naturally lends to the elucidation of trends relevant to management of arable land for the optimisation of biocontrol. Several key findings provide evidence which could refine management for optimisation of the benefits of conservation biocontrol.

6.4.1. Implications for biocontrol and intraguild predation

This thesis identified several spider groups that exhibit enhanced biocontrol potential, namely *Bathyphantes*, *Tenuiphantes* and juvenile spiders. The dietary results that identified the benefit of these groups were further contextualised through prey choice analyses which indicated that predation of pest species by these groups was often disproportionate to their abundance in the field, confirming a strong potential for biocontrol by these spiders. Predation of aphids by spiders was previously known to vary between taxa, with the subfamily Linyphiinae feeding on more aphids than spiders of Erigoninae (Harwood *et al.* 2004); this is largely ratified by the results of this thesis, despite the possibility of itinerant Erigoninae spiders encountering more aphids whilst actively present on the ground (Harwood *et al.* 2004; Gavish-Regev *et al.* 2009). The disproportionate predation of aphids by juvenile spiders, which are thought to be of low nutritional value and contain toxins (Bilde and Toft 2001; White *et al.* 2017), as discussed above, could indicate that the negative consequences of aphid predation are effaced due to aphids better resembling the nutritional needs of juveniles, with important implications for biocontrol. This juvenile tolerance of aphids would suggest that adults could

be encouraged to feed more on these prey if their nutritional needs were skewed by an imperfect diet that nutritionally complements carbohydrate-rich aphids. Ultimately, predation of aphids can elicit negative, positive or neutral consequences for the predator, dependent on the taxa comprising the remainder of the recent diet (Toft 2005). This highlights the importance of alternative prey in sustaining generalist predator-mediated biocontrol of aphids (Agustí *et al.* 2003; Bell, Traugott, *et al.* 2008). Different alternative prey taxa can, however, differentially affect biocontrol, with some taxa possibly detracting from predation of pest taxa (Symondson *et al.* 2006). A situational understanding of community ecology is thus fundamental to the efficacious management of conservation biocontrol (Chailleux *et al.* 2014).

The identification of spiders for which intraguild predation is lower, similarly to the elucidation of those with a higher propensity for predation of pests, could benefit agriculture. The significantly lower rate of intraguild predation in *Bathyphantes*, when considered in the context of their greater predation of pests, confirms their substantial overall benefit to agricultural productivity, similarly true of juvenile spiders. The detrimental extent of intraguild predation is determined by the predisposition of a given species to not only attack other predator species, but also conspecifics, the ratio of which can vary even between closely-related species, largely based on the coincidence of inter-specific phenologies (Rypstra and Samu 2005). Since this thesis used metabarcoding to analyse diet, which neglects conspecific predation, this potentially important compartment of intraguild predation needs further exploration in the spider groups concerned to fully confirm the relevance of the findings for agricultural management. For example, juveniles are known to cannibalise conspecifics, sometimes at a high rate, during development (Lesne *et al.* 2016), which could nullify the benefit of their relatively low tendency toward intraguild predation.

6.4.2. Implications for habitat and community management

With new UK agricultural policy having come into effect just days before the submission of this thesis that will further incentivise sustainable agricultural practices such as habitat creation for biodiversity and ecosystem service enhancement (Department for Environment Food & Rural Affairs 2020a; Department for Environment Food & Rural Affairs 2020b), concepts such as conservation biocontrol are becoming increasingly pertinent. The evidence for nutrient-specific foraging presented in this thesis, by further ratifying this ecological theory, could facilitate more efficacious management of predators by enhancing our understanding of the mechanisms underlying their foraging behaviour, whilst supporting biodiversity enhancement. In synergy with the novel findings on biocontrol and intraguild predation, this can facilitate the formulation of broad management guidelines for the reduction of intraguild predation and encouragement

of biocontrol. Given the likely year-round occurrence of nutrient-specific foraging (Whitney *et al.* 2018), sustaining consistently nutritionally diverse prey communities is important for the population health of generalist predators, thus beckoning persistent methods for the promotion of natural enemy populations. Among the management actions that can influence trophic interactions, habitat diversification is demonstrably impactful, persistent and relatively easily executed (M. Alderweireldt 1994; Gurr *et al.* 2017; Gontijo 2019).

Habitat diversification increases the abundance of spiders and their alternative prey though provision of a broader range of microhabitats and, in many cases, establishment of permanent refugia; this ultimately enhances biological control of pests (Sunderland and Samu 2000; Gurr *et al.* 2016). This diversification can, however, take many forms, including the establishment of flowering field margins (Mansion-Vaquie *et al.* 2017), beetle banks (MacLeod *et al.* 2004) and intercropping (Trenbath 1993). Habitat diversification via nectar-producing flower margins increases crop yields by up to 5% even with decreases in pesticide use of up to 70%; in these scenarios, predators, parasitoids and detritivores were more abundant, suggesting a greater top-down control of pests via provision of alternative prey (Gurr *et al.* 2016). Similarly, beetle banks, through provision of permanent refugia, sustain significantly larger populations of natural enemies such as ground beetles, even throughout winter (MacLeod *et al.* 2004).

Another method, intercropping, can be achieved by planting rows of alternative species or cultivars between rows of the focal crop (Martin and Snaydon 1982). One of the primary motives behind intercropping is the enhanced nutrient uptake and biomass accumulation of the primary crop if intercropped with a complementary species or cultivar, such as legumes between rows of barley (Pappa *et al.* 2012; Darch *et al.* 2018). This method does, however, also facilitate beneficial outcomes for the suppression of pests by multiple means, including the promotion of natural enemies of crop pests (Trenbath 1993). Through encouragement of natural enemies such as hoverfly larvae, ground beetles and ladybirds, intercropping is thought to reduce populations of aphids and other pests (Butts *et al.* 2003; Wang *et al.* 2009; Seidenglanz *et al.* 2011).

Habitat diversification often provides refuge for predators from the ecological instability of crop harvesting demonstrated in Chapter 3, but also presents suitable habitat for a greater diversity of alternative prey through microhabitat heterogeneity. An increase in the nutritional diversity of available prey naturally grants a greater autonomy to generalist predators to enact nutrient-specific foraging. Given the frequency of inter-spider predation where microhabitats and niches overlap, increased habitat diversity may also partly mitigate intraguild predation, resulting in net gains of spider-mediated biocontrol (Harwood and Obrycki 2005). Shorter term strategies such as application of organic matter to arable fields can elicit similar effects, with

predator abundance increasing alongside detritivorous alternative prey such as springtails following compost application (Agustí *et al.* 2003; Bell, Traugott, *et al.* 2008). The effects of habitat manipulation may not always be immediate though, with benefits such as soil moisture retention and nutrient enrichment typically arriving first, followed by alternative prey abundance and subsequently predator enrichment (Bell, Traugott, *et al.* 2008).

The identification of *Bathyphantes* and *Tenuiphantes* as proficient predators of crop pests also has implications for habitat management. Since these genera typically build larger webs higher in the crop than other spiders such as *Erigone* (Sunderland *et al.* 1986; Chapter 4.4), sufficient crop stem height (>40 mm) could be ensured, possibly through winter cropping, to accommodate their webs early in spring before most pest populations arrive. Inspection of the additional prey preferences exhibited by these genera could also identify alternative prey to encourage through appropriate habitat provision in order to sustain healthy populations of these predators.

6.4.3. Implications for conservation

Alongside agricultural management, the findings of this thesis, through their broader ecological context, bear relevance to other management frameworks, including those of conservation. Predation pressure is a key consideration in the management of species of conservation concern, particularly when the focal species already has a low survival rate (Driesche *et al.* 2010; Goldstein *et al.* 2018). Invertebrate-mediated biocontrol can also be an important tool for the maintenance of habitat structure and the safeguarding of ecosystem services, and against the establishment of invasive species (both plants and animals; Driesche *et al.* 2010). By understanding the mechanisms underlying prey choice, such as nutrient-specific foraging, increasingly robust predictions can be made surrounding the trophic interactions that may threaten or benefit a given conservation focus, and better-informed manipulations of these interactions are more likely to be successful.

During this study, widespread invertebrate declines were reported (Bell *et al.* 2020; Cardoso *et al.* 2020), broadly considered a consequence of climate change and agricultural intensification (Habel *et al.* 2019; Morrison *et al.* 2020). Such declines are already reportedly affecting populations of predators that depend on the declining taxa, including spiders (Nyffeler and Bonte 2020). Importantly, temperature extremes exacerbate the developmental detriments associated with poor nutrition (Lee and Roh 2010), which could lead to more extreme exhibition of nutrient-specific foraging, particularly in immature predators. Nutritional stability may thus be an important management consideration for ecosystem health when

mitigating the ecological effects of rising global temperatures and otherwise changing climates.

6.5. Knowledge gaps arising

Despite the aims of this thesis having been met, this area of research contains an abundance of unanswered questions and theories supported by a dearth of appropriate evidence. Through the course of the individual studies within this thesis, several tangential questions of broader ecological relevance arose, some of which were preliminarily explored experimentally.

6.5.1. How is nutrient-specific foraging physiologically enacted?

In determining the importance of prey nutrient content for predator choices, a critical question remains: how do predators differentiate between prey based on their nutrient contents? Predators may exhibit enhanced proficiency in foraging for prey that they have experienced previously, indicative of a learned association between sensory cues and nutritional provision. This would, however, preclude juvenile or naïve spiders from redressing nutritional deficiencies and would make the spatiotemporal dynamics of many spiders, particularly in their first weeks or following ballooning, nutritionally chaotic. Many animals do indeed have an innate sensory response to certain cues (e.g. habitat cues, pheromones; Webster and Card 2017; Cuff *et al.* 2020) which may factor into the detection of prey and, more specifically, prey nutrients, through slight kairomonal differences.

Within this project, preliminary attempts were made to identify differences in sensory cues from individuals of the same species with different macronutrient contents to ascertain whether nutrient content could be sensorially identified beyond basic inter-specific differentiation. *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) flies were reared in polystyrene *Drosophila* vials (28.5 x 95 mm) on four different growth media: control (composition: 750 ml distilled water, 8.4 g agar powder, 10 ml nipagin (10%), 67 g maize starch, 17.3 g autolysed whole yeast powder, 72 g dextrose powder, 3.5 ml propionic acid (99%)), protein-enriched (50 ml water substituted with 50 g casein power), carbohydrate-enriched (50 ml water substituted with 50 g casein power) and lipid-enriched (50 ml water substituted with 50 g lard). To first ascertain whether these dietary treatments had affected the nutrient content of the flies, an early variation of the MEDI protocol (differing from that described in Chapter 2 in the consistent use of chloroform/methanol solvent without switching to NaOH, and BCA protein assay used rather than Lowry) was used to determine the

macronutrient content of the flies. Fly volatile profiles were determined by thermal desorption gas-chromatography time-of-flight mass-spectrometry (Supplementary Information 6.1), and cuticular hydrocarbons (CHCs) by gas-chromatography mass-spectrometry (Supplementary Information 6.2). Multivariate linear models (MLMs) indicated that flies reared on different diets had significantly different proportional macronutrient contents (MLM: $F_{3,48} = 12.17$, $p = 0.005$; Figure 6.1); specifically differing in their protein (MLM: $F_{3,48} = 3.898$, $p = 0.025$; Figure 6.1) carbohydrate (MLM: $F_{3,48} = 2.738$, $p = 0.049$; Figure 6.1) and lipid (MLM: $F_{3,48} = 5.538$, $p = 0.007$; Figure 6.1) content. Permutational multivariate analysis of variance (PerMANOVA) indicated that volatile profiles were significantly different between flies reared on different diets (PerMANOVA: R^2=0.341, df=2, pseudo-F=1.551, p=0.021; Figure 6.2), as were cuticular hydrocarbons (PerMANOVA: R^2=0.333, df=3, pseudo-F=1.835, p=0.010). Via canonical analysis of principal components (CAP), however, only 66.7% of volatile profiles were correctly classified (p=0.109; Figure 6.2) despite their significant difference between these classifications.

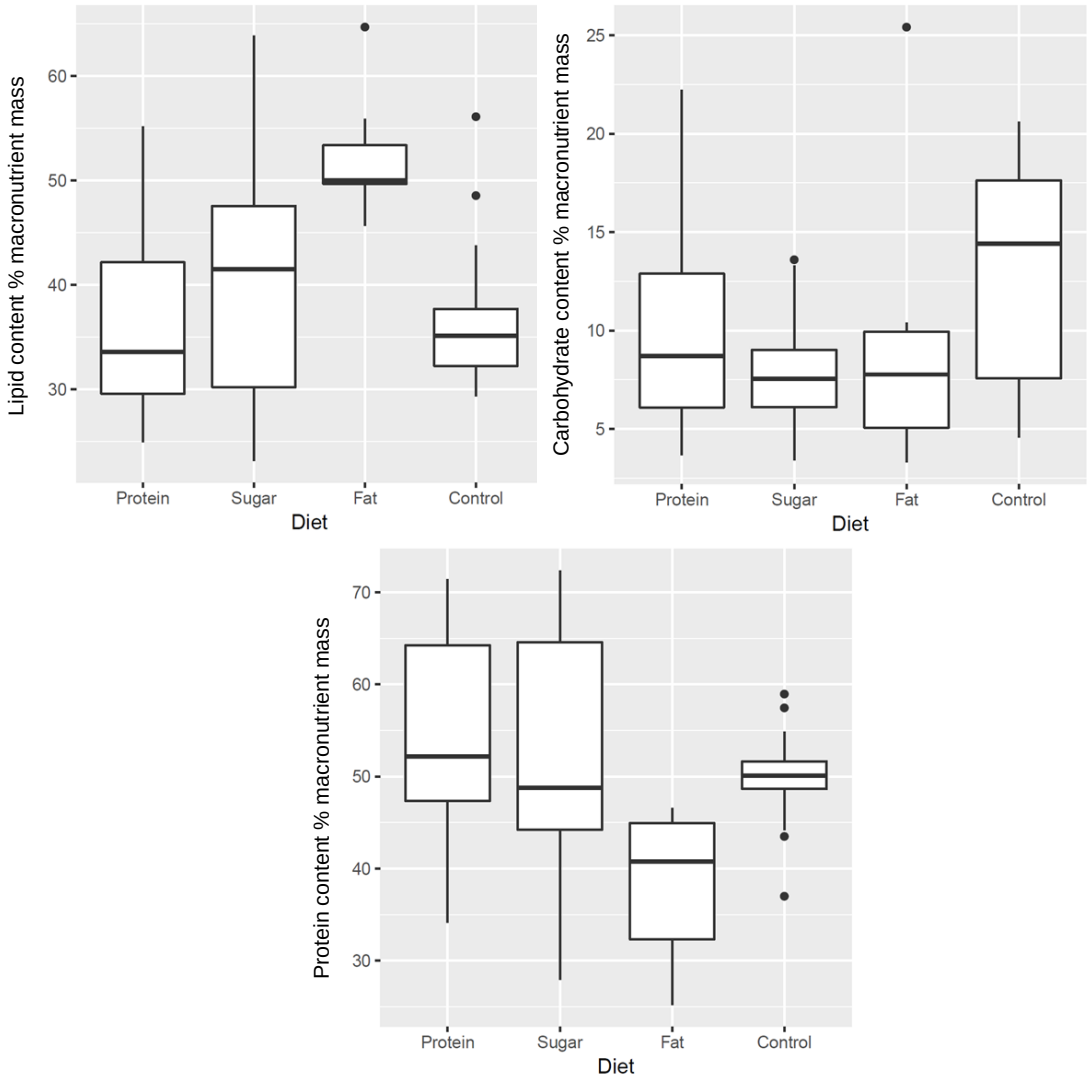

Figure 6.1: Macronutrient content of *Drosophila melanogaster* reared on different diets.

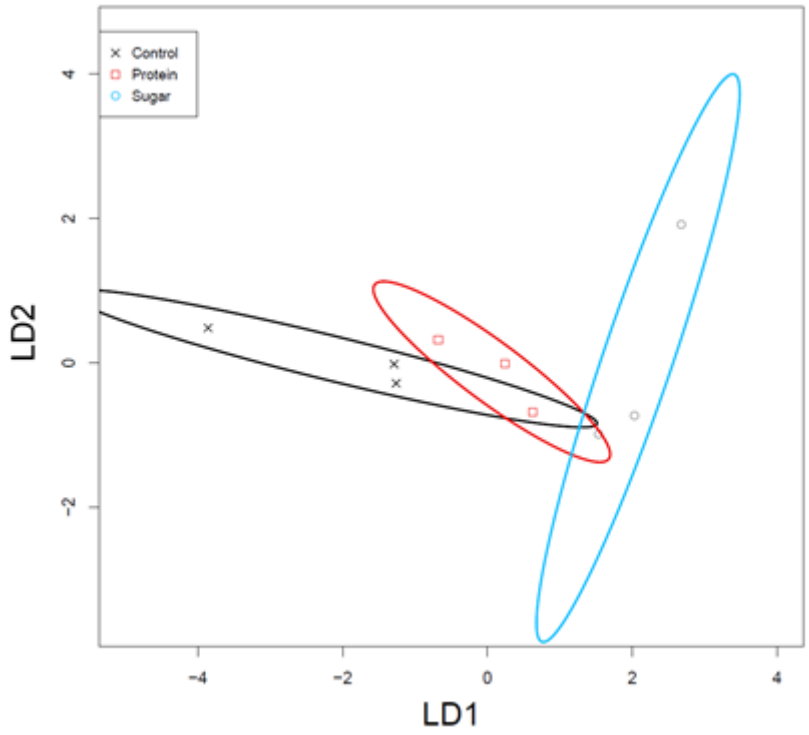

Figure 6.2: Canonical analysis of principal components of *Drosophila melanogaster* volatile profiles. Axes represent linear discriminants. Black, red and blue denote flies reared on control, protein and sugar diets, respectively. Insufficient repeats of lipid-reared fly volatiles were collected for accurate comparison due to high rates of mortality in flies fed this diet. Via CAP, 66.7% of volatile profiles were correctly classified (p=0.109).

This significant difference in both volatile and cuticular hydrocarbon profiles between flies reared on different diets is indicative of the potential for predators to differentiate between prey of differing nutritional value, even within a species, through odour detection or touch-sensing (Delury *et al.* 1999; Bhasin *et al.* 2000; Barth 2015; Manubay and Powell 2020). Importantly, this relies upon the dominant senses of the predator, although spiders can detect flying insect prey from proximate airflow through the complex mechanisms of either the slit sensilla or trichobothria (Barth 2020). If such signals of nutrient enrichment are consistent between taxa (i.e. the abundance of particular compounds associated with specific nutrient enrichments), this would be a very likely method of discernment between taxa of different nutritional profiles in order to enact nutrient-specific foraging. That such intra-specific variation can be observed is, however, an indication that intra-specific nutrient-specific foraging (i.e. predators redressing nutritional needs by predating different individuals within a species enriched with complementary nutrients) could be an additional pathway to nutritional balance in predators. This mechanism would not be detected by the methods used in this thesis: presence/absence representation of metabarcoding data and the family-level clustering of macronutrient contents

(although the impossibility of individual-based macronutrient content determination post-predation is noteworthy here). Lab-based feeding trials have studied intra-specific nutrient-specific foraging (e.g. Mayntz *et al.* 2005), and the observation that some predators will feed on specific tissues to possibly redress nutritional needs aligns with this (Pekár *et al.* 2010; Wilder and Eubanks 2010; Kohl *et al.* 2015). That signals of nutrient-specific foraging were nonetheless detected in this study suggests that the likely greater variation in macronutrients between taxa than within a given taxon renders this a more parsimonious means of acquiring a balanced intake of nutrients than relying on the variability of a given taxon. Regardless, further research is required to determine the reliability of volatile and cuticular hydrocarbon cues in ascertaining nutrient content, both within and between species.

In species with greater visual acuity than that of linyphiids, such as jumping spiders (Araneae: Salticidae), prey choice can be exhibited based on visual cues alone. Nelson and Jackson (2012) demonstrated a preference in jumping spiders for nutritionally superior blood-fed *Anopheles* Meigen, 1818 (Diptera: Culicidae) mosquitoes over those which had not fed on blood. This observation of visual-based nutrient-specific foraging exemplifies an additional pathway to nutrient sensing, highlighting the predisposition of different predators to use their most acute senses to facilitate nutrient-specific foraging. The visually-based *ex situ* prey choice tests employed by Nelson and Jackson address many of the critiques discussed in Chapter 5.5.6 regarding the *ex situ* prey choice assays employed in this thesis; the use of artificial visual cues removes the natural stochasticity of an animal model, facilitating fully standardised presentation of prey options, subject, of course, to sufficient visual acuity in the focal predator. Similar spiders can, however, differentiate between conspecifics, the prey for which they are Batesian mimics, and models (Nelson and Jackson 2007), suggesting that the design of appropriate visual cues for prey must be thorough. As briefly eluded to in Chapter 5.5.6, vibrational cues, being a critical aspect of prey detection and sexual communication in web-building spiders (Hergenröder and Barth 1983; Virant-Doberlet *et al.* 2011), could also be presented via vibrometer in prey choice assay experiments to similarly reduce the stochasticity associated with animal models for choice tests.

This thesis has focused wholly on nutrient assimilation at the point of choice, with predators differentiating between their prey and preferentially choosing one over another based on macronutrient content. There are, however, other stages at which this differential assimilation of nutrients can be achieved. Post-digestion nutrient balancing can be achieved through gastrointestinal tract plasticity, observed in locusts (Clissold *et al.* 2010). Similarly, since digestion begins externally for spiders, they can differentially assimilate nutrients from their prey post-digestion, but prior to ingestion (Mayntz *et al.* 2005), akin to solid-feeding predators

consuming different tissues (Kohl *et al.* 2015). Such factors further confound study of nutrient-specific foraging and could render the discernment of prey based on nutrient profiles redundant altogether, although the results of this thesis and other studies evidencing nutrient-specific foraging suggest that this is insufficient at least as a sole means for redressing nutritional deficits, at least in those predators studied.

6.5.2. Do endosymbionts alter nutrient-specific foraging?

To comprehensively understand the mechanisms underlying trophic interactions, all behaviour-modifying agents must first be understood and disentangled. Endosymbionts are increasingly known to affect the behaviour of their hosts (Lei *et al.* 2020), thus having the potential to alter foraging behaviour such as prey choice in generalist predators. Endosymbionts are highly prevalent in agricultural spiders (Curry *et al.* 2015; White *et al.* 2020), some even detected during the gut content analysis of the spiders in this thesis. Isolation of endosymbionts in gut content extractions does not, however, determine whether they were present in the predator, or in ingested prey tissues (Yun *et al.* 2011). An external collaboration was originally intended to investigate the effect of endosymbionts on the diet of those spiders for which gut content DNA had been sequenced in this thesis, but this work was curtailed by the COVID-19 pandemic. Preliminary results confirmed the presence of endosymbionts in many of the linyphiids studied.

The horizontal transfer of endosymbionts from prey to predator (Yun *et al.* 2011) also has implications for their involvement in trophic interactions. Taxon-specific prey preferences by spiders for endosymbiont-affected prey could alter spider population dynamics rapidly through concentrated uptake of behaviour-modifying endosymbionts. That endosymbionts can affect transmission of viruses from vectors (Lei *et al.* 2020) also introduces implications for biocontrol, with preference by linyphiids for or against endosymbiont-affected vectors possibly affecting the probability of successful virus transmission by non-affected vectors. Further research should thus explore the effects of both predator-borne endosymbionts and prey-borne endosymbionts on prey preference; such data would have significant implications for conservation biocontrol and trophic ecology more broadly.

6.5.3. Do parasites/parasitoids in prey alter predator choice?

That macronutrient content is a determinant of prey choice, as demonstrated in this thesis, confounds several key trophic interactions, notably secondary predation of parasitoid wasps within their aphid hosts. By hosting parasitoids, aphids are observably less desirable prey, at least to coccinellids (Bilu and Coll 2009). This naturally has implications for intraguild predation and biocontrol (Traugott *et al.* 2012), effectively ensuring reduction of the former for the benefit of the latter, but the underlying rationale remains unclear. Given the profound morphological changes underway during the assimilation of aphid tissue in the development of the parasitoids, particularly conversion of soft tissue into chitin, this will undoubtedly impact the nutritional composition of the aphid (and encapsulated parasitoids). It is thus possible that this avoidance of parasitised aphids is, in fact, an artefact of nutrient-specific foraging.

Experiments were formulated to address this hypothesis, first hindered by failure of the parasitoids to produce a second generation, and secondly by the COVID-19 pandemic, but future research could elucidate a nutritional basis for this relationship with relative ease. This parasitised-aphid-avoidance phenomenon could, of course, instead relate to the alteration of aphid cuticular hydrocarbons or pheromonal profile, but these too could reflect compositional, and thus nutritional, changes, as discussed above in Chapter 6.5.1. The implications of this relationship between parasitism and macronutrient content extend beyond aphids and parasitoid wasps, fundamentally affecting all trophic networks containing parasites (arguably even endosymbionts, as discussed above in Chapter 6.5.2).

During the development of the MEDI protocol, preliminary experiments using the early version of MEDI described above (Chapter 6.5.1.) determined, through multivariate linear models, that German cockroaches *Blattella germanica* containing the lipid-dependent gut protozoan *Gregarina blattarum* von Siebold, 1839 (Apicomplexa: Eugregarinida) had significantly different macronutrient contents (only lipid and carbohydrate were analysed due to inaccuracies in protein determination) than those from cultures deemed ‘specific parasite-free’ (SPF; MLM: $F_{1,38}$=24.065, p<0.001). Macronutrients also differed based on the sex of the cockroach (MLM: $F_{1,37}$=8.768, p=0.008), but not an interaction between sex and parasitism. Parasitised cockroaches had a significantly higher lipid content than SPF cockroaches (ANOVA: $F_{1,38}$ = 9.328, p = 0.004; Figure 6.3) but a significantly lower carbohydrate content (ANOVA: $F_{1,38}$=26.7, p<0.001; Figure 6.3).

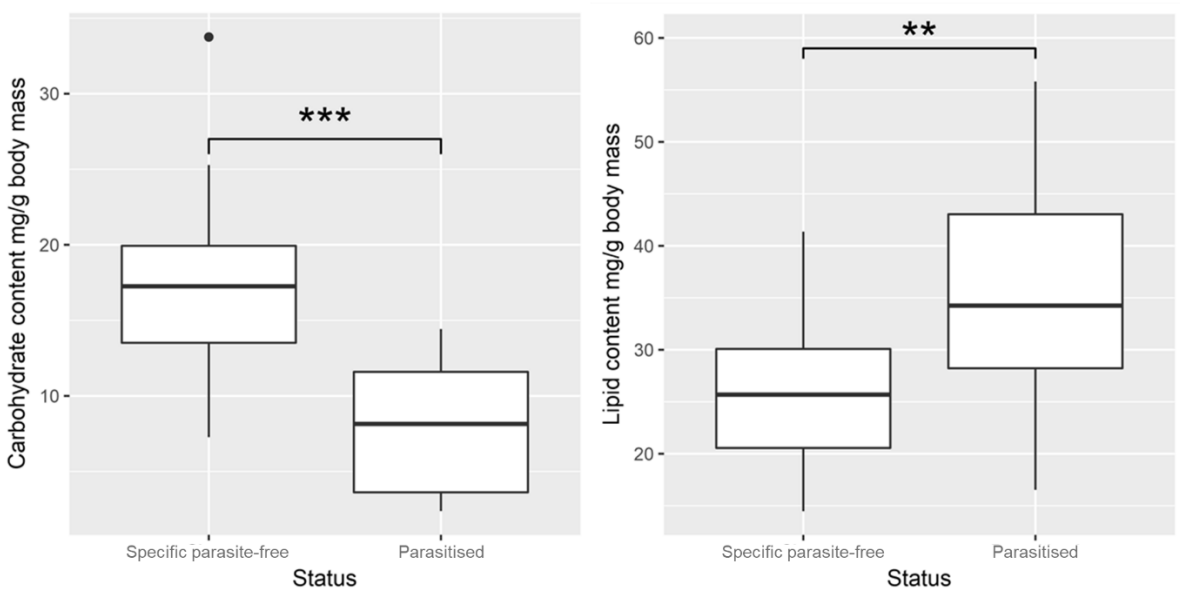

Figure 6.3: Comparison of lipid and carbohydrate content between SPF and parasitised cockroaches. Cockroach carbohydrate and lipid content were significantly different between parasite status. Specifically, parasitised cockroaches had a significantly higher lipid content than SPF cockroaches. Conversely, parasitised cockroaches had a significantly lower carbohydrate content than SPF cockroaches.

The difference in lipid content between parasitised and SPF cockroaches was hypothesised prior to these experiments based on the known accumulation of host lipid resources by the gregarine parasites (Randall *et al.* 2013). This difference may, however, indicate that the parasite-mediated lipid loss is redressed by the cockroach, resulting in this net gain of lipids rather than simple redistribution. It may be that this lipid compensation is metabolically-linked but could also be propagated by nutrient-specific foraging by the cockroach. Randall (2011) showed that gregarine-infected hosts consume more food than SPF, possibly to increase overall lipid intake by over-feeding, as has been observed in other invertebrates (Jensen *et al.* 2011). The difference in macronutrient content between parasitised and SPF cockroaches has implications for its attractiveness to a nutrient-specific foraging predator and thus could affect the survival of the parasitised host, consequently impacting further parasite transmission. Irrespective, these preliminary findings and the literature suggest that parasitism, both by affecting prey macronutrient content and by eliciting nutritional deficits in foragers, has substantial implications for nutrient-specific foraging. Similar trends would be expected based on disease, both in predators and prey. Similarly, virus vectors, such as aphids carrying plant viruses, can experience substantial impairment to body condition, increased susceptibility to parasitoids and altered interactions with predators (Dupont *et al.* 2020), possibly suggesting similar modifications to host macronutrient profile, and thus attractiveness to predators and parsitoids.

6.5.4. Is prey choice transcriptionally regulated?

This thesis demonstrates that physiological factors such as nutritional deficiency can underpin and influence trophic interactions to a large extent. Prey choice is dynamically controlled by biological, ecological and physiological changes (Holling 1966; Mayntz *et al.* 2005; Řezáč and Pekár 2007), but, as with other behaviours, this may be fundamentally modulated by gene transcription. Other choice-based behaviours, such as the choice of oviposition host in parasitoid wasps (Pannebakker *et al.* 2013), are transcriptionally regulated. The transcriptome of predators exhibiting distinct prey choice behaviours, however, remains uncharacterised. Elucidating any such genetic mechanisms underpinning prey choice could facilitate manipulation of predator-prey systems using novel methods already under investigation for agricultural pest management, such as RNA interference (RNAi; Zotti *et al.* 2017; Andongma *et al.* 2020; Kunte *et al.* 2020). If candidate genes could be identified which are upregulated whilst specific foraging choices are enacted, RNAi could be designed to disrupt regulation of these genes, possibly affecting this choice behaviour in a targeted and sustainable manner. If this were achieved, it would provide a pathway to enhanced agricultural productivity by biocontrol with negligible environmental impact and a possibly high degree of accuracy.

6.6. Concluding remarks

Through the synergy of its individual components, this thesis provides field-based evidence toward invertebrate nutrient-specific foraging, the first of its kind. The methodological advancements presented and utilised throughout have provided and will continue to provide a foundation for further research directly linked to the focal topics presented, but also for broader research relating to micro-scale macronutrient dynamics, spider trophic interactions and wider ecology.

The identification of key dietary dynamics in cereal crop spiders, particularly those concerning biocontrol and intraguild predation, provide a basis from which to refine management of generalist predators for biocontrol. By promotion of those spiders most beneficial for agriculture, namely *Bathyphantes*, *Tenuiphantes* and juvenile spiders, conservation biocontrol can be enhanced. Predators can be promoted through habitat manipulation, but also by exploiting their prey preferences to ensure that these populations are appropriately provisioned for effective enactment of nutrient-specific foraging.

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Supplementary Material

Chapter 2 Supplementary Material

Supplementary Information 2.1: Simplified instructions for colorimetric assays

BCA Protein Determination

1. Prepare a standard dilution series of 0-2 mg ml^{-1} BSA diluted with water in nine increments for calibration of absorbance readings.
2. For each sample and standard, prepare 200 µl of BCA working reagent by mixing 50 parts BCA reagent A with 1 part BCA reagent B.
3. Of each standard and sample, add 25 µl to a 96-well microplate with 200 µl of the working reagent.
4. Mix in a thermo-mixer at room temperature for 30 seconds at 450 rpm.
5. Incubate at 37°C for 30 minutes.
6. Cool the plate to room temperature.
7. Measure absorbance at 562 nm.

Anthrone carbohydrate Determination:

1. Prepare a standard dilution series of 0-2 mg ml^{-1} corn starch diluted with ethanol in nine increments for calibration of absorbance readings.
2. Prepare the anthrone reagent by dissolving 1 mg of anthrone in 1 ml of concentrated H_2SO_4 (keep the solution in the dark and use within 12 hours).
3. From each standard and sample, add 40 µl to a 96-well microplate and mix with 160 µl anthrone reagent.
4. Mix in a thermo-mixer at room temperature for 30 seconds at 450 rpm.
5. Incubate the plate at 92 °C for 10 minutes and cool to room temperature.
6. Measure absorbance at 620 nm.

Lipid Determination

1. Prepare astandard dilution series of 0-2 mg ml^{-1} lard oil diluted with ethanol in nine increments for calibration of absorbance readings.
2. From each sample and standard, place 50 µl in a heating block at 100°C for approximately 10 minutes to evaporate the solvent.
3. Add 10 µl concentrated sulfuric acid, vortex and incubate at 100 °C for 10 minutes.

4. Prepare 240 µl vanillin reagent per sample (1.2 mg dissolved in 0.2 ml hot water and 0.8 ml 85% phosphoric acid).
5. Cool the samples to room temperature and add 240 µl vanillin reagent and vortex.
6. After 5 minutes, add 200 µl of each sample and standard into a 96-well microplate.
7. Measure absorbance at 490 nm.

Lowry Protein Determination

1. Prepare a standard dilution series of 0-2 mg ml^{-1} BSA diluted with water in nine increments for calibration of absorbance readings.
2. Prepare 1X (1N) Folin-Ciocalteu Reagent by diluting the supplied 2X (2N) reagent 1:1 with ultrapure water. Because the diluted reagent is unstable, prepare 1X Folin-Ciocalteu Reagent on the same day of use. Each test replicate requires 20µL of 1X Folin-Ciocalteu Reagent.
3. Pipette 40µL of each standard and sample replicate into a microplate.
4. Add 200µL of Modified Lowry Reagent to each well at nearly the same moment using a multi-channel pipettor. Immediately mix microplate on plate mixer for 30 seconds.
5. Cover (e.g., Sealing Tape for 96-Well Plates, Product No.15036) and incubate microplate at room temperature (RT) for exactly 10 minutes.
6. Add 20µL of prepared 1X Folin-Ciocalteu Reagent to each well using a multi-channel pipettor. Immediately mix microplate on plate mixer for 30 seconds.
7. Cover and incubate microplate at RT for 30 minutes.
8. Measure the absorbance at or near 750nm on a plate reader.

Bradford Protein Determination

- Prepare a standard dilution series of 0-2 mg ml^{-1} BSA diluted with water in nine increments for calibration of absorbance readings.
- Pipette 50µL of each standard or unknown sample into the appropriate microplate wells.
- Add 150µL of the Coomassie Plus Reagent to each well and mix with plate shaker for 30 seconds.
- Remove plate from shaker. For the most consistent results, incubate plate for 10 minutes at room temperature (RT).
- Measure the absorbance at or near 595nm on a plate reader.

Table S2.1: Standard dilution series preparation for assay standards. The standard (stock 2 mg ml^{-1}) was diluted with polished water (BSA or corn starch) or chloroform/methanol solution (lard oil) to prepare a dilution series of standards for calibration.

Solution	Diluent volume (µl)	Standard volume (µl), source	Final concentration (µg/ml)
A	0	300, stock	2000
B	125	375, stock	1500
C	325	325, stock	1000
D	175	175, solution B	750
E	325	325, solution C	500
F	325	325, solution E	250
G	325	325, solution F	125
H	400	100, solution G	25
I	400	0	0

Table S2.2: Protein determined via the three trialled assays from each of the five species expressed as absolute protein mass (mass mg), percentage of body mass (%mass) and percentage of total macronutrient mass (%macronutrients). Values were calculated from eight individuals of each species. Values for %mass of *F. candida* and *M. dirhodum* are absent due to inaccuracies associated with determining the body mass of such small specimens.

Species	BCA			Bradford			Lowry		
	Mass (mg)	%mass	%macronutrients	Mass (mg)	%mass	%macronutrients	Mass (mg)	%mass	%macronutrients
Acheta domesticus	11.02 ± 2.60	50.30 ± 6.06	86.73 ± 2.05	1.46 ± 0.42	6.58 ± 0.48	46.49 ± 3.19	8.05 ± 3.30	35.35 ± 5.15	81.97 ± 3.55
Blattella germanica	22.76 ± 6.99	111.89 ± 55.42	91.65 ± 4.61	1.16 ± 0.41	5.26 ± 1.90	38.69 ± 7.12	10.25 ± 4.56	48.34 ± 26.18	82.18 ± 8.86
Folsomia candida	0.20 ± 0.10	20.20 ± 11.40	76.26 ± 10.15	0.04 ± 0.03	4.74 ± 3.09	37.85 ± 15.30	0.010 ± 0.05	8.75 ± 3.98	60.76 ± 15.68
Metopolophium dirhodum	0.37 ± 0.07	12.72 ± 4.42	67.33 ± 7.25	0.07 ± 0.02	2.50 ± 0.86	29.65 ± 6.77	0.17 ± 0.09	5.62 ± 2.63	47.01 ± 12.26
Tenebrio molitor	12.36 ± 2.22	42.63 ± 14.39	84.60 ± 2.04	1.26 ± 0.49	4.12 ± 1.31	35.55 ± 7.31	9.99 ± 5.18	28.74 ± 6.50	78.91 ± 6.16

Chapter 3 Supplementary Material

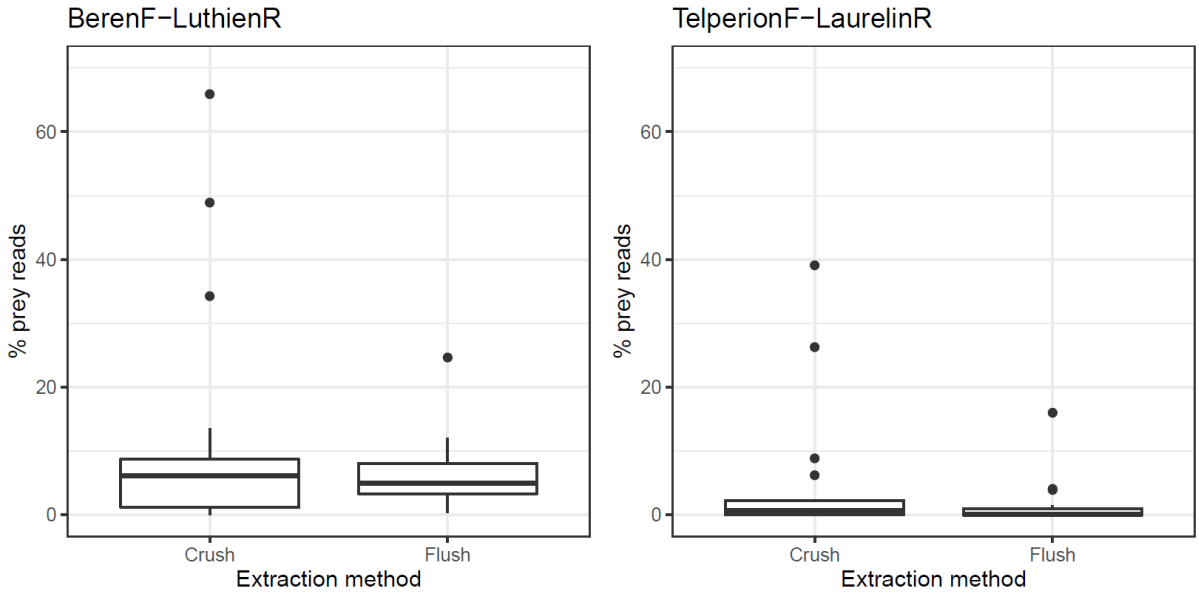


Figure S3.1: Comparison of % prey reads (of total reads per sample) recovered via the two extractions methods for instances in which the predator was amplified: crush (abdomens disrupted and lysed in solution) and flush (abdomen disrupted and removed before lysis). Left = Beren-Luthien (general primers); right = Telperion-Laurelin (spider-exclusion primers). All samples amplified via Beren-Luthien were compared, but only those in which the linyphiid predator was amplified via Telperion-Laurelin were compared. Neither method recovered a significantly greater % prey reads, although "crushing" recovered a higher mean % prey reads (Beren-Luthien: crush = 10.67%, flush = 6.10%; Telperion-Laurelin: crush = 4.55%, flush = 1.43%).

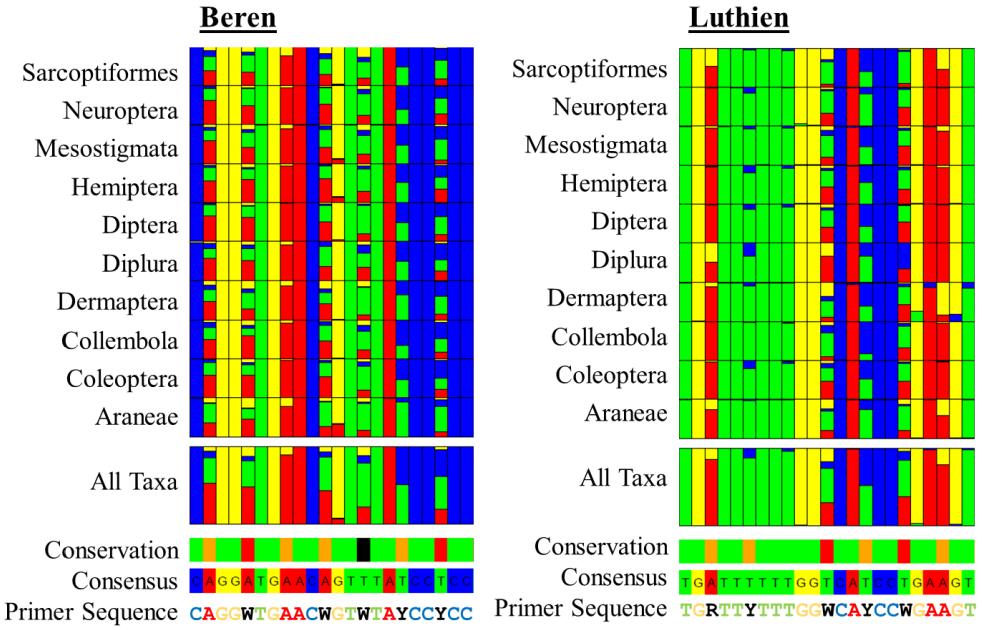


Figure S3.2: PrimerMiner mass alignments of spiders and common cereal crop species for Beren and Luthien. Both primers utilise degenerate bases to increase amplification of a broad taxonomic range. The conserved primer sites enable broad amplification with few degenerate bases.

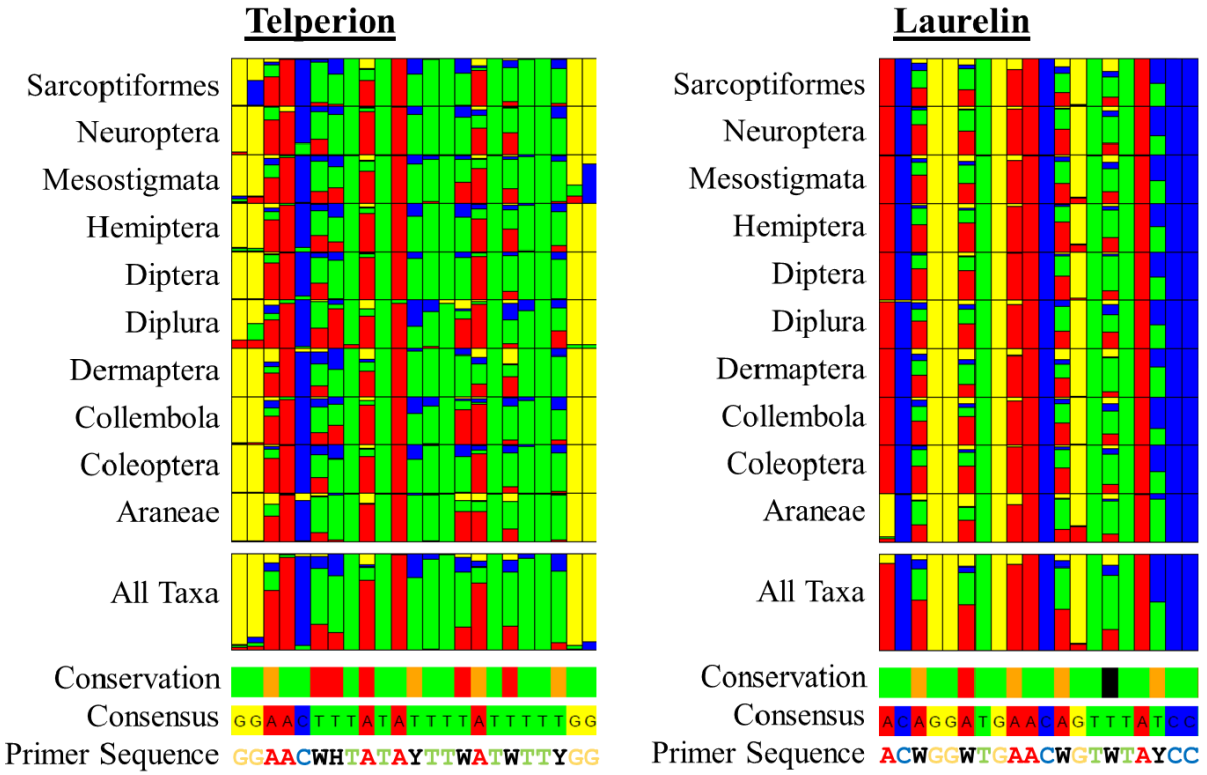


Figure S3.3: PrimerMiner mass alignments of spiders and common cereal crop species for Telperion and Laurelin. Both primers utilise degenerate bases to increase amplification of a broad taxonomic range. The terminal base at the 3' end of Laurelin is a critical mismatch for almost all spiders, making the primer pair efficacious for the amplification of all but spiders (G, rather than A).

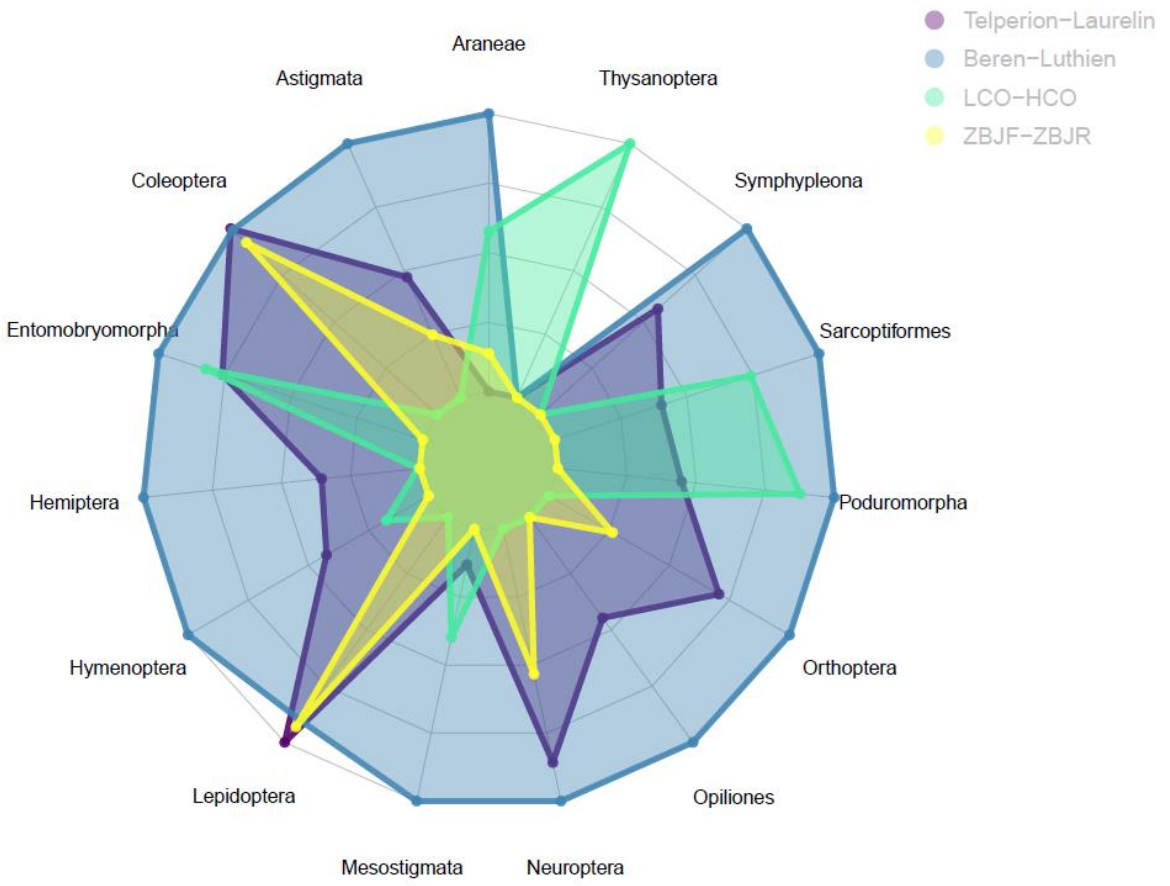


Figure S3.4: *In silico* analysis of primer bias for novel primer pairs compared against standard animal barcoding primers LCO1490 and HCO2198 (Folmer *et al.* 1994), and other primers used for linyphiid dietary analysis ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale *et al.* 2011). The further from the centre that each coloured polygon extends at each anchor point reflects the relative performance of that primer for the respective taxon. Distances are relative, not absolute. Beren-Luthien show almost universally strong amplification potential for most taxa, except for thrips (Thysanoptera). Telperion-Laurelin show the expected bias against spiders (Araneae), but also weaker amplification of mesostigmatic mites and thrips; despite this, the amplification potential for most other groups is stronger than that of ZBJ, which has been used in other studies of linyphiid diet. The reduced amplification potential for thrips by both primer pairs illustrates a potential under-representation of them when paired for dietary analyses.

Figure S3.5: Primer bias ascertained using mock community positive control and high-throughput sequencing. The distance of each point radially from the centre is the proportional success in amplifying the mock community component taxa. The "Expected" amplification is based on the proportion of each taxon's DNA in the mock community. Beren-Luthien shows some bias toward Lepidoptera (*Euproctis similis*) and some Diptera (*Melieria crassipennis*), but reduced amplification of linyphiids, springtails, wasps and aphids. Telperion-Laurelin expectedly avoids amplification of spiders in most cases, although they are still amplified to some extent. The two primer pairs show moderately complementary biases, but both show some bias against linyphiids, and some springtails, flies and wasps.

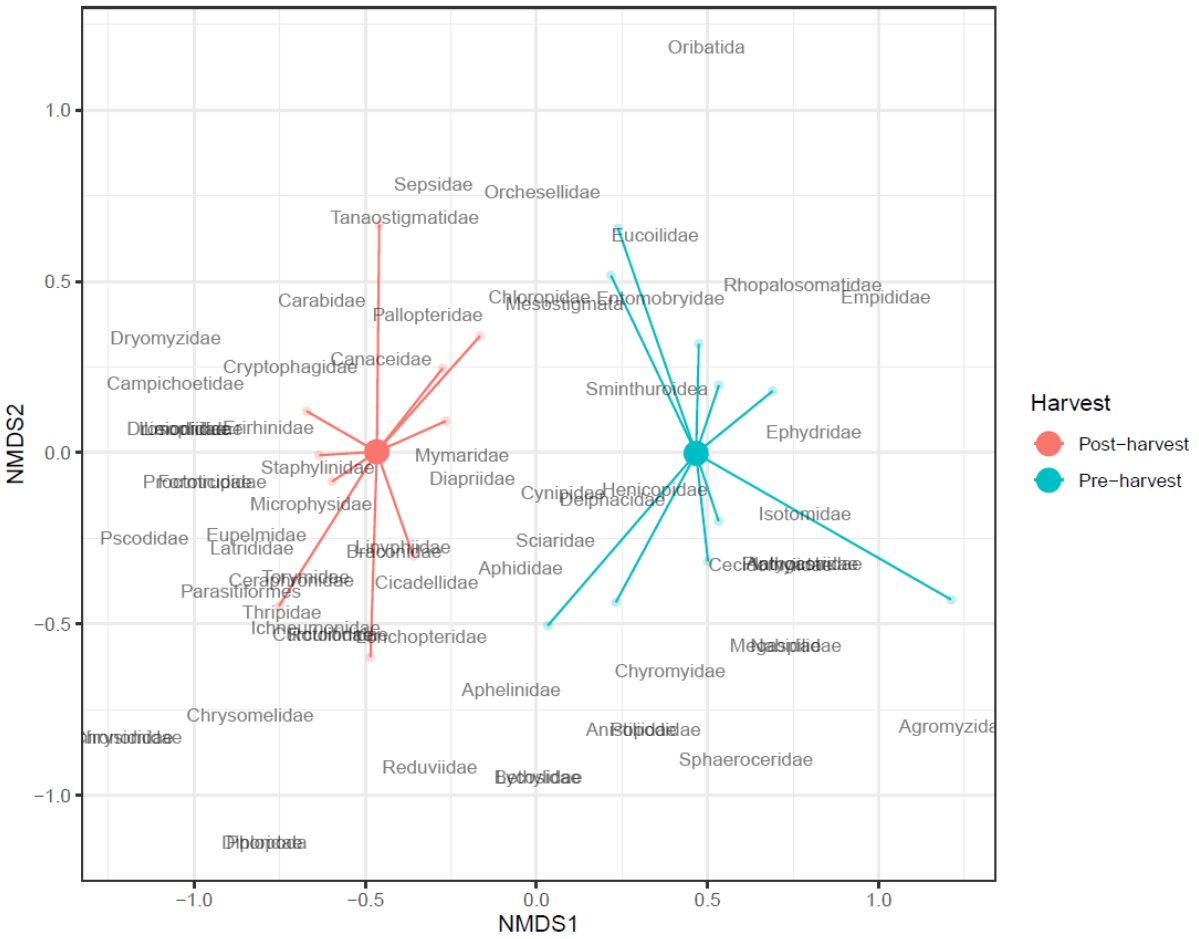


Figure S3.6: Spider plot of invertebrate communities pre- and post-harvest showing distinction between communities (smaller nodes) and centroids of communities (larger nodes, mean coordinates for each category) within each category. Species present in the communities are plotted according to their alignment with sample dissimilarities.

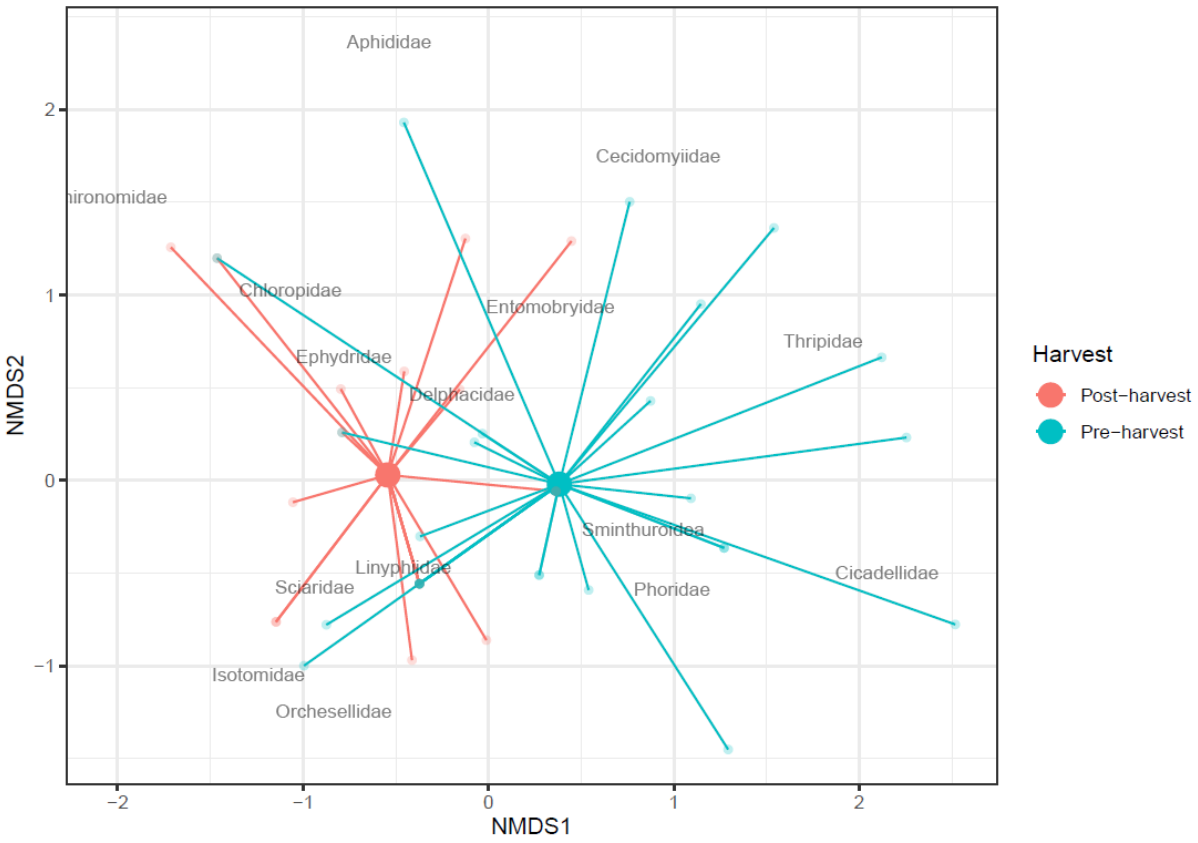


Figure S3.7: Spider plot of spider diets pre- and post-harvest showing some distinction with a degree of overlap between the prey families in the diets (smaller nodes) and centroids of diets (larger nodes) within both categories. Species present in the diets are plotted according to their alignment with sample dissimilarities.

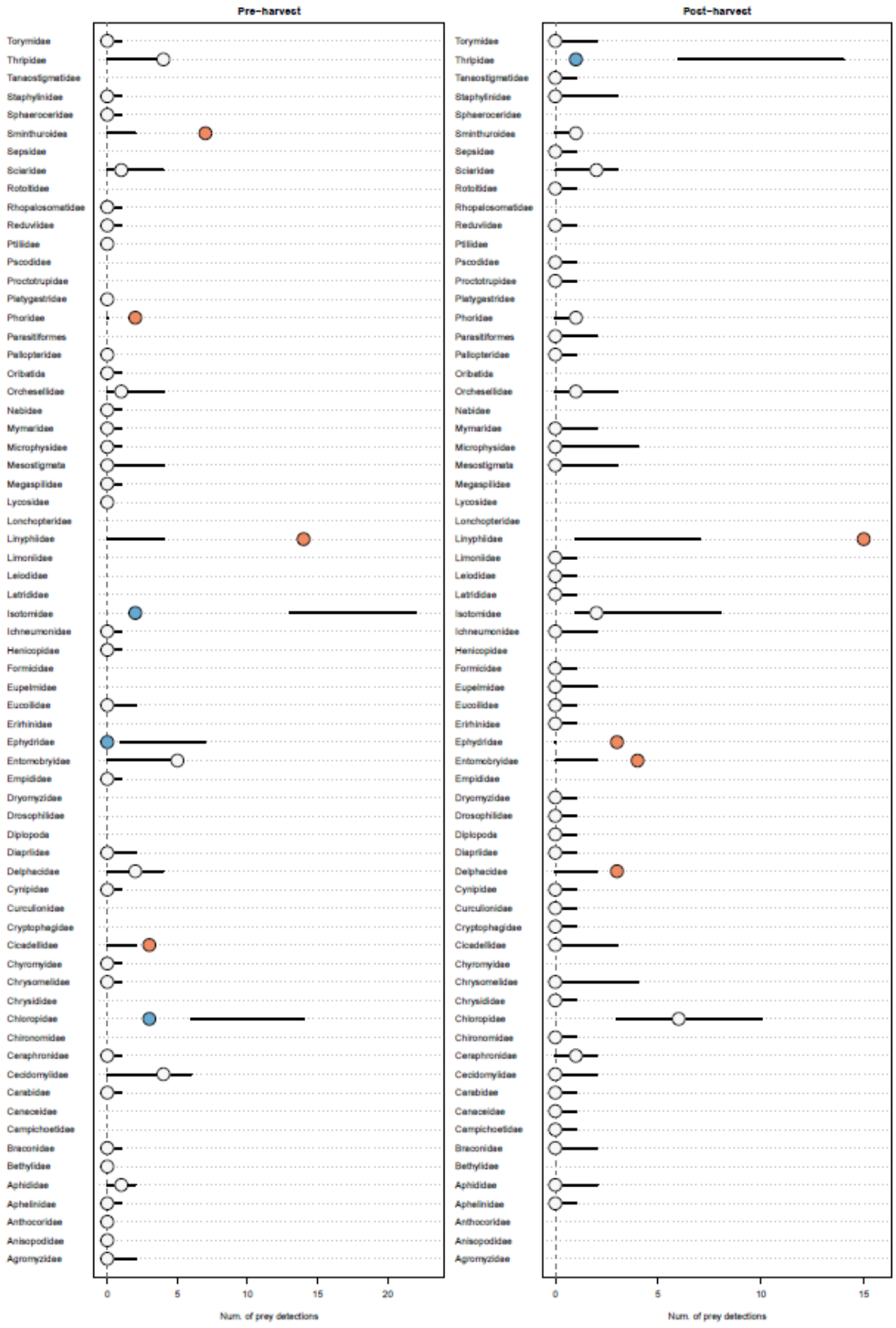


Figure S3.8: Full prey choice plot. Blue = lower consumption than expected (avoidance), white = as expected (in proportion to relative abundance), red = higher than expected (consumed more frequently than predicted from relative abundance). Horizontal lines denote 95% confidence limits of the observed frequency of predation.

Table S3.1: Mock community DNA mixtures.The volume of each 0.1 ng µl^{-1} sample added to each mock community mixture. Mock communities include some uniformly-distributed volumes and others weighted in favour of specific taxa (most often spiders to reflect the abundance of spider DNA in spider diet extracts).

Taxon	Sample	Mix1 µl	Mix2 µl	Mix3 µl	Mix4 µl	Mix5 µl
Springtail	*Sminthurus viridis*	0	1	1	1	1
Springtail	*Orchesella villosa*	1	1	1	1	2
Aphid	*Utamphorophora* sp.	0	1	1	1	1
Aphid	*Metopolophium dirhodum*	1	1	1	1	2
Lacewing	*Chrysoperla carnea*	1	1	1	1	1
Fly	*Melieria crassipennis*	1	1	1	1	2
Fly	*Tvetenia calvescens*	0	1	1	1	1
Wasp	*Trichopria* sp.	1	1	1	1	2
Wasp	*Promethes sulcator*	0	1	1	1	1
Moth	*Euproctis similis*	1	1	1	1	1
Bug	*Anthocoris nemorum*	0	1	1	1	1
Spider	*Erigone dentipalpis*	3	0	4	1	0
Spider	*Tenuiphantes tenuis*	3	4	0	1	0
Spider	*Bathyphantes nigrinus*	3	0	0	1	0
Spider	*Pardosa palustris*	0	0	0	1	0

Table S3.2: The 66 spiders included in dietary screening included 37 pre-harvest, 29 post-harvest, 16 Erigoninae, 50 Linyphiinae, 33 adult, 17 sub-adult, 16 juvenile, 19 female and 31 male spiders.

Pre-harvest	**Erigoninae**	Adult	Female	5
			Male	3
		Sub-adult	Female	0
			Male	0
		Juvenile		4
	Linyphiinae	Adult	Female	6
			Male	6
		Sub-adult	Female	1
			Male	6
		Juvenile		6
Post-harvest	**Erigoninae**	Adult	Female	1
			Male	1
		Sub-adult	Female	0
			Male	1
		Juvenile		1
	Linyphiinae	Adult	Female	4
			Male	7
		Sub-adult	Female	2
			Male	7
		Juvenile		5

Table S3.3: *In vitro* results for novel primer pairs. A) Arachnids, B) other invertebrates, C) vertebrates and marine. Both primer pairs show a broad amplification of many groups. Beren-Luthien proves to be very general, whilst Telperion-Laurelin avoids amplification of some spiders, but also a few other invertebrate species tested.

A)

Primer pair	*Erigone atra*	*Tenuiphantes tenuis*	*Eratigena atrica*	*Psilochorus simoni*	*Amaurobius similis*	*Clubiona comta*	*Dysdera crocata*	*Philodromus* sp.	*Bathyphantes nigrinus*	*Oedothorax fuscus*	*Tapinopa longidens*	*Pardosa pullata*	*Chernes cimicoides*	*Chthonius ischnocheles*	Epiriidae sp.	Phthiracaidae sp.
Beren-Luthien	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Telperion-Laurelin	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	-

B)

Primer pair	*Anthocoris nemorum*	*Pterostichus melanarius*	*Folsomia candida*	*Protophorura armata*	*Chrysoperla carnea*	*Porcellio scaber*	*Sminthurus viridis*	*Orchesella villosa*	*Lasius brunneus*	*Geophilus truncorum*	*Cryptops hortensis*	*Lithobius variegatus*	*Cylindroiulus punctatus*	*Brachydesmus superus*	*Coccinella 7-punctata*	*Oxychilus navarricus*	*Promethus sulcator*	*Trichopria* sp.	*Loricera pilicornis*	*Episyrphus balteatus*	*Utamphorophora* sp.	*Nossidium pillosellum*
Beren-Luthien	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Telperion-Laurelin	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	+	+

C)

Primer pair	*Turdus merula*	*Sylvia atricapilla*	*Ficedula hypoleuca*	*Sylvia borin*	*Alcedo atthis*	*Carduelis carduelis*	*Erithacus rubecula*	*Fringilla coelebs*	*Passer domesticus*	*Phylloscopus collybita*	*Phylloscopus trochilus*	Patellogastropoda sp.	Brachyura sp.	Bivalvia sp.	Decapoda sp.	Actiniaria sp.	Nudibranchia sp.	Gastropod sp.	*Zeus faber*
Beren-Luthien	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Telperion-Laurelin	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table S3.4: Invertebrate taxa identified from vacuum samples pre- and post-harvest. Vacuum samples were collected for 30 secs over 4 m^2. Each period represents the total from 10 samples evenly split between two adjacent fields.

Taxon Order	Taxon Family	Pre-harvest	Post-harvest
Araneae	Linyphiidae	68	124
Araneae	Lycosidae	1	0
Coleoptera	Carabidae	1	8
Coleoptera	Chrysomelidae	8	55
Coleoptera	Cryptophagidae	0	2
Coleoptera	Curculionidae	0	1
Coleoptera	Erirhinidae	0	5
Coleoptera	Latrididae	0	2
Coleoptera	Leiodidae	0	2
Coleoptera	Ptiliidae	1	0
Coleoptera	Staphylinidae	5	27
Diplopoda	Julidae	0	1
Diptera	Agromyzidae	10	0
Diptera	Anisopodidae	1	0
Diptera	Campichoetidae	0	2
Diptera	Canaceidae	0	1
Diptera	Cecidomyiidae	80	12
Diptera	Chironomidae	0	1
Diptera	Chloropidae	354	289
Diptera	Chyromyidae	2	0
Diptera	Drosophilidae	0	6
Diptera	Dryomyzidae	0	2
Diptera	Empididae	1	0
Diptera	Ephydridae	94	0
Diptera	Limoniidae	0	1
Diptera	Lonchopteridae	26	38
Diptera	Pallopteridae	1	8
Diptera	Phoridae	0	2
Diptera	Sciaridae	56	27
Diptera	Sepsidae	0	1
Diptera	Sphaeroceridae	3	0
Entomobryomorpha	Entomobryidae	56	20
Entomobryomorpha	Isotomidae	847	156
Entomobryomorpha	Orchesellidae	34	37
Hemiptera	Anthocoridae	1	0
Hemiptera	Aphididae	22	22
Hemiptera	Cicadellidae	18	31
Hemiptera	Delphacidae	41	22
Hemiptera	Microphysidae	5	36

Hemiptera	Nabidae	1	0
Hemiptera	Reduviidae	3	3
Hymenoptera	Aphelinidae	5	1
Hymenoptera	Bethylidae	1	0
Hymenoptera	Braconidae	4	10
Hymenoptera	Ceraphronidae	3	13
Hymenoptera	Chrysididae	0	1
Hymenoptera	Cynipidae	6	3
Hymenoptera	Diapriidae	8	6
Hymenoptera	Eucoilidae	12	3
Hymenoptera	Eupelmidae	0	15
Hymenoptera	Formicidae	0	1
Hymenoptera	Ichneumonidae	4	9
Hymenoptera	Megaspilidae	1	0
Hymenoptera	Mymaridae	5	9
Hymenoptera	Platygastridae	1	0
Hymenoptera	Proctotrupidae	0	1
Hymenoptera	Rhopalosomatidae	1	0
Hymenoptera	Rotoitidae	0	3
Hymenoptera	Tanaostigmatidae	0	2
Hymenoptera	Torymidae	2	13
Lithobiomorpha	Henicopidae	2	0
Mesostigmata	Mesostigmata	35	23
Oribatida	Oribatida	4	0
Parasitiformes	Parasitiformes	0	13
Psocoptera	Pscocidae	0	2
Symphypleona	Sminthuroidea	13	8
Thysanoptera	Thripidae	49	496

Table S3.5: Percentage of spiders in each category (pre- or post-harvest, sex, subfamily and age class) that had consumed each of the families detected in gut content analysis.

	Aphididae	Sminthuroidea	Cecidomyiidae	Chironomidae	Chloropidae	Cicadellidae	Delphacidae	Entomobryidae	Ephydridae	Isotomidae	Linyphiidae	Orchesellidae	Phoridae	Sciaridae	Thripidae
Pre-harvest	3.7	25.9	14.8	0.0	11.1	11.1	7.4	18.5	0.0	7.4	51.9	3.7	7.4	3.7	14.8
Post-harvest	0.0	5.3	0.0	5.3	31.6	0.0	15.8	21.1	15.8	10.5	78.9	5.3	5.3	10.5	5.3
Female	0.0	18.2	0.0	0.0	18.2	0.0	18.2	18.2	9.1	9.1	63.6	9.1	9.1	9.1	0.0
Male	3.7	18.5	14.8	3.7	22.2	3.7	11.1	18.5	7.4	7.4	63.0	3.7	7.4	7.4	15.4
Erigoninae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.1	0.0	22.2	100.0	11.1	0.0	0.0	0.0
Linyphiinae	2.7	21.6	10.8	2.7	24.3	8.1	13.5	21.6	8.1	5.4	54.1	2.7	8.1	8.1	13.5
Adult	0.0	20.0	0.0	0.0	20.0	4.0	16.0	16.0	8.0	8.0	72.0	4.0	4.0	8.0	4.0
Sub-adult	7.7	15.4	30.8	7.7	23.1	0.0	7.7	23.1	7.7	7.7	46.2	7.7	15.4	7.7	23.1
Juvenile	0.0	12.5	0.0	0.0	12.5	25.0	0.0	25.0	0.0	12.5	62.5	0.0	0.0	0.0	12.5

Table S3.6: Each spider from which a dietary sample was taken is given alongside the raw dietary data and the other information associated with that sample.

Spider sample code	Harvest stage	Subfamily	Genus	Sex	Age	Extraction method	Aphididae	Sminthuroidea	Cecidomyiidae	Chironomidae	Chloropidae	Cicadellidae	Delphacidae	Entomobryidae	Ephydridae	Isotomidae	Linyphiidae	Orchesellidae	Phoridae	Sciaridae	Thripidae
33C	Post-harvest	Erigoninae	*Dicymbium*	NA	Juvenile	Crush	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
28F	Post-harvest	Erigoninae	*Erigone*	Male	Adult	Flush	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
24C	Post-harvest	Linyphiinae	*Microlinyphia*	Male	Sub	Crush	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
15C	Post-harvest	Linyphiinae	*Tenuiphantes*	Female	Adult	Crush	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
14C	Post-harvest	Linyphiinae	*Tenuiphantes*	Female	Adult	Crush	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
15F	Post-harvest	Linyphiinae	*Tenuiphantes*	Female	Adult	Flush	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
18C	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Adult	Crush	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0
16C	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Adult	Crush	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0
17C	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Adult	Crush	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
16F	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Adult	Flush	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0
17F	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Adult	Flush	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
14F	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Adult	Flush	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
18F	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Adult	Flush	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
25C	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Sub	Crush	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
22C	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Sub	Crush	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0
23C	Post-	Linyphiinae	*Tenuiphantes*	Male	Sub	Crush	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0

	harvest																				
25F	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Sub	Flush	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
23F	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Sub	Flush	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
24F	Post-harvest	Linyphiinae	*Tenuiphantes*	Male	Sub	Flush	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0
32C	Pre-harvest	Erigoninae	*Dicymbium*	NA	Juvenile	Crush	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
30F	Pre-harvest	Erigoninae	*Dicymbium*	NA	Juvenile	Flush	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
26C	Pre-harvest	Erigoninae	*Erigone*	Female	Adult	Crush	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
27C	Pre-harvest	Erigoninae	*Erigone*	Female	Adult	Crush	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
26F	Pre-harvest	Erigoninae	*Erigone*	Female	Adult	Flush	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
28C	Pre-harvest	Erigoninae	*Erigone*	Male	Adult	Crush	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
27F	Pre-harvest	Erigoninae	*Erigone*	Male	Adult	Flush	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
32F	Pre-harvest	Linyphiinae	*Agyneta*	NA	Juvenile	Flush	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
02C	Pre-harvest	Linyphiinae	*Tenuiphantes*	Female	Adult	Crush	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
04C	Pre-harvest	Linyphiinae	*Tenuiphantes*	Female	Adult	Crush	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
02F	Pre-harvest	Linyphiinae	*Tenuiphantes*	Female	Adult	Flush	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
03F	Pre-harvest	Linyphiinae	*Tenuiphantes*	Female	Adult	Flush	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
04F	Pre-harvest	Linyphiinae	*Tenuiphantes*	Female	Adult	Flush	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0
05C	Pre-harvest	Linyphiinae	*Tenuiphantes*	Male	Adult	Crush	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
06C	Pre-harvest	Linyphiinae	*Tenuiphantes*	Male	Adult	Crush	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
06F	Pre-harvest	Linyphiinae	*Tenuiphantes*	Male	Adult	Flush	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0
05F	Pre-harvest	Linyphiinae	*Tenuiphantes*	Male	Adult	Flush	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
10C	Pre-harvest	Linyphiinae	*Tenuiphantes*	Male	Sub	Crush	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
11C	Pre-harvest	Linyphiinae	*Tenuiphantes*	Male	Sub	Crush	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0

12C	Pre-harvest	Linyphiinae	*Tenuiphantes*	Male	Sub	Crush	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
11F	Pre-harvest	Linyphiinae	*Tenuiphantes*	Male	Sub	Flush	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1
12F	Pre-harvest	Linyphiinae	*Tenuiphantes*	Male	Sub	Flush	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
13F	Pre-harvest	Linyphiinae	*Tenuiphantes*	Male	Sub	Flush	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
07C	Pre-harvest	Linyphiinae	*Tenuiphantes*	NA	Juvenile	Crush	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
09C	Pre-harvest	Linyphiinae	*Tenuiphantes*	NA	Juvenile	Crush	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
08F	Pre-harvest	Linyphiinae	*Tenuiphantes*	NA	Juvenile	Flush	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
09F	Pre-harvest	Linyphiinae	*Tenuiphantes*	NA	Juvenile	Flush	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0

Chapter 4 Supplementary Material

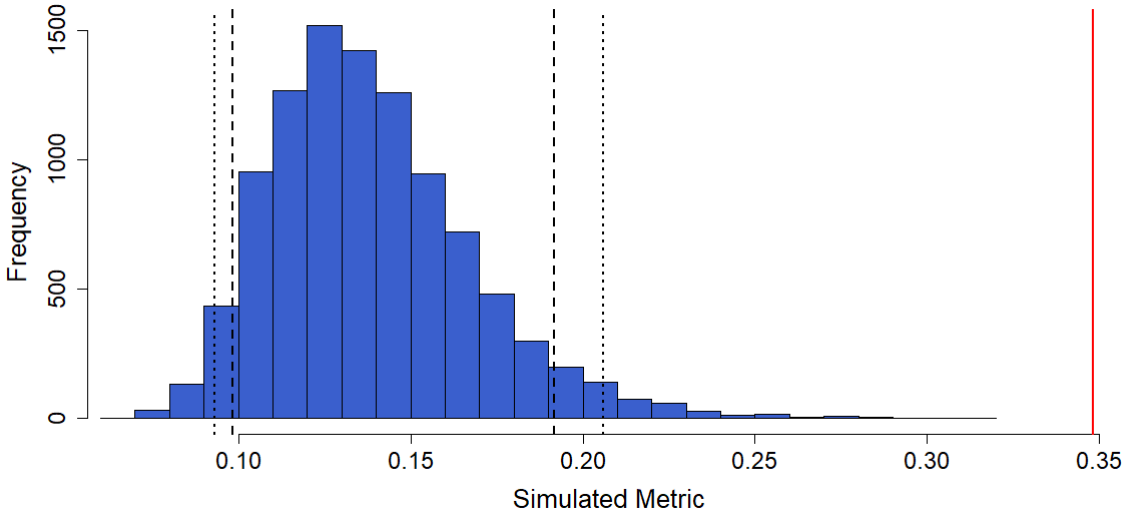


Figure S4.1: Pianka niche overlap for different spider genera. Blue bars indicate niche overlap indices from random simulations, and the red line denotes observed niche overlap. Black lines indicate 95% confidence limits, with the coarser-dashed line representative for the 1-tailed testing pertinent to this case.

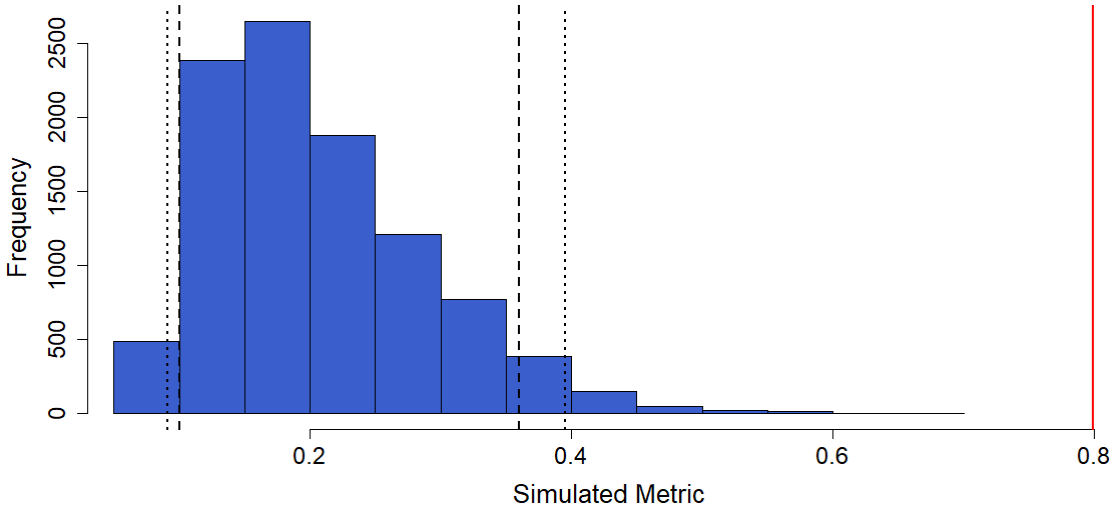


Figure S4.2: Pianka niche overlap for different spider life stages. Blue bars indicate niche overlap indices from random simulations, and the red line denotes observed niche overlap. Black lines indicate 95% confidence limits, with the coarser-dashed line representative for the 1-tailed testing pertinent to this case. An additional output is available as Figure S4.2.

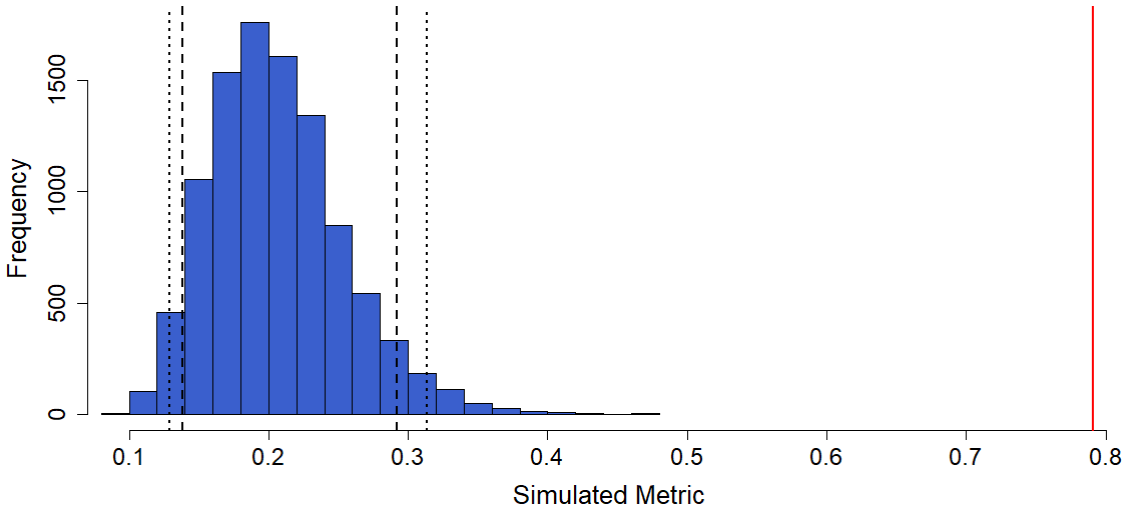


Figure S4.3: Pianka niche overlap for different spider sexes. Blue bars indicate niche overlap indices from random simulations, and the red line denotes observed niche overlap. Black lines indicate 95% confidence limits, with the coarser-dashed line representative for the 1-tailed testing pertinent to this case. An additional output is available as Figure S4.3.

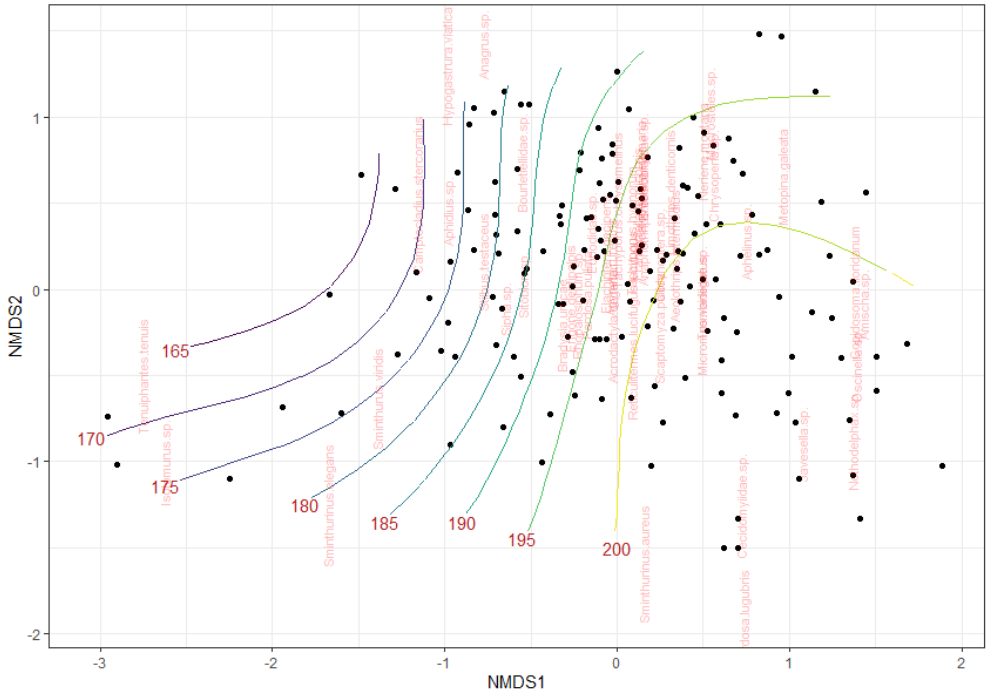


Figure S4.4: Surf plot based on NMDS of spider diets with contours representing Julian days throughout the sampling period (purple and yellow denoting the earliest and latest days, respectively, with a gradual scaling between). Prey species are overlaid in red text to denote their mean occurrence in spider diets across the sampling period.

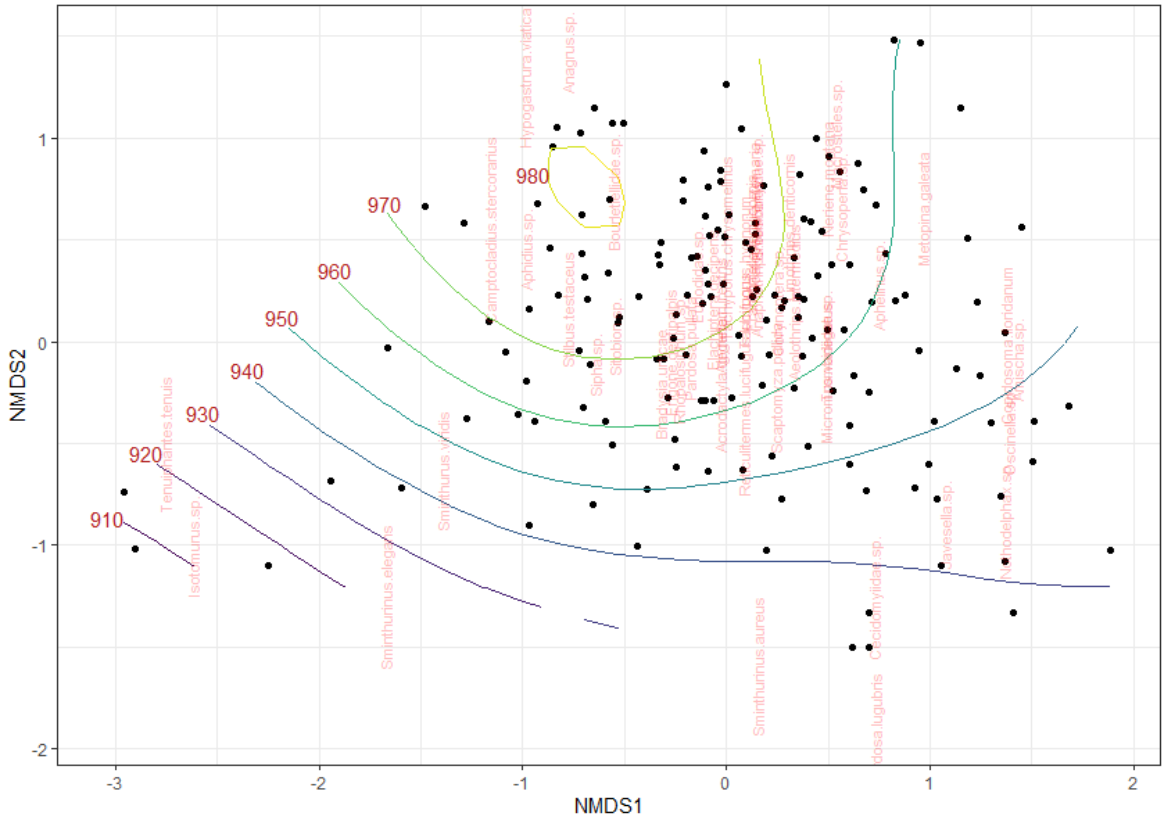


Figure S4.5: Surf plot based on NMDS of spider diets with contours representing mean week daylength across the sampling period (purple and yellow denoting the lowest and highest daylengths, respectively, and a gradual scaling between). Prey species are overlaid in red text to denote their mean occurrence in spider diets across the sampling period.

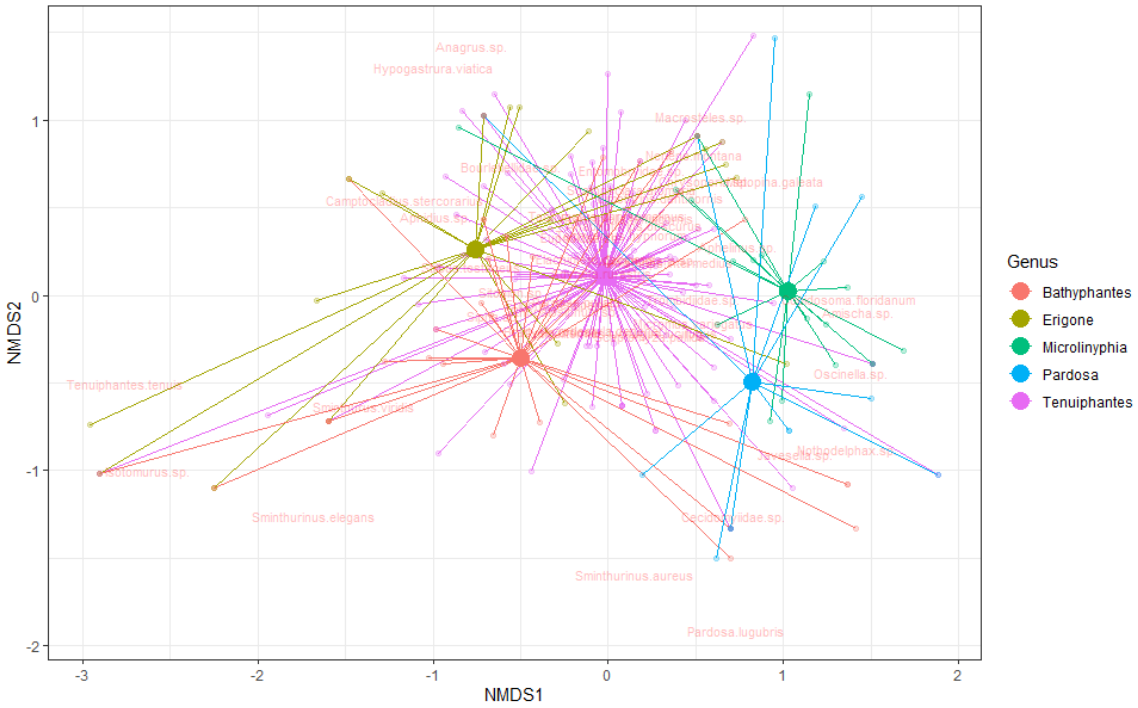


Figure S4.6: Spider plot based on NMDS of spider diets with colours denoting spider genera (red, gold, green, blue and purple denoting Bathyphantes, Erigone, Microlinyphia, Pardosa and Tenuiphantes, respectively). Large central nodes represent the mean coordinates for the individual diets (small points) of each genus. Prey species are overlaid in red text to denote their mean occurrence in spider diets across the sampling period.

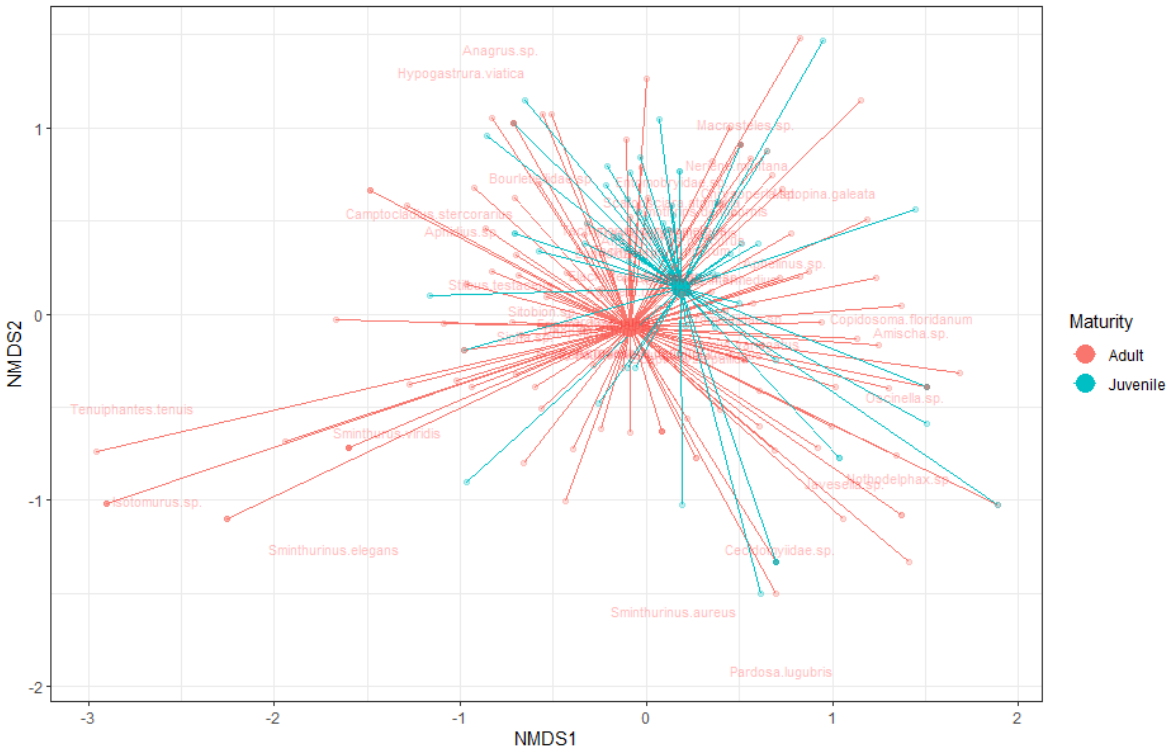


Figure S4.7: Spider plot based on NMDS of spider diets with colours denoting spider life stages (red and blue denoting adult and juvenile, respectively). Large central nodes represent the mean coordinates for the individual diets (small points) of each genus. Prey species are overlaid in red text to denote their mean occurrence in spider diets across the sampling period.

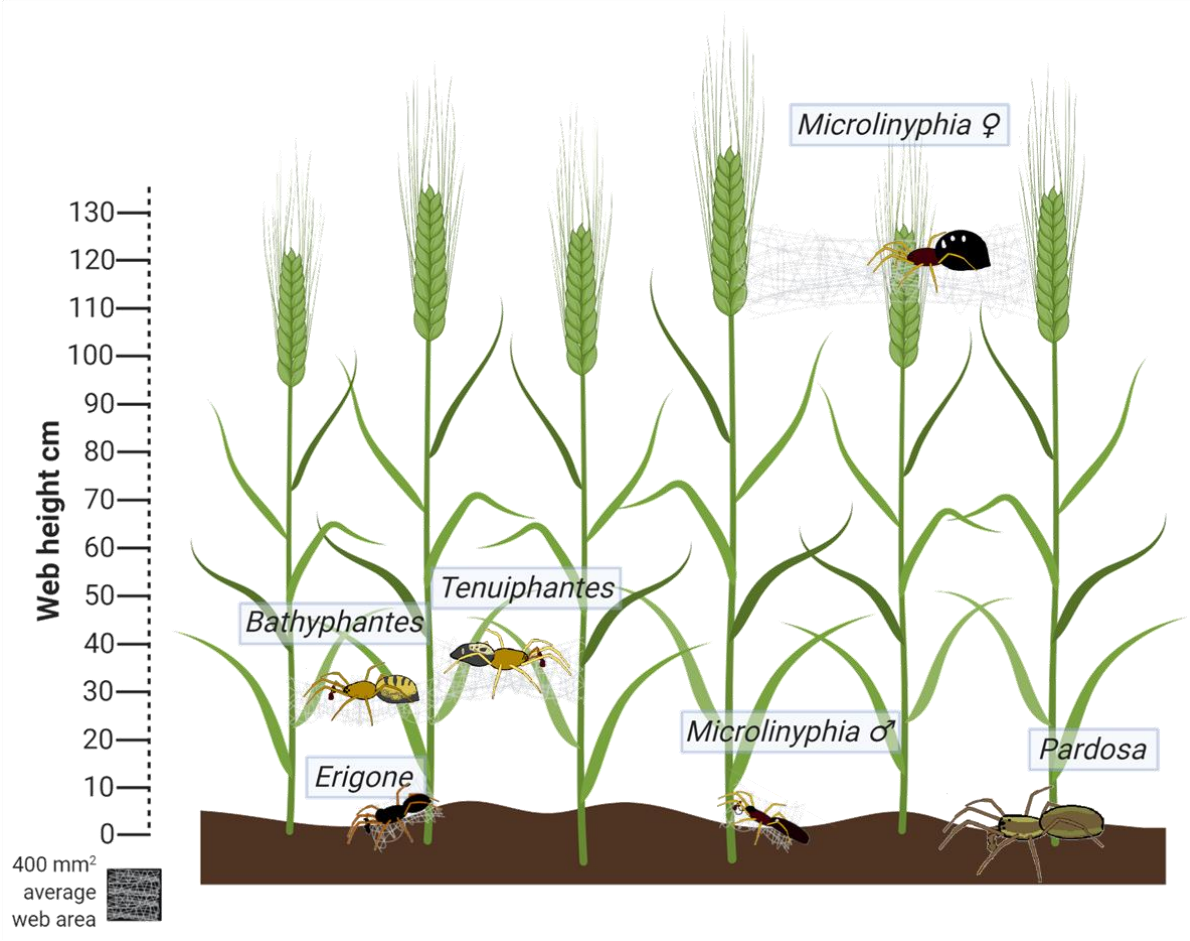


Figure S4.8: An approximate visual representation of web height and area of the five genera studied: *Bathyphantes*, *Tenuiphantes*, *Erigone* and *Microlinyphia*, with ground-hunting *Pardosa* shown without a web. Figure created in Biorender with spiders imported from custom MS paint drawings.

Table S4.1: Positive control composition. All DNA concentrations were ascertained via Qubit dsDNA High-sensitivity Assay Kits and diluted to standardise at 4 ng µl-1. All samples were taken from an existing collection of invertebrate DNA at Cardiff University from Round Island, Mauritius.

Species	Mix 1	Mix 2	Mix 3	Mix 4
Pheidole megacephala	9	12	6	12
O. ancestor	9	6	12	6
Oligotoma saundersii	9	12	6	6
Diptera 4	9	6	12	12
Coleoptera 2	9	12	6	12
Hemiptera 6	9	6	12	6

Table S4.2: Classification of prey as pests and predators of pests. Excluded prey were not deemed a pest or predator/parasitoid of pests.

Taxon	Classification	Rationale
Acrodactyla degener	Parasitoid	Spider parasitoid
Aeolothrips intermedius	Predator	Predator of other thrips and pollen
Agyneta rurestris	Predator	Web-building predator
Amischa sp.	Predator	Active predator
Anagrus sp.	Parasitoid	Leafhopper parasitoid
Anaphothrips obscurus	Pest	Feed on cereals
Anotylus tetracarinatus	Predator	Active predator
Aphelinus sp.	Parasitoid	Aphid parasitoid
Aphidius sp.	Parasitoid	Aphid parasitoid
Bourletiellidae sp.	Pest	Feed on emerging seedlings
Bradysia urticae	Pest	Feed on plants
Cecidomyiidae sp.	Pest	Galls in cereals
Centromerita bicolor	Predator	Web-building predator
Chrysoperla sp.	Predator	Larval active predators
Copidosoma floridanum	Parasitoid	Moth parasitoid
Corynoptera sp.	Pest	Larval root pest
Elachiptera decipens	Pest	Feed on grasses
Erigone dentipalpis	Predator	Web-building predator
Eupodidae sp.	Pest	Some pest species (e.g. *Halotydeus destructor*)
Frankliniella tenuicornis	Pest	Breeds in cereal crops and can damage
Javesella sp.	Pest	Feed on cereals, cause hopperburn and spread disease
Limothrips denticornis	Pest	Feed on cereals
Macrosteles sp.	Pest	Feed on cereals, cause hopperburn and spread disease
Micromus variegatus	Predator	Both adult and larval predators of pests
Neriene montana	Predator	Web-building predator
Nothodelphax sp.	Pest	Feed on cereals, cause hopperburn and spread disease
Oscinella sp.	Pest	Bore into grasses
Pardosa amentata	Predator	Active predator
Pardosa lugubris	Predator	Active predator
Pardosa pullata	Predator	Active predator
Reticulitermes lucifugus lucifugus	Pest	Damage to infrastructure
Rhopalosiphum sp.	Pest	Common cereal pest
Scaptomyza pallida	Pest	Brassica pest
Scatopsciara atomaria	Pest	Larval root pest
Sipha sp.	Pest	Feed on grasses
Sitobion sp.	Pest	Common cereal pest
Sminthurinus aureus	Pest	Pest of grasses
Sminthurinus elegans	Pest	Pest of grasses
Sminthurus viridis	Pest	Pest of grasses
Tachyporus chrysomelinus	Predator	Active predator
Tachyporus hypnorum	Predator	Active predator
Tenuiphantes tenuis	Predator	Active predator

Trombidiidae sp.	Predator	Feed on pests

Table S4.3: Spider dietary contents for all spiders and each of the five focal genera represented as the number of spiders that consumed each prey taxon ('# Spiders'), the percentage of spiders that consumed each prey taxon ('% Spiders') and the minimum number of individuals of that taxon that were predated ('# Prey'; accounting for different species within the same family). Percentages are coloured with darker green denoting higher percentages relative to the largest percentage.

	All Spiders			*Bathyphantes*			*Erigone*			*Microlinyphia*			*Pardosa*			*Tenuiphantes*		
Family	**# Spiders**	**% Spiders**	**# Prey**	**# Spiders**	**% Spiders**	**# Prey**	**# Spiders**	**% Spiders**	**# Prey**	**# Spiders**	**% Spiders**	**# Prey**	**# Spiders**	**% Spiders**	**# Prey**	**# Spiders**	**% Spiders**	**# Prey**
Aeolothripidae	4	1.64	4	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	**4**	3.23	4
Anthocoridae	1	0.41	1	0	0.00	0	1	2.86	1	0	0.00	0	0	0.00	0	**0**	0.00	0
Anthomyiidae	1	0.41	1	0	0.00	0	0	0.00	0	1	3.85	1	0	0.00	0	**0**	0.00	0
Aphelinidae	12	4.92	12	0	0.00	0	2	5.71	2	3	11.54	3	1	4.76	1	**6**	4.84	6
Aphididae	47	19.26	53	10	26.32	12	5	14.29	6	1	3.85	1	0	0.00	0	**31**	25.00	34
Bourletiellidae	38	15.57	38	1	2.63	1	6	17.14	6	1	3.85	1	1	4.76	1	**29**	23.39	29
Braconidae	15	6.15	15	5	13.16	5	3	8.57	3	0	0.00	0	0	0.00	0	7	5.65	7
Cecidomyiidae	22	9.02	23	4	10.53	4	0	0.00	0	3	11.54	4	9	42.86	9	6	4.84	6
Chironomidae	4	1.64	4	1	2.63	1	0	0.00	0	1	3.85	1	0	0.00	0	2	1.61	2
Chloropidae	34	13.93	35	1	2.63	1	3	8.57	3	17	65.38	18	3	14.29	3	10	8.06	10
Chrysopidae	2	0.82	2	0	0.00	0	0	0.00	0	1	3.85	1	0	0.00	0	1	0.81	1
Cicadellidae	12	4.92	12	2	5.26	2	0	0.00	0	2	7.69	2	1	4.76	1	7	5.65	7
Damaeidae	1	0.41	1	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	1	0.81	1
Delphacidae	16	6.56	23	3	7.89	5	1	2.86	1	1	3.85	1	2	9.52	2	9	7.26	14
Dolichopodidae	1	0.41	1	1	2.63	1	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0
Drosophilidae	4	1.64	4	1	2.63	1	0	0.00	0	1	3.85	1	0	0.00	0	2	1.61	2
Encyrtidae	2	0.82	2	0	0.00	0	0	0.00	0	1	3.85	1	0	0.00	0	1	0.81	1
Entomobryidae	7	2.87	7	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	7	5.65	7
Ephydridae	1	0.41	1	0	0.00	0	1	2.86	1	0	0.00	0	0	0.00	0	0	0.00	0
Eupodidae	41	16.80	41	9	23.68	9	0	0.00	0	0	0.00	0	0	0.00	0	32	25.81	32
Figitidae	1	0.41	1	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	1	0.81	1
Hemerobiidae	2	0.82	2	0	0.00	0	0	0.00	0	1	3.85	1	0	0.00	0	1	0.81	1
Hemipteran Family	2	0.82	2	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	2	1.61	2
Hypogastruridae	3	1.23	3	0	0.00	0	2	5.71	2	0	0.00	0	0	0.00	0	1	0.81	1
Ichneumonidae	4	1.64	4	1	2.63	1	0	0.00	0	2	7.69	2	0	0.00	0	1	0.81	1
Isotomidae	12	4.92	12	4	10.53	4	4	11.43	4	0	0.00	0	0	0.00	0	4	3.23	4
Katiannidae	8	3.28	8	5	13.16	5	1	2.86	1	0	0.00	0	0	0.00	0	2	1.61	2

Linyphiidae	17	6.97	18	1	2.63	1	5	14.29	6	0	0.00	0	0	0.00	0	11	8.87	11
Lycosidae	10	4.10	10	0	0.00	0	0	0.00	0	1	3.85	1	5	23.81	5	4	3.23	4
Mymaridae	2	0.82	2	0	0.00	0	1	2.86	1	0	0.00	0	0	0.00	0	1	0.81	1
Nabidae	1	0.41	1	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	1	0.81	1
Noctuidae	1	0.41	1	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	1	0.81	1
Phalacridae	2	0.82	2	2	5.26	2	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0
Phoridae	8	3.28	8	0	0.00	0	2	5.71	2	3	11.54	3	1	4.76	1	2	1.61	2
Psychodidae	1	0.41	1	1	2.63	1	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0
Rhinotermitidae	45	18.44	45	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	45	36.29	45
Sciaridae	25	10.25	26	3	7.89	3	2	5.71	2	1	3.85	1	1	4.76	1	18	14.52	19
Sminthuridae	18	7.38	18	10	26.32	10	2	5.71	2	0	0.00	0	0	0.00	0	6	4.84	6
Sphaeroceridae	3	1.23	3	2	5.26	2	0	0.00	0	0	0.00	0	0	0.00	0	1	0.81	1
Staphylinidae	12	4.92	13	1	2.63	1	3	8.57	3	2	7.69	2	1	4.76	1	5	4.03	6
Syrphidae	2	0.82	2	0	0.00	0	1	2.86	1	0	0.00	0	0	0.00	0	1	0.81	1
Thripidae	88	36.07	143	7	18.42	9	8	22.86	8	8	30.77	11	1	4.76	1	64	51.61	114
Tomoceridae	1	0.41	1	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	1	0.81	1
Triozidae	1	0.41	1	0	0.00	0	1	2.86	1	0	0.00	0	0	0.00	0	0	0.00	0
Trombidiidae	9	3.69	9	0	0.00	0	1	2.86	1	1	3.85	1	1	4.76	1	6	4.84	6

Table S4.4: Spider dietary contents for all spiders and the two sexes represented as the number of spiders that predated each prey taxon ('# Spiders'), the percentage of spiders that predated each prey taxon ('% Spiders') and the minimum number of individuals of that taxon that were predated ('# Prey'; accounting for different species within the same family). Percentages are coloured with darker blue denoting higher percentages relative to the largest percentage.

	All Spiders			Female			Male		
Family	# Spiders	% Spiders	# Prey	# Spiders	% Spiders	# Prey	# Spiders	% Spiders	# Prey
Aeolothripidae	4	1.64	4	4	3.81	4	0	0.00	0
Anthocoridae	1	0.41	1	1	0.95	1	0	0.00	0
Anthomyiidae	1	0.41	1	1	0.95	1	0	0.00	0
Aphelinidae	12	4.92	12	6	5.71	6	5	5.38	5
Aphididae	47	19.26	53	14	13.33	16	27	29.03	30
Bourletiellidae	38	15.57	38	10	9.52	10	21	22.58	21
Braconidae	15	6.15	15	10	9.52	10	5	5.38	5
Cecidomyiidae	22	9.02	23	6	5.71	7	6	6.45	6
Chironomidae	4	1.64	4	2	1.90	2	1	1.08	1
Chloropidae	34	13.93	35	21	20.00	22	9	9.68	9
Chrysopidae	2	0.82	2	0	0.00	0	2	2.15	2
Cicadellidae	12	4.92	12	4	3.81	4	3	3.23	3
Damaeidae	1	0.41	1	1	0.95	1	0	0.00	0
Delphacidae	16	6.56	23	10	9.52	13	3	3.23	6
Dolichopodidae	1	0.41	1	1	0.95	1	0	0.00	0
Drosophilidae	4	1.64	4	4	3.81	4	0	0.00	0
Encyrtidae	2	0.82	2	2	1.90	2	0	0.00	0
Entomobryidae	7	2.87	7	2	1.90	2	3	3.23	3
Ephydridae	1	0.41	1	0	0.00	0	1	1.08	1
Eupodidae	41	16.80	41	11	10.48	11	15	16.13	15
Figitidae	1	0.41	1	1	0.95	1	0	0.00	0
Hemerobiidae	2	0.82	2	0	0.00	0	2	2.15	2
Hemipteran Family	2	0.82	2	0	0.00	0	2	2.15	2
Hypogastruridae	3	1.23	3	2	1.90	2	1	1.08	1
Ichneumonidae	4	1.64	4	2	1.90	2	1	1.08	1
Isotomidae	12	4.92	12	7	6.67	7	5	5.38	5
Katiannidae	8	3.28	8	4	3.81	4	3	3.23	3
Linyphiidae	17	6.97	18	9	8.57	10	5	5.38	5
Lycosidae	10	4.10	10	5	4.76	5	2	2.15	2
Mymaridae	2	0.82	2	2	1.90	2	0	0.00	0
Nabidae	1	0.41	1	1	0.95	1	0	0.00	0
Noctuidae	1	0.41	1	0	0.00	0	0	0.00	0
Phalacridae	2	0.82	2	1	0.95	1	1	1.08	1
Phoridae	8	3.28	8	6	5.71	6	1	1.08	1
Psychodidae	1	0.41	1	1	0.95	1	0	0.00	0
Rhinotermitidae	45	18.44	45	17	16.19	17	21	22.58	21

Sciaridae	25	10.25	26	14	13.33	15	8	8.60	8
Sminthuridae	18	7.38	18	13	12.38	13	5	5.38	5
Sphaeroceridae	3	1.23	3	3	2.86	3	0	0.00	0
Staphylinidae	12	4.92	13	9	8.57	10	2	2.15	2
Syrphidae	2	0.82	2	1	0.95	1	1	1.08	1
Thripidae	88	36.07	143	23	21.90	32	44	47.31	74
Tomoceridae	1	0.41	1	0	0.00	0	1	1.08	1
Triozidae	1	0.41	1	1	0.95	1	0	0.00	0
Trombidiidae	9	3.69	9	3	2.86	3	6	6.45	6

Table S4.5: Spider dietary contents for all spiders and the two life stages represented as the number of spiders that predated each prey taxon ('# Spiders'), the percentage of spiders that predated each prey taxon ('% Spiders') and the minimum number of individuals of that taxon that were predated ('# Prey'; accounting for different species within the same family). Percentages are coloured with darker blue denoting higher percentages relative to the largest percentage.

Family	All Spiders			Adult			Juvenile		
	# Spiders	% Spiders	# Prey	# Spiders	% Spiders	# Prey	# Spiders	% Spiders	# Prey
Aeolothripidae	4	1.64	4	3	1.79	3	1	1.32	1
Anthocoridae	1	0.41	1	1	0.60	1	0	0.00	0
Anthomyiidae	1	0.41	1	1	0.60	1	0	0.00	0
Aphelinidae	12	4.92	12	7	4.17	7	5	6.58	5
Aphididae	47	19.26	53	35	20.83	40	12	15.79	13
Bourletiellidae	38	15.57	38	22	13.10	22	16	21.05	16
Braconidae	15	6.15	15	14	8.33	14	1	1.32	1
Cecidomyiidae	22	9.02	23	12	7.14	13	10	13.16	10
Chironomidae	4	1.64	4	3	1.79	3	1	1.32	1
Chloropidae	34	13.93	35	27	16.07	28	7	9.21	7
Chrysopidae	2	0.82	2	2	1.19	2	0	0.00	0
Cicadellidae	12	4.92	12	5	2.98	5	7	9.21	7
Damaeidae	1	0.41	1	1	0.60	1	0	0.00	0
Delphacidae	16	6.56	23	13	7.74	19	3	3.95	4
Dolichopodidae	1	0.41	1	1	0.60	1	0	0.00	0
Drosophilidae	4	1.64	4	4	2.38	4	0	0.00	0
Encyrtidae	2	0.82	2	2	1.19	2	0	0.00	0
Entomobryidae	7	2.87	7	1	0.60	1	6	7.89	6
Ephydridae	1	0.41	1	1	0.60	1	0	0.00	0
Eupodidae	41	16.80	41	16	9.52	16	25	32.89	25
Figitidae	1	0.41	1	1	0.60	1	0	0.00	0
Hemerobiidae	2	0.82	2	2	1.19	2	0	0.00	0
Hemipteran Family	2	0.82	2	2	1.19	2	0	0.00	0
Hypogastruridae	3	1.23	3	3	1.79	3	0	0.00	0
Ichneumonidae	4	1.64	4	4	2.38	4	0	0.00	0
Isotomidae	12	4.92	12	11	6.55	11	1	1.32	1
Katiannidae	8	3.28	8	6	3.57	6	2	2.63	2
Linyphiidae	17	6.97	18	13	7.74	14	4	5.26	4
Lycosidae	10	4.10	10	5	2.98	5	5	6.58	5
Mymaridae	2	0.82	2	1	0.60	1	1	1.32	1
Nabidae	1	0.41	1	1	0.60	1	0	0.00	0
Noctuidae	1	0.41	1	0	0.00	0	1	1.32	1
Phalacridae	2	0.82	2	1	0.60	1	1	1.32	1
Phoridae	8	3.28	8	5	2.98	5	3	3.95	3

Psychodidae	1	0.41	1	1	0.60	1	0	0.00	0
Rhinotermitidae	45	18.44	45	29	17.26	29	16	21.05	16
Sciaridae	25	10.25	26	18	10.71	19	7	9.21	7
Sminthuridae	18	7.38	18	18	10.71	18	0	0.00	0
Sphaeroceridae	3	1.23	3	3	1.79	3	0	0.00	0
Staphylinidae	12	4.92	13	11	6.55	12	1	1.32	1
Syrphidae	2	0.82	2	2	1.19	2	0	0.00	0
Thripidae	88	36.07	143	46	27.38	63	42	55.26	80
Tomoceridae	1	0.41	1	0	0.00	0	1	1.32	1
Triozidae	1	0.41	1	1	0.60	1	0	0.00	0
Trombidiidae	9	3.69	9	9	5.36	9	0	0.00	0

Table S4.6: Species co-occurrence across spider diets. The probability corresponds to the probability that the respective species co-occur more or less than expected (listed as 'relationship', with '+' and '-' denoting positive and negative co-occurrences, respectively).

Species 1	Species 2	Observed	Expected	Probability	Relationship
Anaphothrips obscurus	Cecidomyiidae sp.	0	4.7	0.0039	-
Anaphothrips obscurus	Entomobryidae sp.	5	1.5	0.00552	+
Anaphothrips obscurus	Eupodidae sp.	23	8.7	0	+
Anaphothrips obscurus	*Frankliniella tenuicornis*	18	4.9	0	+
Anaphothrips obscurus	*Limothrips denticornis*	34	14.1	0	+
Anaphothrips obscurus	*Macrosteles* sp.	7	2.3	0.00228	+
Anaphothrips obscurus	*Oscinella* sp.	2	6.4	0.02379	-
Anaphothrips obscurus	*Reticulitermes lucifugus*	16	9.6	0.01076	+
Anaphothrips obscurus	*Sminthurus viridis*	0	3.8	0.01118	-
Aphelinus sp.	*Frankliniella tenuicornis*	4	1.1	0.01801	+
Aphelinus sp.	*Limothrips denticornis*	9	3.2	0.00053	+
Aphidius sp.	Bourletiellidae sp.	6	2.3	0.01661	+
Bourletiellidae sp.	*Oscinella* sp.	1	4.7	0.0323	-
Bourletiellidae sp.	*Sitobion* sp.	9	5.1	0.04727	+
Cecidomyiidae sp.	Eupodidae sp.	0	3.7	0.01424	-
Cecidomyiidae sp.	*Limothrips denticornis*	2	6	0.03337	-
Eupodidae sp.	*Frankliniella tenuicornis*	11	3.9	0.00025	+
Eupodidae sp.	*Limothrips denticornis*	18	11.1	0.00823	+
Eupodidae sp.	*Reticulitermes lucifugus*	14	7.6	0.0063	+
Frankliniella tenuicornis	*Limothrips denticornis*	14	6.2	0.00035	+
Isotomurus sp.	*Limothrips denticornis*	0	3.2	0.02047	-
Limothrips denticornis	*Nothodelphax* sp.	0	3.2	0.02047	-
Limothrips denticornis	*Oscinella* sp.	2	8.1	0.00366	-
Limothrips denticornis	*Reticulitermes lucifugus*	19	12.2	0.01097	+
Limothrips denticornis	*Sminthurus viridis*	0	4.9	0.00268	-
Reticulitermes lucifugus	*Rhopalosiphum* sp.	5	2.2	0.04914	+

Chapter 5 Supplementary Material

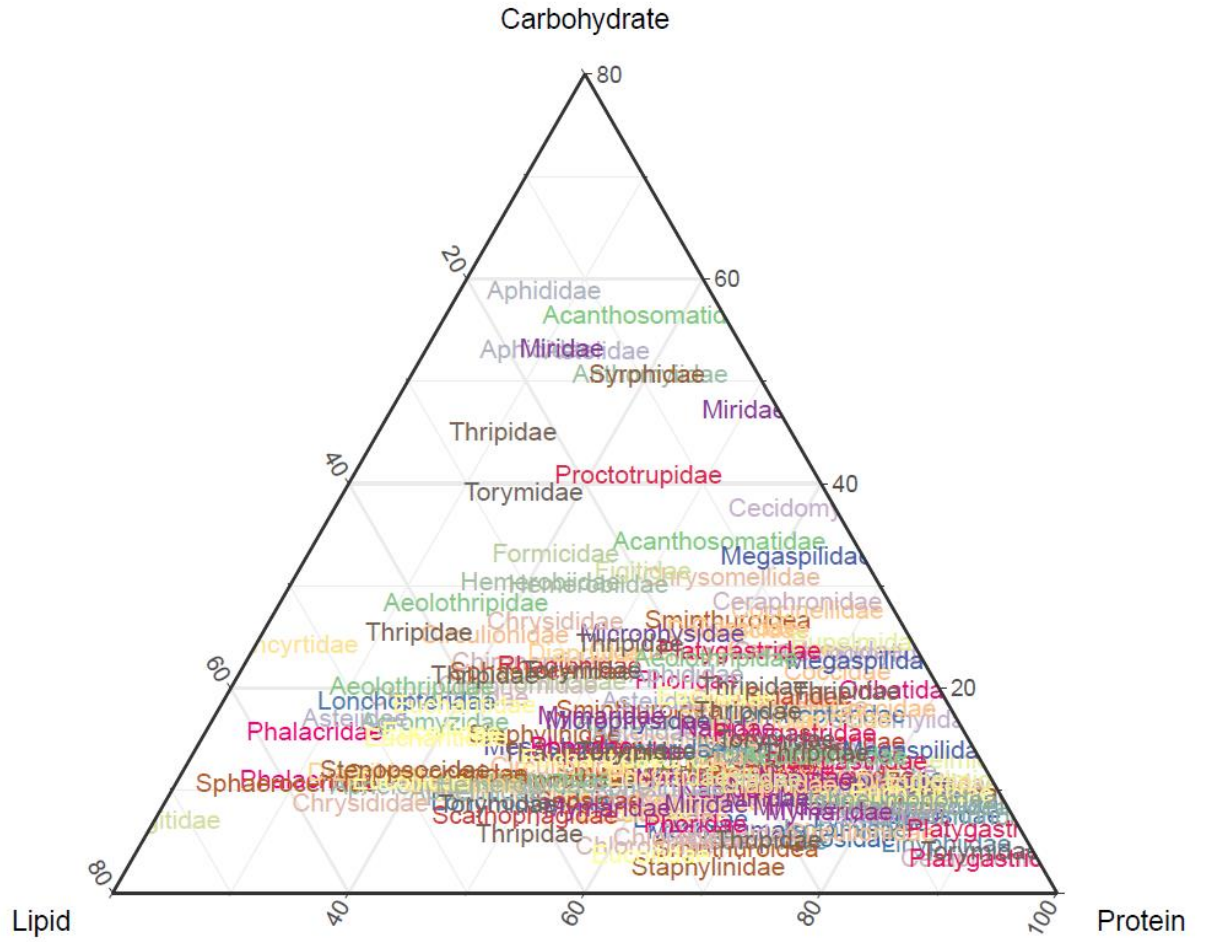


Figure S5.1: Ternary plot of macronutrient content of the taxa (each family label) for which macronutrient content was determined, coloured by family. Proximity to each labelled point of the triangle corresponds to the proportion of that macronutrient in the body of the invertebrate.

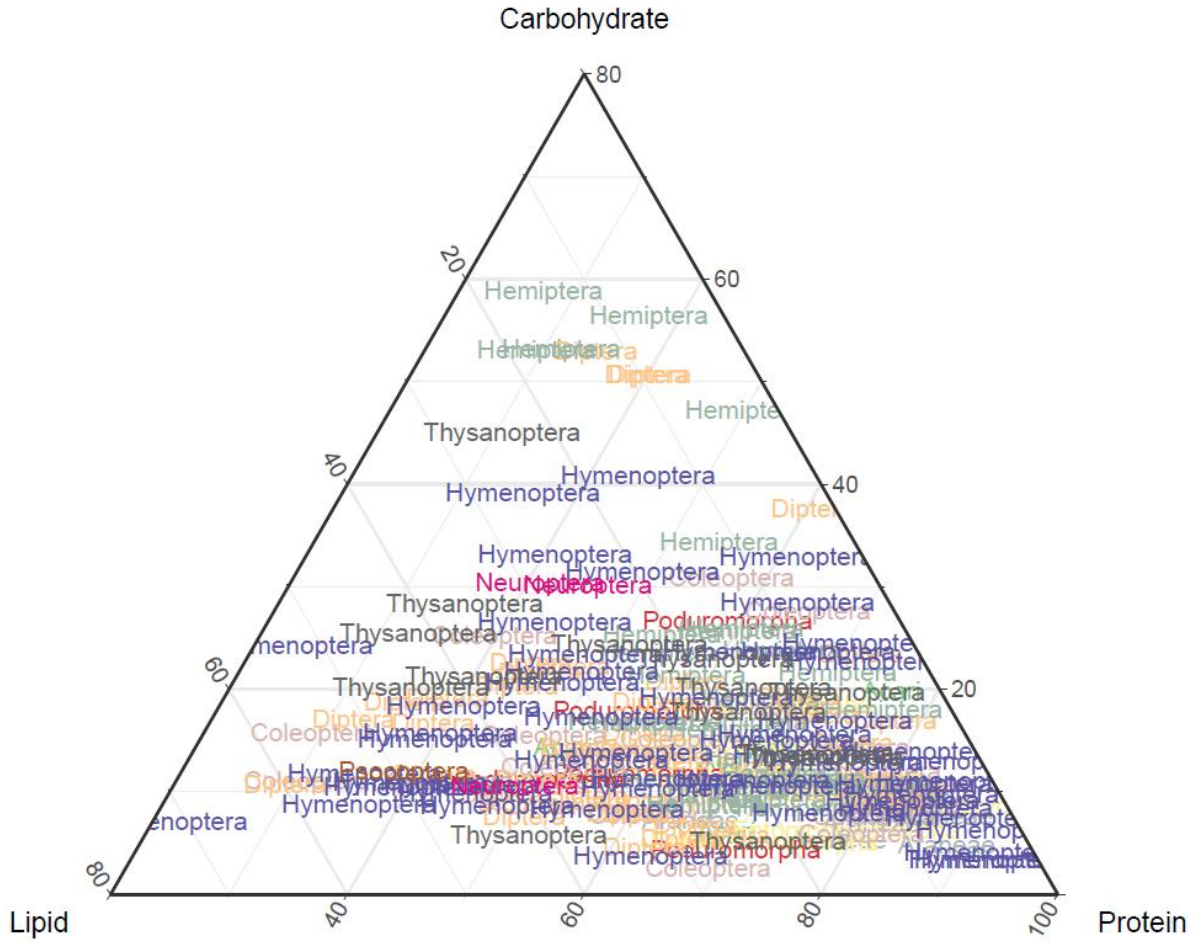


Figure S5.2: Ternary plot of macronutrient content of the taxa (each taxonomic order label) for which macronutrient content was determined, coloured by order. Proximity to each labelled point of the triangle corresponds to the proportion of that macronutrient in the body of the invertebrate.

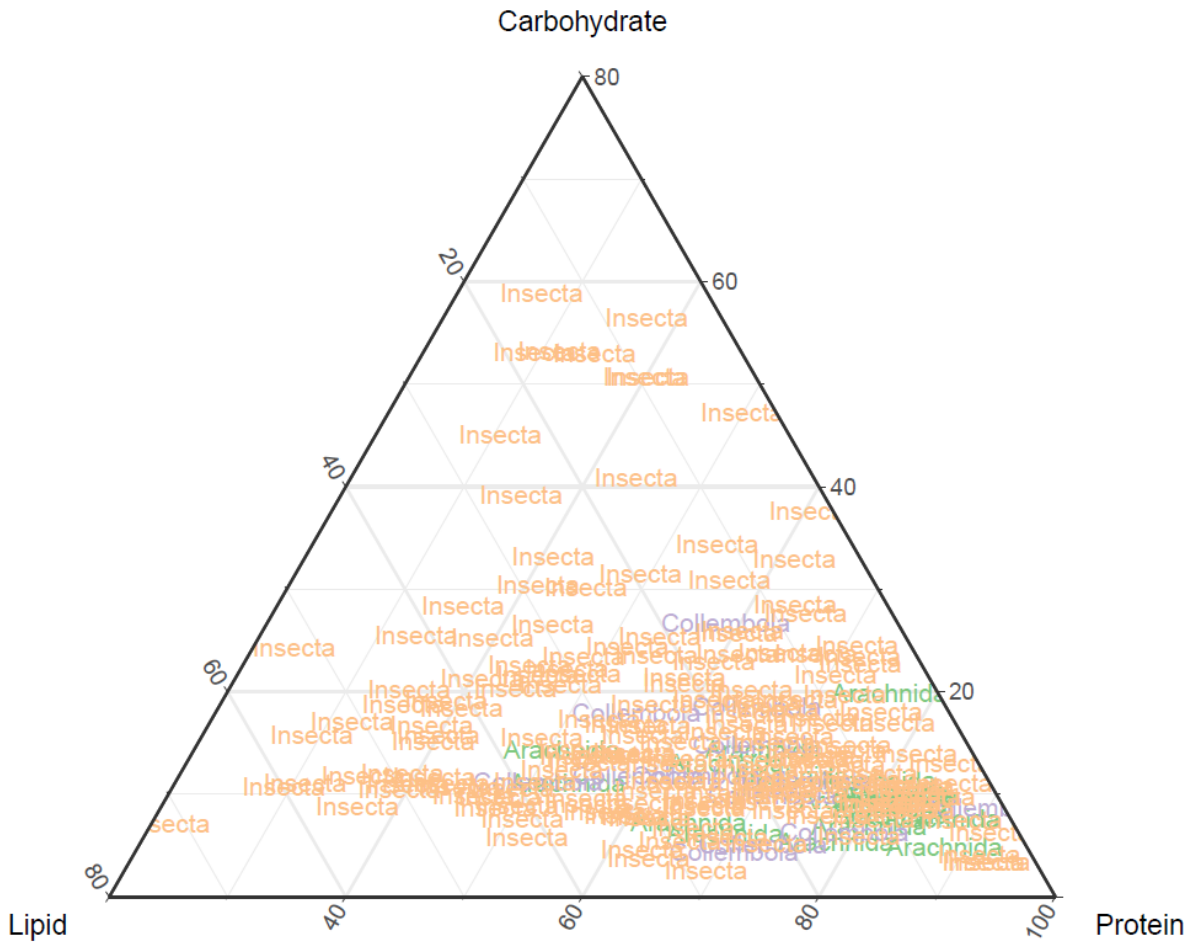


Figure S5.3: Ternary plot of macronutrient content of the taxa (each class label) for which macronutrient content was determined, coloured by class. Proximity to each labelled point of the triangle corresponds to the proportion of that macronutrient in the body of the invertebrate.

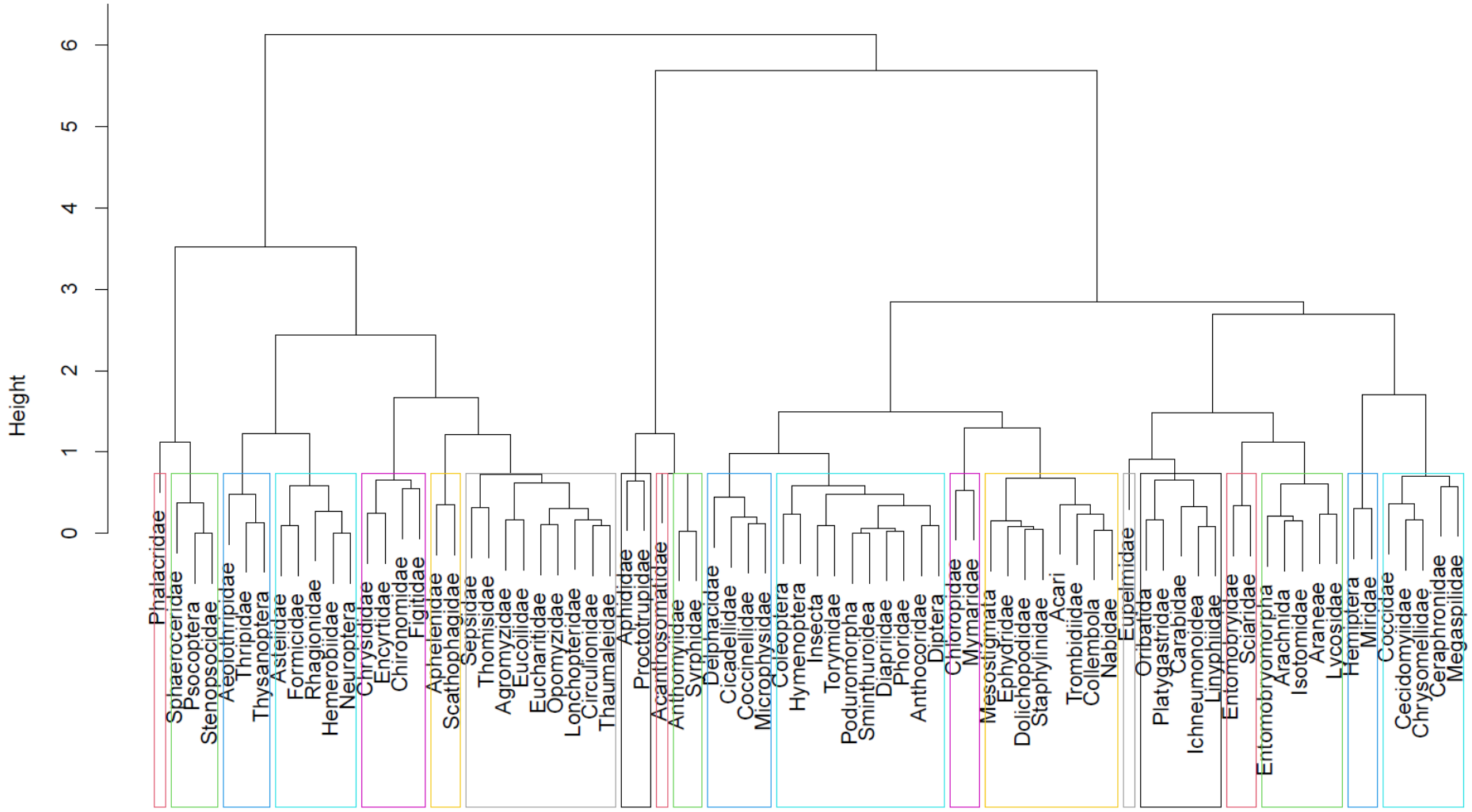


Figure S5.4: Hierarchical clustering dendrogram of macronutrient content for 64 taxa. Coloured rectangles denote separation of taxa into 20 clusters, termed "tropho-species".

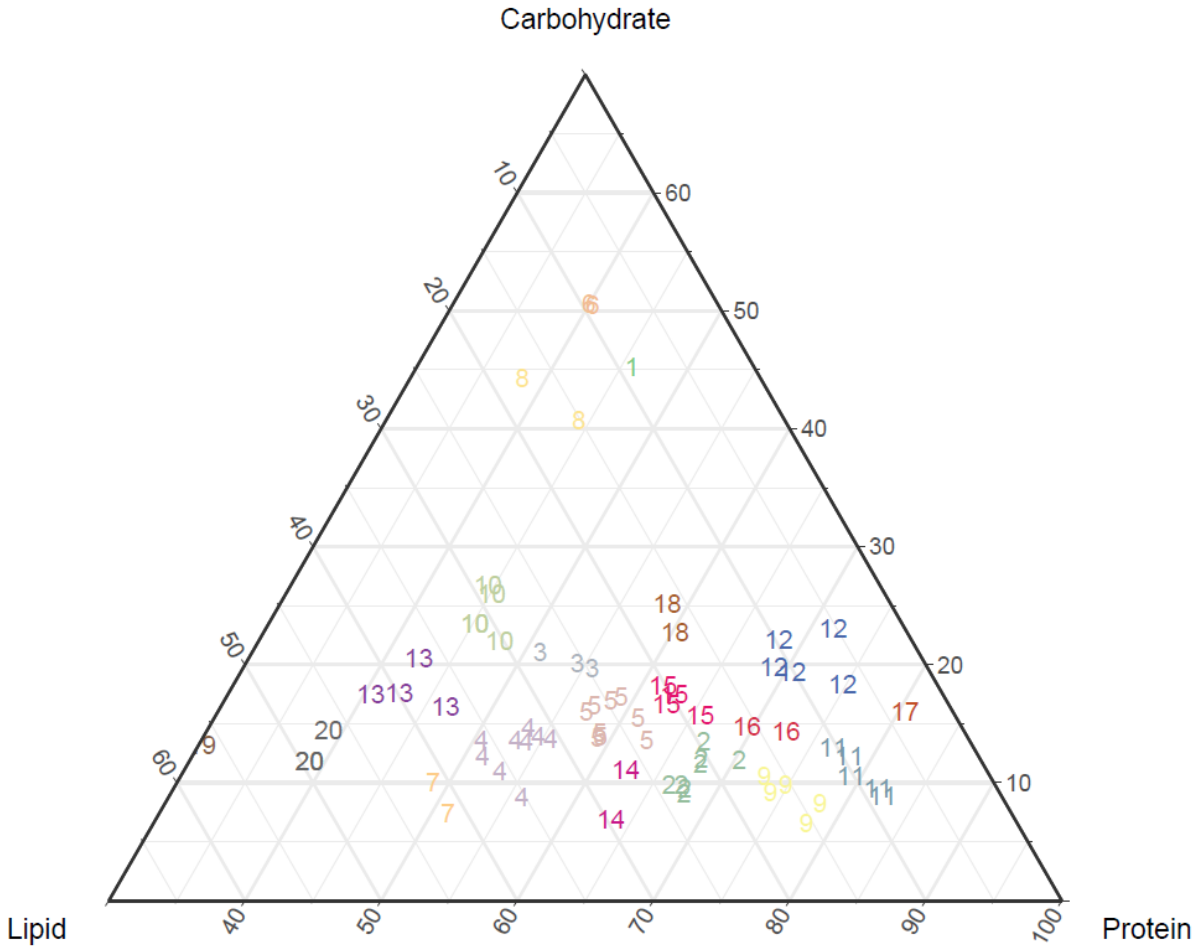
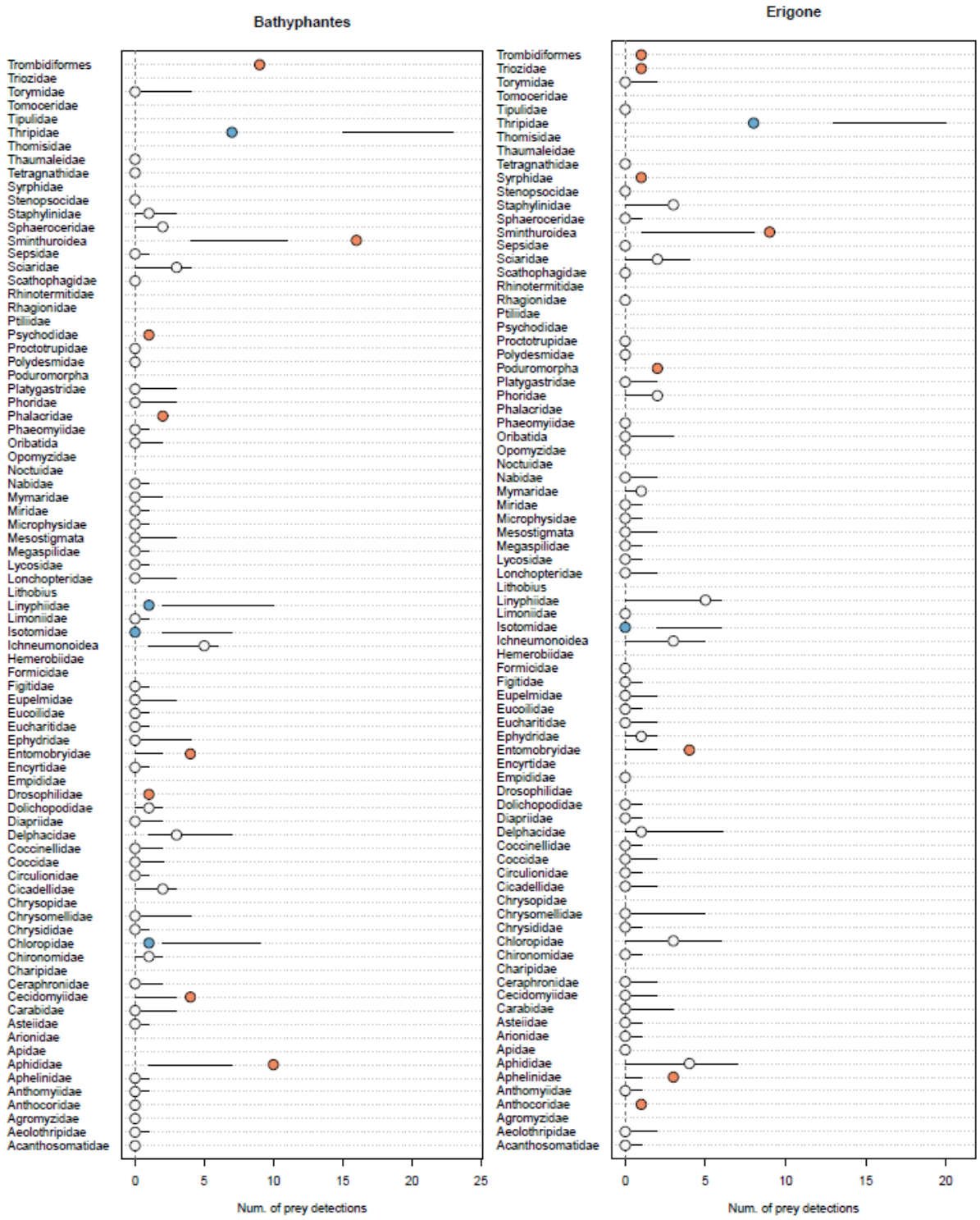
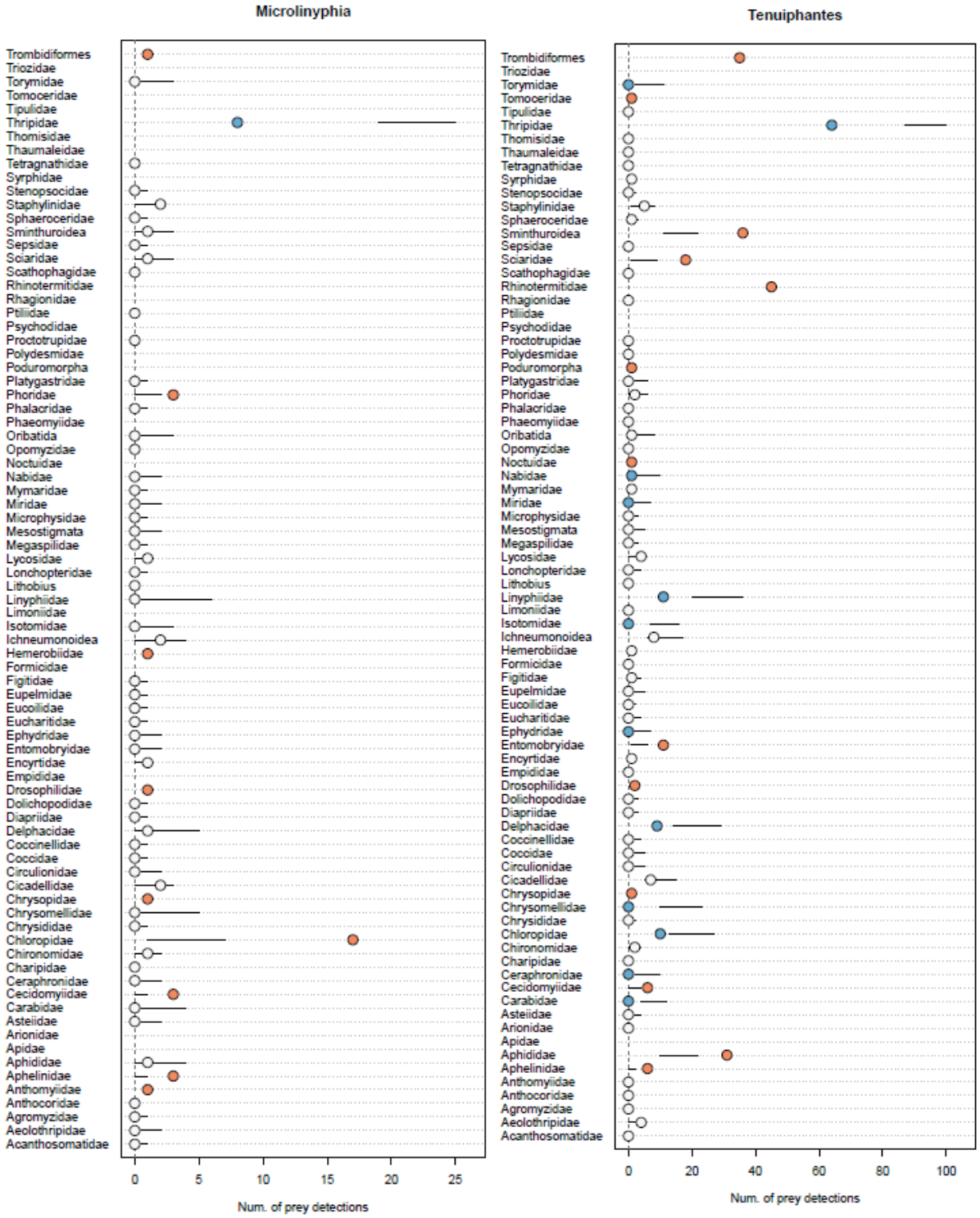


Figure S5.5: Ternary plot of macronutrient content of the taxa (each numerical label) assigned to each of the 20 clusters (each colour and cluster number), equating to tropho-species. Proximity to each labelled point of the triangle corresponds to the proportion of that macronutrient in the body of the invertebrate.


Bathyphantes
Trombidiformes
Triozidae
Torymidae
Tomoceridae
Tipulidae
Thripidae
Thomisidae
Thaumaleidae
Tetragnathidae
Syrphidae
Stenopsocidae
Staphylinidae
Sphaeroceridae
Sminthuroidea
Sepsidae
Sciaridae
Scathophagidae
Rhinotermitidae
Rhagionidae
Ptiliidae
Psychodidae
Proctotrupidae
Polydesmidae
Poduromorpha
Platygastridae
Phoridae
Phalacridae
Phaeomyiidae
Oribatida
Opomyzidae
Noctuidae
Nabidae
Mymaridae
Miridae
Microphysidae
Mesostigmata
Megaspilidae
Lycosidae
Lonchopteridae
Lithobius
Linyphiidae
Limoniidae
Isotomidae
Ichneumonoidea
Hemerobiidae
Formicidae
Figitidae
Eupelmidae
Eucoilidae
Eucharitidae
Ephydridae
Entomobryidae
Encyrtidae
Empididae
Drosophilidae
Dolichopodidae
Diapriidae
Delphacidae
Coccinellidae
Coccidae
Circulionidae
Cicadellidae
Chrysopidae
Chrysomellidae
Chrysididae
Chloropidae
Chironomidae
Charipidae
Ceraphronidae
Cecidomyiidae
Carabidae
Asteiidae
Arionidae
Apidae
Aphididae
Aphelinidae
Anthomyiidae
Anthocoridae
Agromyzidae
Aeolothripidae
Acanthosomatidae
0
5
10
15
20
25
Num. of prey detections
Erigone
Trombidiformes
Triozidae
Torymidae
Tomoceridae
Tipulidae
Thripidae
Thomisidae
Thaumaleidae
Tetragnathidae
Syrphidae
Stenopsocidae
Staphylinidae
Sphaeroceridae
Sminthuroidea
Sepsidae
Sciaridae
Scathophagidae
Rhinotermitidae
Rhagionidae
Ptiliidae
Psychodidae
Proctotrupidae
Polydesmidae
Poduromorpha
Platygastridae
Phoridae
Phalacridae
Phaeomyiidae
Oribatida
Opomyzidae
Noctuidae
Nabidae
Mymaridae
Miridae
Microphysidae
Mesostigmata
Megaspilidae
Lycosidae
Lonchopteridae
Lithobius
Linyphiidae
Limoniidae
Isotomidae
Ichneumonoidea
Hemerobiidae
Formicidae
Figitidae
Eupelmidae
Eucoilidae
Eucharitidae
Ephydridae
Entomobryidae
Encyrtidae
Empididae
Drosophilidae
Dolichopodidae
Diapriidae
Delphacidae
Coccinellidae
Coccidae
Circulionidae
Cicadellidae
Chrysopidae
Chrysomellidae
Chrysididae
Chloropidae
Chironomidae
Charipidae
Ceraphronidae
Cecidomyiidae
Carabidae
Asteiidae
Arionidae
Apidae
Aphididae
Aphelinidae
Anthomyiidae
Anthocoridae
Agromyzidae
Aeolothripidae
Acanthosomatidae
0
5
10
15
20
Num. of prey detections


Microlinyphia
Trombidiformes
Triozidae
Torymidae
Tomoceridae
Tipulidae
Thripidae
Thomisidae
Thaumaleidae
Tetragnathidae
Syrphidae
Stenopsocidae
Staphylinidae
Sphaeroceridae
Sminthuroidea
Sepsidae
Sciaridae
Scathophagidae
Rhinotermitidae
Rhagionidae
Ptiliidae
Psychodidae
Proctotrupidae
Polydesmidae
Poduromorpha
Platygastridae
Phoridae
Phalacridae
Phaeomyiidae
Oribatida
Opomyzidae
Noctuidae
Nabidae
Mymaridae
Miridae
Microphysidae
Mesostigmata
Megaspilidae
Lycosidae
Lonchopteridae
Lithobius
Linyphiidae
Limoniidae
Isotomidae
Ichneumonoidea
Hemerobiidae
Formicidae
Figitidae
Eupelmidae
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Eucharitidae
Ephydridae
Entomobryidae
Encyrtidae
Empididae
Drosophilidae
Dolichopodidae
Diapriidae
Delphacidae
Coccinellidae
Coccidae
Circulionidae
Cicadellidae
Chrysopidae
Chrysomelidae
Chrysididae
Chloropidae
Chironomidae
Charipidae
Ceraphronidae
Cecidomyiidae
Carabidae
Asteiidae
Arionidae
Apidae
Aphididae
Aphelinidae
Anthomyiidae
Anthocoridae
Agromyzidae
Aeolothripidae
Acanthosomatidae
0
5
10
15
20
25
Num. of prey detections
Tenuiphantes
Trombidiformes
Triozidae
Torymidae
Tomoceridae
Tipulidae
Thripidae
Thomisidae
Thaumaleidae
Tetragnathidae
Syrphidae
Stenopsocidae
Staphylinidae
Sphaeroceridae
Sminthuroidea
Sepsidae
Sciaridae
Scathophagidae
Rhinotermitidae
Rhagionidae
Ptiliidae
Psychodidae
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Phalacridae
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Oribatida
Opomyzidae
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Nabidae
Mymaridae
Miridae
Microphysidae
Mesostigmata
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Lycosidae
Lonchopteridae
Lithobius
Linyphiidae
Limoniidae
Isotomidae
Ichneumonoidea
Hemerobiidae
Formicidae
Figitidae
Eupelmidae
Eucoilidae
Eucharitidae
Ephydridae
Entomobryidae
Encyrtidae
Empididae
Drosophilidae
Dolichopodidae
Diapriidae
Delphacidae
Coccinellidae
Coccidae
Circulionidae
Cicadellidae
Chrysopidae
Chrysomelidae
Chrysididae
Chloropidae
Chironomidae
Charipidae
Ceraphronidae
Cecidomyiidae
Carabidae
Asteiidae
Arionidae
Apidae
Aphididae
Aphelinidae
Anthomyiidae
Anthocoridae
Agromyzidae
Aeolothripidae
Acanthosomatidae
0
20
40
60
80
100
Num. of prey detections

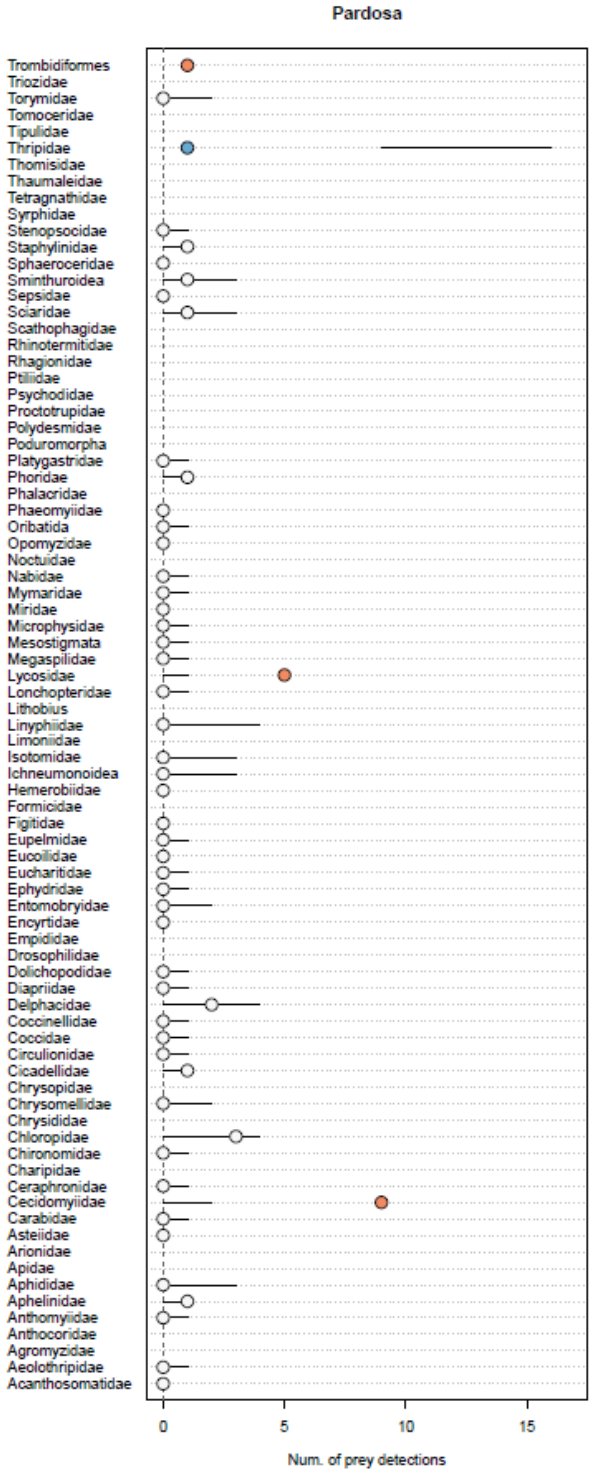


Figure S5.6: Significant deviations from expected frequencies of trophic interactions for spider genera predating prey taxa in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), white = as expected (in proportion to relative abundance), red = higher than expected (consumed more frequently than predicted from relative abundance).

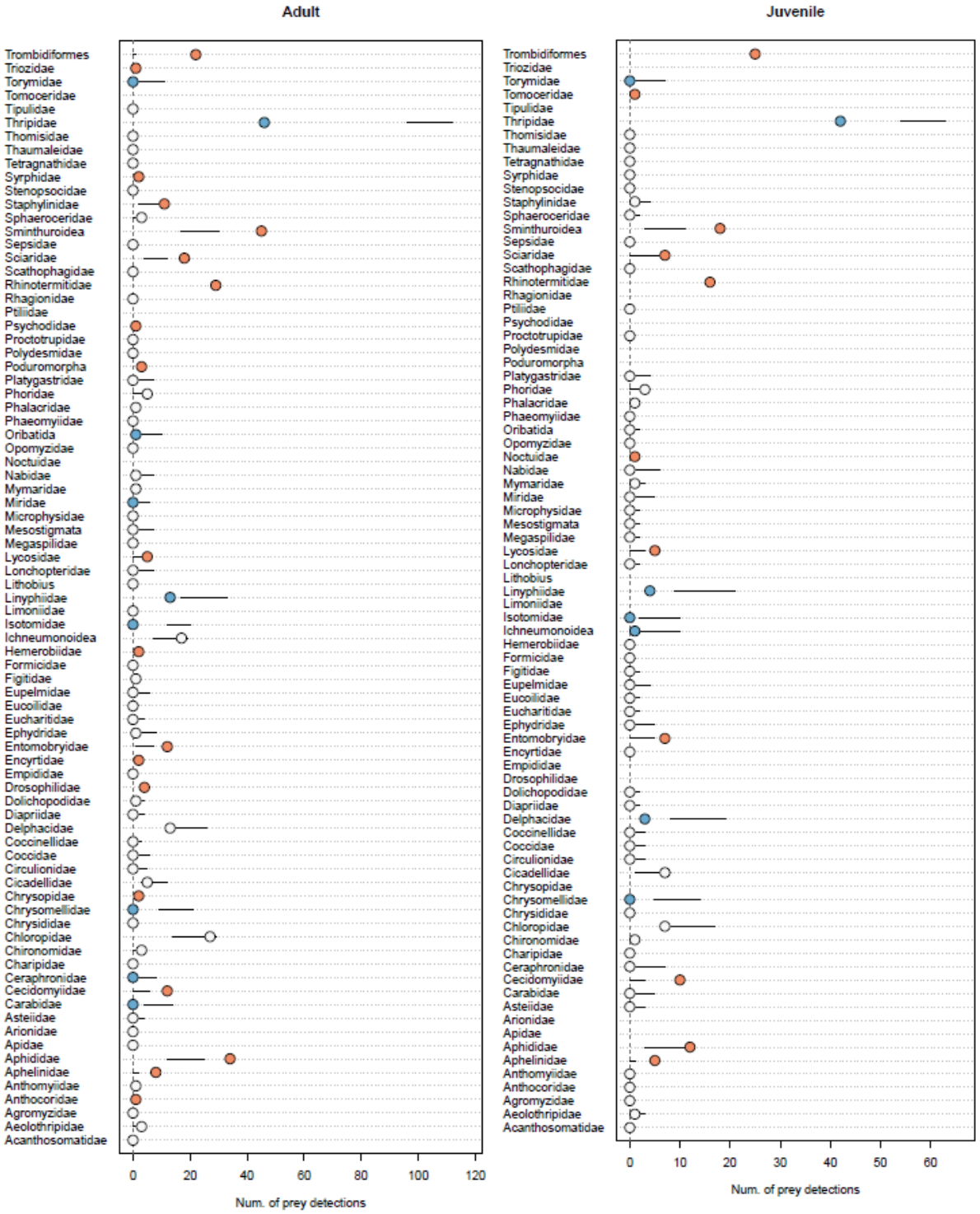


Figure S5.7: Significant deviations from expected frequencies of trophic interactions for spider life stages predating prey taxa in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), white = as expected (in proportion to relative abundance), red = higher than expected (consumed more frequently than predicted from relative abundance).

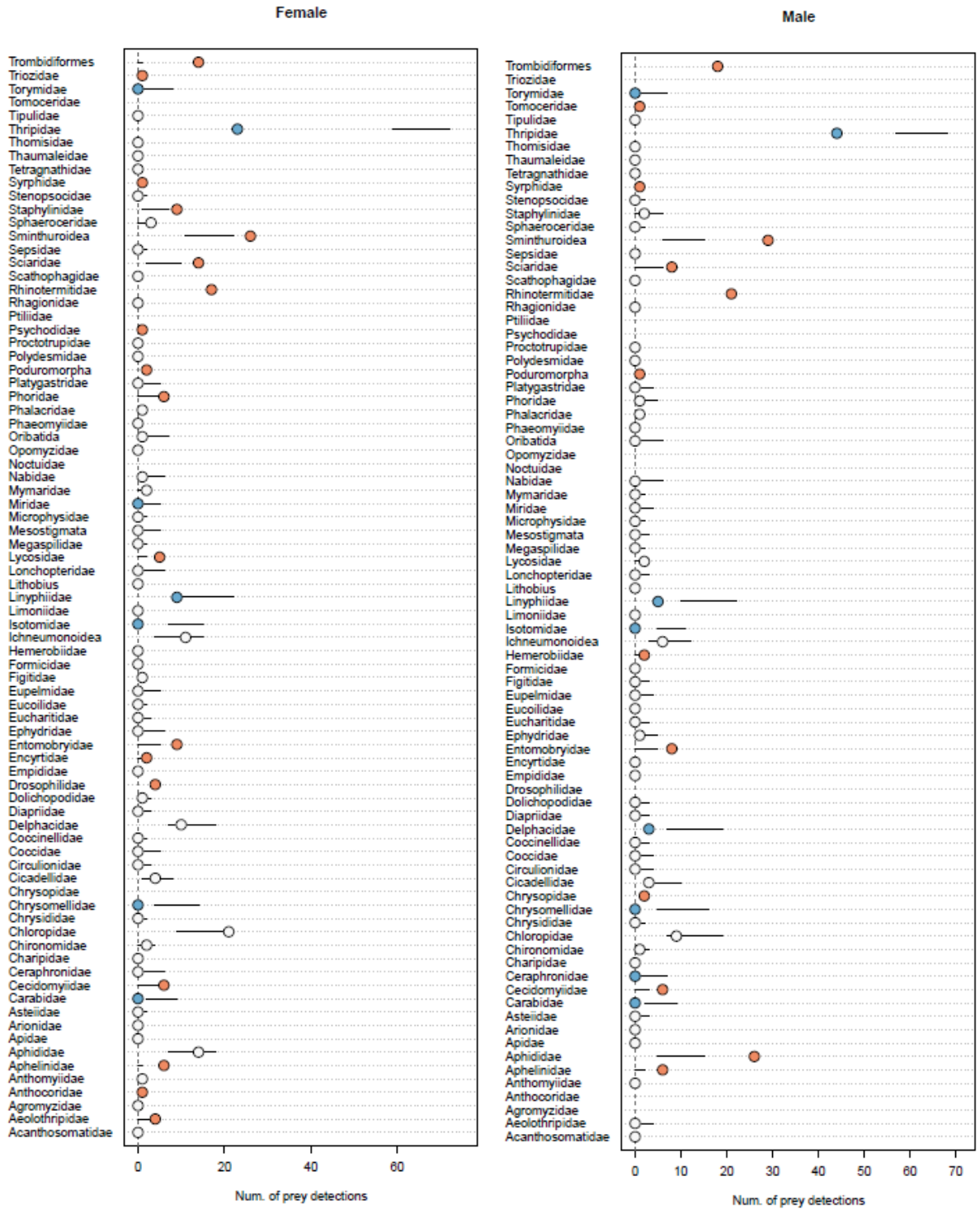
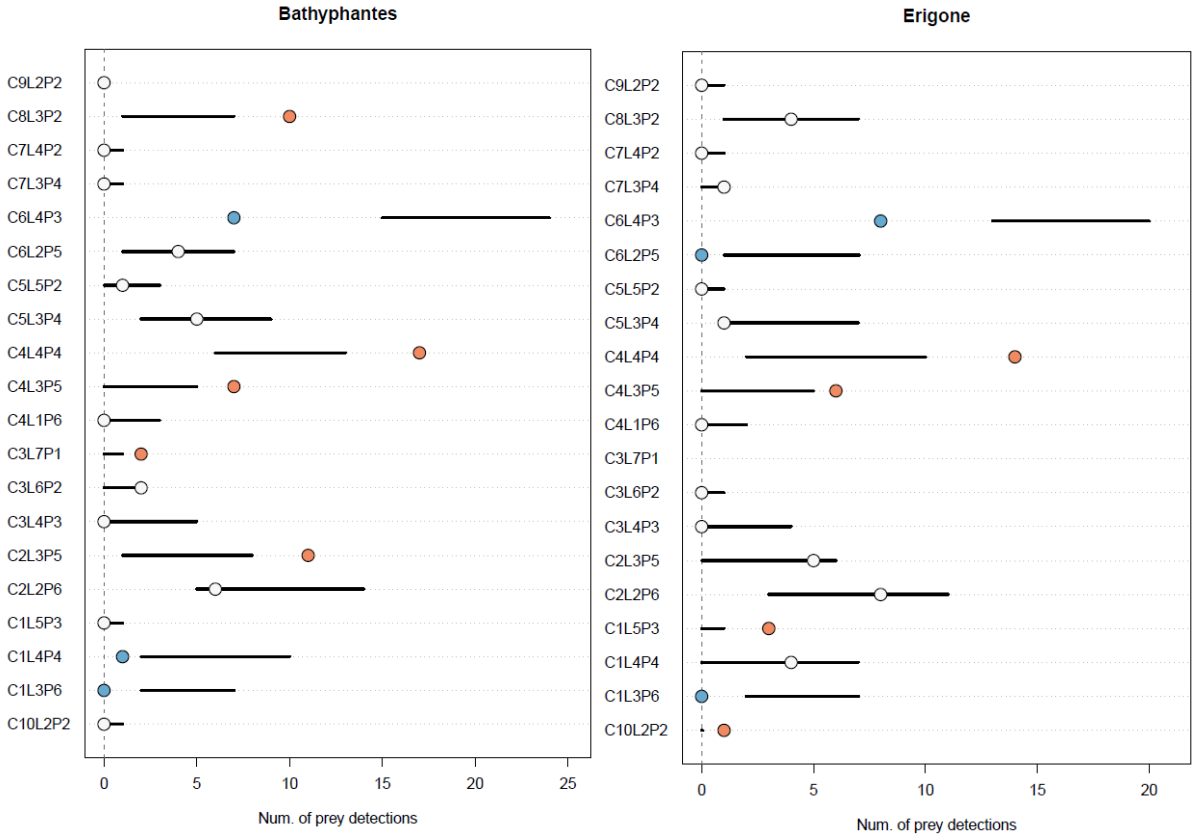


Figure S5.8: Significant deviations from expected frequencies of trophic interactions for spider sexes predating prey taxa in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), white = as expected (in proportion to relative abundance), red = higher than expected (consumed more frequently than predicted from relative abundance).


Bathyphantes
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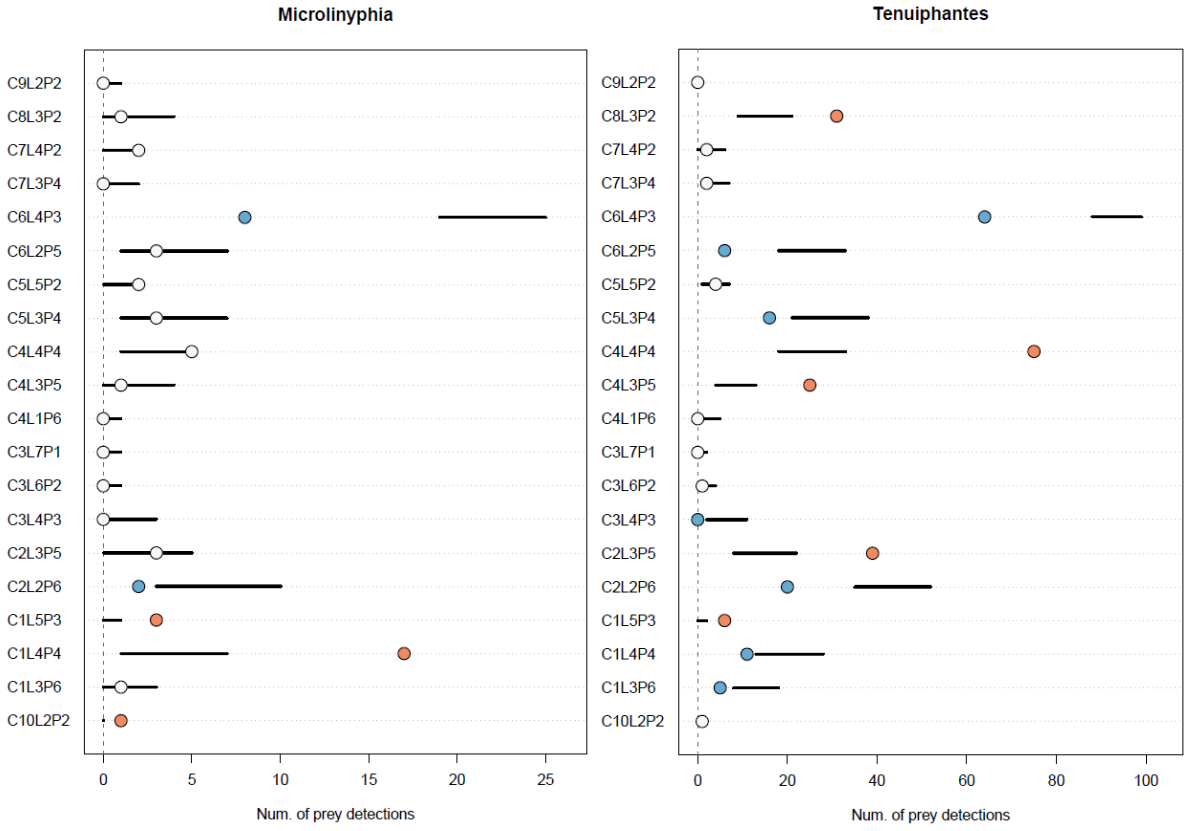
C8L3P2
C7L4P2
C7L3P4
C6L4P3
C6L2P5
C5L5P2
C5L3P4
C4L4P4
C4L3P5
C4L1P6
C3L7P1
C3L6P2
C3L4P3
C2L3P5
C2L2P6
C1L5P3
C1L4P4
C1L3P6
C10L2P2
0
5
10
15
20
25
Num. of prey detections
Erigone
C9L2P2
C8L3P2
C7L4P2
C7L3P4
C6L4P3
C6L2P5
C5L5P2
C5L3P4
C4L4P4
C4L3P5
C4L1P6
C3L7P1
C3L6P2
C3L4P3
C2L3P5
C2L2P6
C1L5P3
C1L4P4
C1L3P6
C10L2P2
0
5
10
15
20
Num. of prey detections


Microlinyphia
C9L2P2
C8L3P2
C7L4P2
C7L3P4
C6L4P3
C6L2P5
C5L5P2
C5L3P4
C4L4P4
C4L3P5
C4L1P6
C3L7P1
C3L6P2
C3L4P3
C2L3P5
C2L2P6
C1L5P3
C1L4P4
C1L3P6
C10L2P2
0
5
10
15
20
25
Num. of prey detections
Tenuiphantes
C9L2P2
C8L3P2
C7L4P2
C7L3P4
C6L4P3
C6L2P5
C5L5P2
C5L3P4
C4L4P4
C4L3P5
C4L1P6
C3L7P1
C3L6P2
C3L4P3
C2L3P5
C2L2P6
C1L5P3
C1L4P4
C1L3P6
C10L2P2
0
20
40
60
80
100
Num. of prey detections

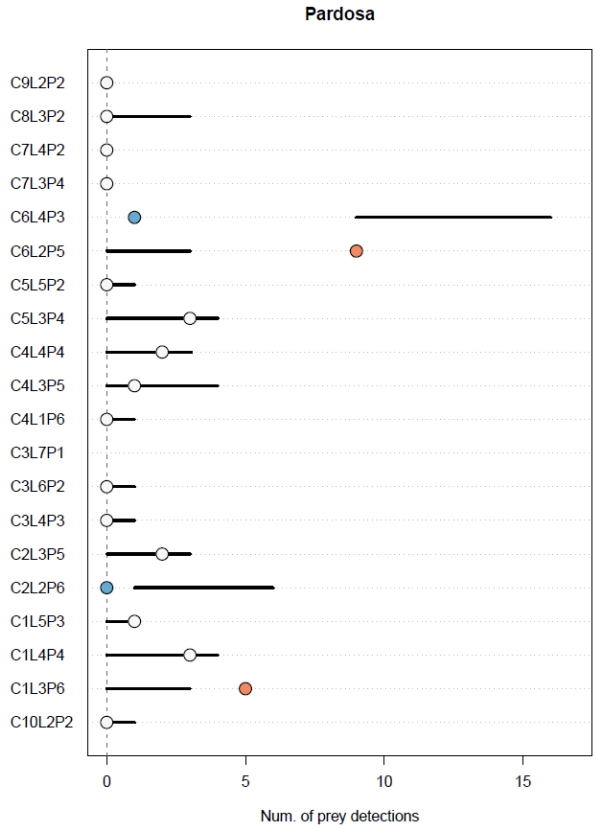


Figure S5.9: Significant deviations from expected frequencies of trophic interactions for spider genera predating prey tropho-species in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), white = as expected (in proportion to relative abundance), red = higher than expected (consumed more frequently than predicted from relative abundance).

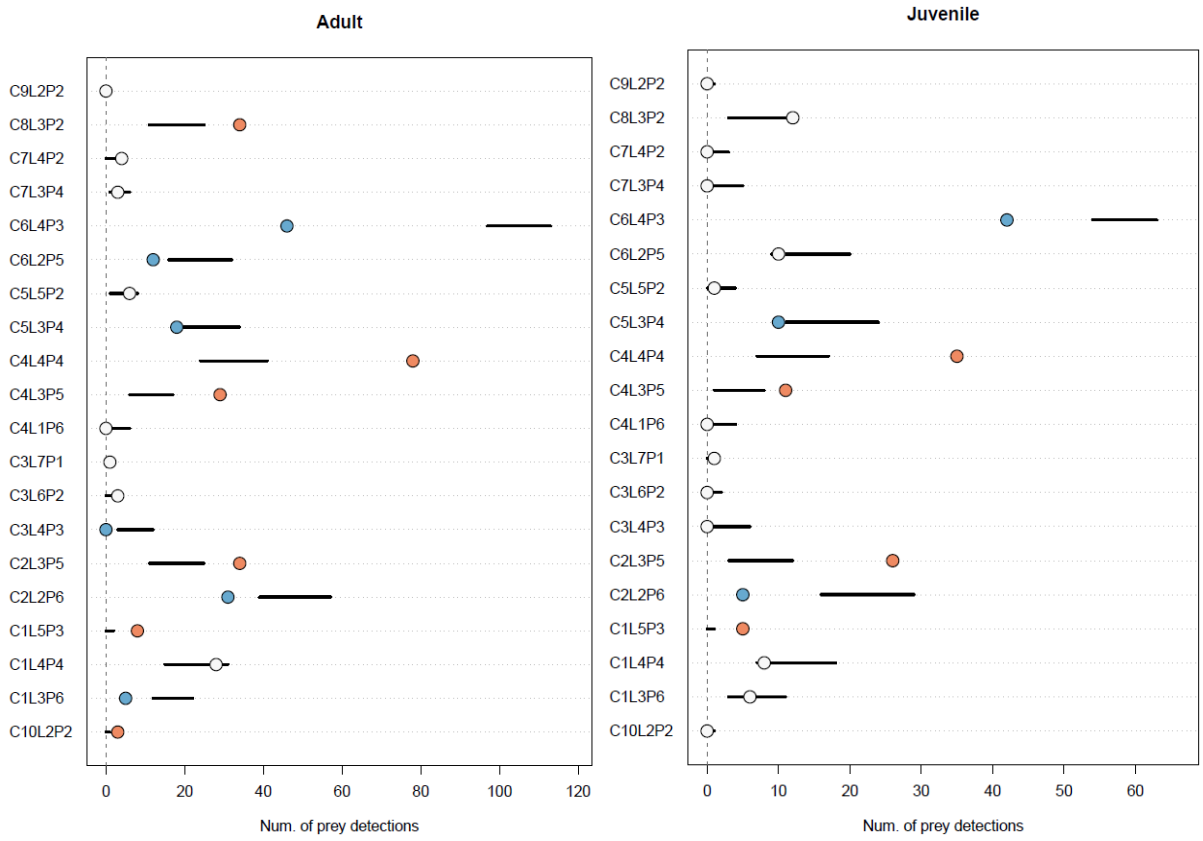


Figure S5.10: Significant deviations from expected frequencies of trophic interactions for spider life stages predating prey tropho-species in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), white = as expected (in proportion to relative abundance), red = higher than expected (consumed more frequently than predicted from relative abundance).

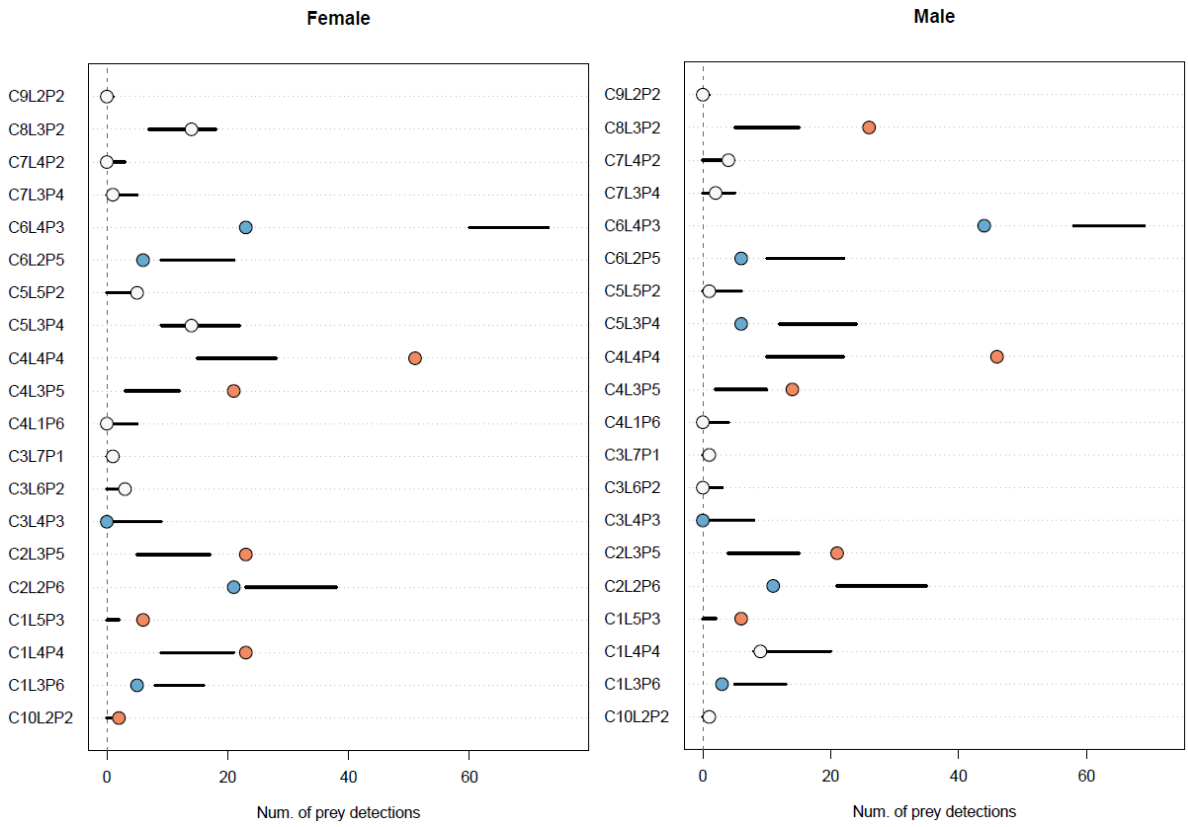


Figure S5.11: Significant deviations from expected frequencies of trophic interactions for spider sexes predating prey tropho-species in the field. Horizontal lines denote 95% confidence limits of the frequency of predation calculated in the null models, whereas circles represent the observed frequency of predation. Blue = lower consumption than expected (avoidance), white = as expected (in proportion to relative abundance), red = higher than expected (consumed more frequently than predicted from relative abundance).

Table S5.1: Tropho-species co-occurrence across spider diets in the field. The probability corresponds to the probability that the respective species co-occur more or less than expected (listed as 'relationship', with '+' in red and '-' in blue, denoting positive and negative co-occurrences, respectively).

Tropho-species 1	Tropho-species 2	Observed	Expected	Probability	Relationship
C1L3P6	C6L4P3	1	4	0.049	-
C1L4P4	C4L3P5	1	5.9	0.009	-
C1L4P4	C4L4P4	9	16.7	0.004	-
C1L4P4	C6L4P3	5	13	0.002	-
C1L4P4	C8L3P2	2	6.8	0.017	-
C1L5P3	C6L4P3	9	4.7	0.013	+
C2L3P5	C6L4P3	32	21.6	0.001	+
C4L4P4	C5L3P4	6	13	0.004	-
C5L3P4	C8L3P2	1	5.3	0.017	-
C6L2P5	C6L4P3	2	7.9	0.003	-
C6L4P3	C8L3P2	24	16.6	0.010	+

Chapter 6 Supplementary Material

Supplementary Information 6.1: Volatile organic compound analysis

Volatile organic compounds (VOCs) were collected from *Drosophila melanogaster* specimens from each rearing substrate by placing them in clear nalorphine bags for 1 h and extracting 300 ml headspace air through SafeLok™ themal desorption tubes (TenaxTA/Sulficarb, Markes International Ltd.) via hand pump (Easy VOC pump, Markes International Ltd.). VOCs were identified using thermal desorption gas-chromatography time-of-flight mass-spectrometry (TD-GC-TOF-MS) with a retention standard of 1 µl of C8-C20 alkane standard solution (Sigma-Aldrich, St. Louis, MO, USA).

The thermal desorption tubes were placed in a Markes International TD-100 Thermal Desorber (Markes International Ltd.) which desorbed the tubes at 280 °C for 10 min with a 40 ml min^{-1} trap flow. Trap desorption and transfer were carried out with a maximum temperature of 300 °C for 6 min, with a split flow of 5 ml min^{-1}. The VOCs were then separated in an Agilent 7890A GC system (Agilent Technologies, Santa Clara, CA, USA) with a helium carrier gas under constant flow conditions for 3 min at 40 °C. The mass spectra of the separated VOCs were then recorded from m/z 35–550 in a time-of-flight ALMSCO BenchTOF-dx (Markes International Ltd.). Data from GC–MS were deconvoluted and quantified using MSD ChemStation (Agilent Technologies Inc. 2005) and AMDIS and verified against the NIST 2011 library. Data were normalised as proportions of each volatile profile and were square root transformed to prevent large values from biasing the results.

Supplementary Information 6.2: Cuticular hydrocarbon analysis

Drosophila melanogaster specimens from each rearing substrate were killed by freezing and soaked in 100 µl of n-hexane for 30 s to solubilise the cuticular hydrocarbons (CHCs). Samples were processed in an AI/AS 1310 Series Autosampler (TGA-MS, Thermo Scientific Ltd.), with an 1 µl injecting volume at 280 °C. Separation occurred under a constant pressure of 92 psi, after which samples were transferred to the GC and kept at 180 °C for 5 mins, with a constant increase of 4°C min^{-1} up to 320 °C for 5 min. The ionisation mode was set for electronic impact (EI) detection, with a mass range of 35-600 m/z, with transfer and ion lines at 250 °C and 200 °C, respectively.