Does invasiveness predict competition outcomes between ecologically similar invasive blowflies?

A thesis
submitted in partial fulfilment
of the requirements for the degree
of

Master of Science (Research) in Ecology and Biodiversity

at

The University of Waikato

by

Daniel Nunn

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

2024

Abstract

Biological invasion is a complex puzzle, and its significance is at an all-time high. Current research highlights that the success of invasive species is partly due to their superior performance in key behavioural traits, such as aggression and boldness, especially when compared to ecologically similar native species. However, less attention has been given to differences in these traits among invasive species themselves. Not all invaders are created equal; they vary in their degree of invasiveness, often characterised by the extent of their global spread. If behavioural traits are crucial for invasiveness, it stands to reason that more invasive species - those with greater success - should outperform less invasive ones.

I tested this hypothesis using larval competition between differentially invasive blowfly species. *Calliphora stygia* has a limited distribution, found only in Australia and New Zealand, whereas *Lucilia sericata* and *Lucilia cuprina* (*Lucilia spp.*) are invaders with almost global distributions. Under direct laboratory competition, the highly invasive *Lucilia spp.* exhibited fewer body size reductions than *C. stygia*, regardless of intra- or interspecific competition levels. These findings suggest that competitive traits may exist along a continuum from native to invasive species, increasing predictably with invasiveness. This insight highlights invasiveness as a potentially reliable metric for predicting competition outcomes between ecologically similar sympatric invaders.

Acknowledgements

I would not be the scientist or the person I am today without the people around me.

To Dr Ang McGaughran, I don’t know if I will ever be able to express the depth of gratitude I have for you. You have exceeded every preconceived notion of what I thought a post-graduate supervisor could be. Your genuine care for my success and development as a scientist motivated me every single day. Beyond your professionalism, you are one of the kindest people I have ever had the pleasure of knowing. I always felt I had a place in your office and lab. Thank you for everything.

To Dr Nathan Butterworth, your guidance and knowledge of blowflies and carrion ecology has been invaluable to this thesis. The enthusiasm you display for science and blowflies is infectious and always inspired me to look at things in a new light.

To the Invasomics lab, you are an amazing group of people. Although we come from diverse cultural backgrounds, our shared love for the natural world brings us together. I wish nothing but the best for each of you, as I know the kind of impact each of you can make on this world. I will never forget any of you.

To Max and Ashleigh, my fellow fools who thought doing a Master’s wouldn’t be that hard: both of you have become some of my closest friends on this earth; I cannot picture this experience without either of you in it. While we may not have been productive at all times (don’t tell Ang), it was always a good time. Your potential as scientists is immeasurable, and your impact on my life is even greater.

To my mother, I don’t even know where to start. You are the reason I am the man I am today. You fostered my love for the natural world every day of my life. From dinosaur toys to nature documentaries as a little boy, you always saw potential in me. I succeed only partly because

of my abilities; the rest comes from my determination to achieve no less than what you believe I’m capable of. You have put me first every day of my life, and the personal cost to you was never too high if it meant putting me in a better position. I only hope that someday I can repay the love you have given me; nothing means more to me in this world than you.

It wouldn't be a complete list of my favourite beings without my dog, Leo. It doesn’t matter what kind of day I'm having; the second I’m home, it all disappears-his wagging tail and happy face fix everything. I love you, my boy.

Table of contents

List of Figures

Appendices:

List of tables

Appendices:

Chapter 1

General Introduction

1.1 Invasive species overview

Fueled by a changing climate and a historical precedent of human-driven species translocation, biological invasion has become one of the largest concerns to ecosystem structure in recent times (Early et al., 2016; Pyšek et al., 2020). Invasive species, in part, succeed due to their adaptability to new conditions. Currently, there are extensive examples of direct comparisons between native and invasive species across traits. Yet, we still understand little about how the expression of these traits may vary between invasive species - particularly with respect to how such variation may facilitate differences in invasion success (i.e., establishment and spread).

An invasive species is one that exists outside its native range, due to anthropogenic activity, where it then has negative impacts on the invaded environment (Blackburn et al., 2014). Invasive species present immense economic, social, and environmental costs. For example, estimated global costs exceed $423 billion annually (IPBES, 2023). Beyond the global figures, individual regions and countries also face significant financial challenges; for example, $USD140 billion was spent on management costs in Europe from 1960 to 2020 (Haubrock et al., 2021). The costs in Aotearoa New Zealand are also high: over the 52 years since 1968, an estimated $NZD97 billion was allocated towards the management of invasive species (Bodey et al., 2022). From a social perspective, native species and habitats that hold significant value to Indigenous peoples are typically adversely affected by invasive species (Pfeiffer & Voeks, 2008). Moreover, invasive species can have significant ecological impacts, primarily driving local species loss across both terrestrial and aquatic communities (Hermoso et al., 2011; Mollot et al., 2017) via predation and/or direct competition (Doherty et al., 2016). However, secondary processes, including food web alteration, niche restructuring, and trophic degradation, also have profound ecological effects (David et al., 2017; Doody et al., 2015; Ferlian et al., 2018) that are often hard to quantify due to their latency with respect to damages incurred (Bellard et al., 2021) (Fig. 1.1).

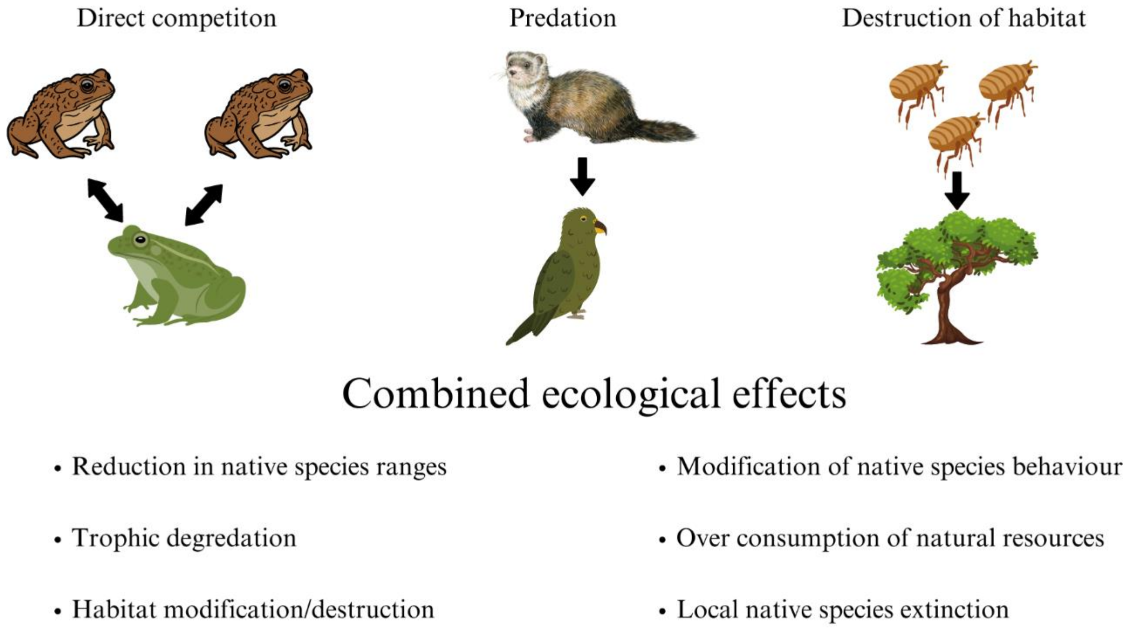

Figure 1.1 Examples of key pressures invasive species can exert on native species and ecosystems, and the associated potential ecological consequences.

1.2 How traits can facilitate invasions

Invasive species are often faced with the challenge of adjusting to novel conditions to which they have not evolved because each environment presents unique challenges and compositions of local flora and fauna (Saul & Jeschke, 2015). The success of an invader is therefore in some part determined by how well it can exploit novel conditions, both biotic and abiotic. Though approaches to overcoming this problem are highly variable among species, a range of traits (e.g., phenotypic, physiological, genetic, behavioural) facilitate the invasion process and these can often aid invasive species to outcompete their native counterparts (Card et al., 2018; Da Silva et al., 2021; Harms & Turingan, 2012; Yeung et al., 2023). For example, physiological traits can provide invasive species with a higher degree of resilience or adaptability to environmental change than their native counterparts (Herrera et al., 2024; Lockwood & Somero, 2011). This is seen in the invasive inland silverside (*Menidia beryllina*), which possesses greater tolerance of thermal stress than its native competitor, delta smelt (*Hypomesus transpacificus*) (Komoroske et al., 2021). Similarly, *Braunsapis puangensis* and *Ceratina dentipes* (two invasive bee species), have higher resistance desiccation and thermal stress tolerance than *Homalictus fijiensis*, their native counterpart in Fiji (Da Silva et al., 2021; Gabel et al., 2024). Such trait differences can also form the basis of variation between an invasive population of a species and populations in its home range (Kosmala et al., 2017). For example, *Sclerophrys gutturalis* (guttural toad) has higher resistance to drought stress in its invasive versus native populations (Barsotti et al., 2021). Moreover, differences in traits between different invasive species likely drive differences in their invasive success (i.e., geographic spread), though this area has been less-studied to date (Chown & McGeoch, 2023).

If a species can get past the initial stage of being transported and introduced outside its native range, it must still then establish itself and spread. High-performing reproductive traits can aid in this process, allowing invasive species to proliferate at a faster rate than their native counterparts and/or their own native range populations. For example, males of the harlequin ladybird (*Harmonia axyridis*) from an invasive population are more likely to copulate first and father a higher proportion of offspring than males from a native population, and invasive female ladybirds lay more eggs and reproduce quicker than native females (Laugier et al., 2013). Highly effective reproductive traits can also aid establishment of invasive populations when the number of invading individuals is low (Deacon et al., 2011). However, investment in reproductive traits is not uniform across all invasive species because there are inherent trade-offs associated with high investment in behavioural, physiological, and/or morphological traits (Roff et al., 2002; Salzman et al., 2018; Somjee, 2021). For example, the African clawed frog (*Xenopus laevis*) displays lower reproductive investment at the invasion front, most likely as a result of investing more energy in dispersal (Courant et al., 2017).

1.3 The role of competition in invasion

Competition underpins the interactions between species with similar niche requirements and therefore has a clear pathway of impact for determining invasion success, though of course, invasive species can use behavioural adaptations or even random chance to avoid competition altogether. At one extreme is the enemy-release hypothesis, whereby species invading a new range succeed due to their release from native predators (Heger & Jeschke, 2014), or at minimum experience lower predation pressures. In the latter case, this can happen when invaders have broad anti-predator strategies that work effectively even on predators not found in their home range (Cisterne et al., 2014). However, invasive species can also avoid competition by modulating their behaviour. For example, they can change their activity patterns to avoid overlap with native species - sometimes showing drastic temporal shifts, including from diurnal to nocturnal activity (Harrington et al., 2009). Alternatively, rapid recognition of native species through environmental cues can facilitate appropriate short-term avoidance responses (Heavener et al., 2014), which are more commonly seen in invasive prey encountering novel predators in the new environment.

In the absence of avoidance, invasive species must engage with any other species with which they have competing interests. In fact, invasive species are more likely to aggressively pursue resources and territory when compared to native species (Yeung et al., 2023; Zheng et al., 2023). Such behaviour is underlain by a suite of behavioural adaptations that can enhance invasion success through competitive advantage (Grangier & Lester, 2012; Szabo et al., 2020). For example, boldness (defined as engaging in risky behaviours, such as those that increase the risk of predation or aggression from other species) can result in the exploration of new habitats while also correlating with a higher propensity to disperse and be aggressive (Myles-Gonzalez et al., 2015). Individuals in invading populations are often characterised as being bold and/or aggressive - both when compared with their native range and when the invaded range core and edge are contrasted (Barry et al., 2020; Gruber et al., 2017a, 2017b; Pettit et al., 2016). The spiny cheek crayfish (*Faxonius limosus*) exemplifies this, showing higher boldness by leaving microterritories in the invaded range at a higher rate than the native Turkish crayfish (*Pontastacus leptodactylus*) (Pârvulescu et al., 2021). Invaders inhabiting edge zones can often habituate to novel conditions faster than those within the inner zones (Eccard et al., 2023), as a result of more rapid acceptance of new food sources (Cohen et al., 2020) or greater or more varied use of exploratory behaviours (Liebl & Martin, 2012). Such traits are often related to resource acquisition and retention, balancing out the potential costs that may be incurred (Chucholl et al., 2008; Polo-Cavia et al., 2011).

Aggressive behaviours can promote invasive success in several ways. For example, *Linepithema humile* (Argentine ant) displays higher rates of aggressive behavior towards native competitors when the initial invading force of ants is higher in abundance (Sagata & Lester, 2009) and when competition for shelter is necessary, suggesting a role for density in determining aggression (Neumann & Pinter-Wollman, 2022). The distribution and abundance of the competed-for resources are also important, with increased aggressive behaviour observed in invasive species when resources are clumped or have low availability (Tanner et al., 2011). Some invasive species can monopolise resources at a higher rate. This includes the invasive common carp (*Cyprinus carpio*), which can consume food at a faster rate than native crucian carp (*Carassius carassius*). Other kinds of traits can work together to facilitate invasion. For example, a dietary switch from piscivorous to generalist necessitated changes in a suite of traits for the pike killifish *(Belonesox belizanus*) in the invasive range, including phenotypic changes in mouth structure, physiological changes in digestion, and behavioural changes associated with new prey (Courant et al., 2017; Harms & Turingan, 2012).

Characterisation of invasive behaviours often assumes uniform performance in key traits by invasive species. However, even when directly comparing invasive species, clear distinctions in levels of aggression and behaviour modification can be observed (Bertelsmeier et al., 2015). To further understand how we might expect specific traits to vary in invasive species, we must directly compare performance in key metrics between species that differ in 'invasiveness' (e.g., differences in geographic extent, incursion frequency) to see whether trait expression corresponds with invasiveness in a predictable manner.

1.4 A model system for examining competition of invasive species

Blowflies (Calliphoridae) are an excellent model system for studying the traits of invasive species because they are highly successful invaders across all continents except Antarctica. The reasons for this are threefold. First, blowflies reproduce rapidly by producing high volumes of eggs (Hayes et al., 1999; Pitts & Wall, 2004), capitalising on ephemeral, but highly productive, carrion within the environment. Carrion is fundamental to the blowfly's life history as it provides necessary resources for the rapid growth of larvae over short time scales (Anderson, 2000; Smith & Wall, 1997). Thus, blowflies can rapidly expand their population size, aiding their ability to colonise new habitats. Second, blowflies are excellent dispersers. Adult blowflies may disperse 1-3 km per day (Lee et al., 2023; Tsuda et al., 2009) and the range at which they may lay eggs during their lifecycle expands far beyond this. For example, *Chrysomya bezziana* (screw worm fly) lays eggs from a release point at median and maximum distances of 10.8 and 100 km, respectively (Spradbery et al., 1995). Third, blowflies are highly diverse and adaptable. Temperature preferences for larval development varies between species, creating a high range of temperatures in which blowflies are capable of thriving (Richards et al., 2009). Some species, such as *Calliphora vicina,* can even adjust the thermal tolerance of their future larvae in response to climatic conditions (Coleman et al., 2014).

Aotearoa New Zealand is home to several invasive blowflies. Among these, based on their sympatry in New Zealand and similar functional roles, but significant difference in global distribution patterns, we characterise *Calliphora stygia* as a moderately invasive blowfly (native to Australia, with an Australasian distribution), and *Lucilia cuprina* and *Lucilia sericata* (hereafter *Lucillia spp.*) as highly invasive species (near-global distributions). *C. stygia* is a large-bodied mottled yellow blowfly, while *Lucillia spp.* is relatively small-bodied and iridescent green and blue (Fig. 1.2). While both species are invasive, their differences in global distribution may be indicative of differences in their competitive abilities. I used this study system as a model to explore the relationship between invasiveness and competitive ability in blowflies. When comparing these two species, I hypothesised that *Lucillia spp.* (more invasive) would outcompete *C. stygia* (less invasive) under direct competition for resources, both inside and outside the laboratory.

Figure 1.2. The three species of interest, from left to right: *Calliphora stygia* (© Nathan Butterworth), *Lucilia cuprina* (© Fir0002/Flagstaffotos), and *Lucilia sericata* (Unattributed). *L. cuprina* and *L. sericata* are extremely similar morphologically, play similar functional roles in the ecosystem, and have overlapping global distributions - on this basis, and as they are hard to identify with taxonomic certainty, they have been pooled as *Lucilia spp*. for analysis.

1.5 Thesis structure

In this thesis, I use laboratory and field methods to examine the competitive capabilities of invasive blowflies in Aotearoa New Zealand, specifically testing whether more invasive species (*Lucilia spp.*) outcompete less invasive species *(C. stygia).* Following this introductory chapter, Chapter 2 explores larval competition between invasive blowflies under resource limitation and variable intra- and interspecific density ratios in both field and laboratory conditions. Chapter 3 provides a critical evaluation of the thesis, including future research implications.

1.6 Literature cited

Anderson, G. S. (2000). Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). *Journal of Forensic Sciences*, *45*(4), 824-832.

Barry, T., Gurarie, E., Cheraghi, F., Kojola, I., & Fagan, W. F. (2020). Does dispersal make the heart grow bolder? Avoidance of anthropogenic habitat elements across wolf life history. *Animal Behaviour*, *166*, 219-231.

Barsotti, A. M. G., Madelaire, C. B., Wagener, C., Titon Jr, B., Measey, J., & Gomes, F. R. (2021). Challenges of a novel range: Water balance, stress, and immunity in an invasive toad. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *253*, 110870.

Bellard, C., Bernery, C., & Leclerc, C. (2021). Looming extinctions due to invasive species: Irreversible loss of ecological strategy and evolutionary history. *Global Change Biology*, *27*(20), 4967-4979.

Bertelsmeier, C., Avril, A., Blight, O., Confais, A., Diez, L., Jourdan, H., Orivel, J., Saint Germès, N., & Courchamp, F. (2015). Different behavioural strategies among seven highly invasive ant species. *Biological Invasions*, *17*, 2491-2503.

Blackburn, T. M., Essl, F., Evans, T., Hulme, P. E., Jeschke, J. M., Kühn, I., Kumschick, S., Marková, Z., Mrugała, A., & Nentwig, W. (2014). A unified classification of alien species based on the magnitude of their environmental impacts. *PLoS One* ,*12*(5), e1001850.

Bodey, T. W., Carter, Z. T., Haubrock, P. J., Cuthbert, R. N., Welsh, M. J., Diagne, C., & Courchamp, F. (2022). Building a synthesis of economic costs of biological invasions in New Zealand. *PeerJ*, *10*, e13580.

Card, D. C., Perry, B. W., Adams, R. H., Schield, D. R., Young, A. S., Andrew, A. L., Jezkova, T., Pasquesi, G. I., Hales, N. R., & Walsh, M. R. (2018). Novel ecological and climatic conditions drive rapid adaptation in invasive Florida Burmese pythons. *Molecular Ecology*, *27*(23), 4744-4757.

Chown, S. L., & McGeoch, M. A. (2023). Functional trait variation along animal invasion pathways. *Annual Review of Ecology, Evolution, and Systematics*, *54*(1), 151-170.

Chucholl, C., Stich, H. B., & Maier, G. (2008). Aggressive interactions and competition for shelter between a recently introduced and an established invasive crayfish: *Orconectes immunis* vs. *O. limosus*. *Fundamental and Applied Limnology*, *172*(1), 27.

Cisterne, A., Vanderduys, E. P., Pike, D. A., & Schwarzkopf, L. (2014). Wary invaders and clever natives: sympatric house geckos show disparate responses to predator scent. *Behavioral Ecology*, *25*(3), 604-611.

Cohen, T. M., Kumar, R. S., Nair, M., Hauber, M. E., & Dor, R. (2020). Innovation and decreased neophobia drive invasion success in a widespread avian invader. *Animal Behaviour*, *163*, 61-72.

Coleman, P. C., Bale, J. S., & Hayward, S. A. (2014). Cross-generation plasticity in cold hardiness is associated with diapause, but not the non-diapause developmental pathway, in the blow fly *Calliphora vicina*. *Journal of Experimental Biology*, *217*(9), 1454-1461.

Courant, J., Secondi, J., Bereiziat, V., & Herrel, A. (2017). Resources allocated to reproduction decrease at the range edge of an expanding population of an invasive amphibian. *Biological Journal of the Linnean Society*, *122*(1), 157-165.

Courant, J., Vogt, S., Marques, R., Measey, J., Secondi, J., Rebelo, R., De Villiers, A., Ihlow, F., De Busschere, C., & Backeljau, T. (2017). Are invasive populations characterized by a broader diet than native populations? *PeerJ*, *5*, e3250.

Da Silva, C. R., Beaman, J. E., Dorey, J. B., Barker, S. J., Congedi, N. C., Elmer, M. C., Galvin, S., Tuiwawa, M., Stevens, M. I., & Alton, L. A. (2021). Climate change and invasive species: a physiological performance comparison of invasive and endemic bees in Fiji. *Journal of Experimental Biology*, *224*(1), jeb230326.

David, P., Thebault, E., Anneville, O., Duyck, P.-F., Chapuis, E., & Loeuille, N. (2017). Impacts of invasive species on food webs: a review of empirical data. *Advances in Ecological Research*, *56*, 1-60.

Deacon, A. E., Ramnarine, I. W., & Magurran, A. E. (2011). How reproductive ecology contributes to the spread of a globally invasive fish. *PLoS One*, *6*(9), e24416.

Doherty, T. S., Glen, A. S., Nimmo, D. G., Ritchie, E. G., & Dickman, C. R. (2016). Invasive predators and global biodiversity loss. *Proceedings of the National Academy of Sciences*, *113*(40), 11261-11265.

Doody, J. S., Soanes, R., Castellano, C. M., Rhind, D., Green, B., McHenry, C. R., & Clulow, S. (2015). Invasive toads shift predator-prey densities in animal communities by removing top predators. *Ecology*, *96*(9), 2544-2554.

Early, R., Bradley, B. A., Dukes, J. S., Lawler, J. J., Olden, J. D., Blumenthal, D. M., Gonzalez, P., Grosholz, E. D., Ibañez, I., & Miller, L. P. (2016). Global threats from invasive alien species in the twenty-first century and national response capacities. *Nature Communications*, *7*(1), 12485.

Eccard, J. A., Mazza, V., Holland, C., & Stuart, P. (2023). The timid invasion: behavioural adjustments and range expansion in a non-native rodent. *Proceedings of the Royal Society B*, *290*(2003), 20230823.

Ferlian, O., Eisenhauer, N., Aguirrebengoa, M., Camara, M., Ramirez-Rojas, I., Santos, F., Tanalgo, K., & Thakur, M. P. (2018). Invasive earthworms erode soil biodiversity: a meta-analysis. *Journal of Animal Ecology*, *87*(1), 162-172.

Gabel, F., Vosshage, A. T., & Meyer, E. I. (2024). Non-native amphipods reach higher maximum swimming velocities than natives. *Science of the Total Environment*, *948*, 174794.

Grangier, J., & Lester, P. J. (2012). Behavioral plasticity mediates asymmetric competition between invasive wasps and native ants. *Communicative & Integrative Biology*, *5*(2), 127-129.

Gruber, J., Brown, G., Whiting, M. J., & Shine, R. (2017a). Geographic divergence in dispersal-related behaviour in cane toads from range-front versus range-core populations in Australia. *Behavioral Ecology and Sociobiology*, *71*, 1-7.

Gruber, J., Brown, G., Whiting, M. J., & Shine, R. (2017b). Is the behavioural divergence between range-core and range-edge populations of cane toads (*Rhinella marina*) due to evolutionary change or developmental plasticity? *Royal Society Open Science*, *4*(10), 170789.

Harms, C. A., & Turingan, R. G. (2012). Dietary flexibility despite behavioral stereotypy contributes to successful invasion of the pike killifish, *Belonesox belizanus*, in Florida, USA. *Aquatic Invasions*, *7*(4).

Harrington, L. A., Harrington, A. L., Yamaguchi, N., Thom, M. D., Ferreras, P., Windham, T. R., & Macdonald, D. W. (2009). The impact of native competitors on an alien invasive: temporal niche shifts to avoid interspecific aggression. *Ecology*, *90*(5), 1207-1216.

Haubrock, P. J., Turbelin, A. J., Cuthbert, R. N., Novoa, A., Taylor, N. G., Angulo, E., Ballesteros-Mejia, L., Bodey, T. W., Capinha, C., & Diagne, C. (2021). Economic costs of invasive alien species across Europe. *NeoBiota*, *67*, 153-190.

Hayes, E., Wall, R., & Smith, K. (1999). Mortality rate, reproductive output, and trap response bias in populations of the blowfly *Lucilia sericata*. *Ecological Entomology*, *24*(3), 300-307.

Heavener, S. J., Carthey, A. J., & Banks, P. B. (2014). Competitive naïveté between a highly successful invader and a functionally similar native species. *Oecologia*, *175*, 73-84.

Heger, T., & Jeschke, J. M. (2014). The enemy release hypothesis as a hierarchy of hypotheses. *Oikos*, *123*(6), 741-750.

Hermoso, V., Clavero, M., Blanco-Garrido, F., & Prenda, J. (2011). Invasive species and habitat degradation in Iberian streams: an analysis of their role in freshwater fish diversity loss. *Ecological Applications*, *21*(1), 175-188.

Herrera, I., de Carvalho-Souza, G. F., & González-Ortegón, E. (2024). Physiological responses of the invasive blue crabs *Callinectes sapidus* to salinity variations: Implications for adaptability and invasive success. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *297*, 111709.

Komoroske, L. M., Jeffries, K. M., Whitehead, A., Roach, J. L., Britton, M., Connon, R. E., Verhille, C., Brander, S. M., & Fangue, N. A. (2021). Transcriptional flexibility during thermal challenge corresponds with expanded thermal tolerance in an invasive compared to native fish. *Evolutionary Applications*, *14*(4), 931-949.

Kosmala, G., Christian, K., Brown, G., & Shine, R. (2017). Locomotor performance of cane toads differs between native-range and invasive populations. *Royal Society Open Science*, *4*(7), 170517.

Laugier, G. J., Le Moguedec, G., Tayeh, A., Loiseau, A., Osawa, N., Estoup, A., & Facon, B. (2013). Increase in male reproductive success and female reproductive investment in invasive populations of the harlequin ladybird *Harmonia axyridis*. *PLoS One*, *8*(10), e77083.

Lee, P. S., Dong, M. H., Yan, X. L., He, T. Y., Yu, S. F., Wee, S. L., & Wilson, J. J. (2023). Blowfly-derived mammal DNA as mammal diversity assessment tool: Determination of dispersal activity and flight range of tropical blowflies. *Biodiversity Data Journal*, *11*.

Liebl, A. L., & Martin, L. B. (2012). Exploratory behaviour and stressor hyper-responsiveness facilitate range expansion of an introduced songbird. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1746), 4375-4381.

Lockwood, B. L., & Somero, G. N. (2011). Invasive and native blue mussels (genus *Mytilus*) on the California coast: the role of physiology in a biological invasion. *Journal of Experimental Marine Biology and Ecology*, *400*(1-2), 167-174.

Mollot, G., Pantel, J., & Romanuk, T. (2017). The effects of invasive species on the decline in species richness: a global meta-analysis. In *Advances in Ecological Research* (Vol. 56, pp. 61-83). Elsevier.

Myles-Gonzalez, E., Burness, G., Yavno, S., Rooke, A., & Fox, M. G. (2015). To boldly go where no goby has gone before: boldness, dispersal tendency, and metabolism at the invasion front. *Behavioral Ecology*, *26*(4), 1083-1090.

Neumann, K., & Pinter-Wollman, N. (2022). The effect of resource availability on interspecific competition between a native and an invasive ant. *Philosophical Transactions of the Royal Society B*, *377*(1851), 20210146.

Pârvulescu, L., Stoia, D. I., Miok, K., Ion, M. C., Puha, A. E., Sterie, M., Vereş, M., Marcu, I., Muntean, M. D., & Aburel, O. M. (2021). Force and boldness: assets of a successful crayfish invader. *Frontiers in Ecology and Evolution*, *9*, 581247.

Pettit, L. J., Greenlees, M. J., & Shine, R. (2016). Is the enhanced dispersal rate seen at invasion fronts a behaviourally plastic response to encountering novel ecological conditions? *Biology Letters*, *12*(9), 20160539.

Pfeiffer, J. M., & Voeks, R. A. (2008). Biological invasions and biocultural diversity: linking ecological and cultural systems. *Environmental Conservation*, *35*(4), 281-293.

Pitts, K., & Wall, R. (2004). Adult mortality and oviposition rates in field and captive populations of the blowfly *Lucilia sericata*. *Ecological Entomology*, *29*(6), 727-734.

Polo-Cavia, N., López, P., & Martín, J. (2011). Aggressive interactions during feeding between native and invasive freshwater turtles. *Biological Invasions*, *13*, 1387-1396.

Pyšek, P., Hulme, P. E., Simberloff, D., Bacher, S., Blackburn, T. M., Carlton, J. T., Dawson, W., Essl, F., Foxcroft, L. C., & Genovesi, P. (2020). Scientists' warning on invasive alien species. *Biological Reviews*, *95*(6), 1511-1534.

Richards, C. S., Price, B. W., & Villet, M. H. (2009). Thermal ecophysiology of seven carrion-feeding blowflies in Southern Africa. *Entomologia Experimentalis et Applicata*, *131*(1), 11-19.

Roff, D. A., Mostowy, S., & Fairbairn, D. J. (2002). The evolution of trade-offs: testing predictions on response to selection and environmental variation. *Evolution*, *56*(1), 84-95.

Sagata, K., & Lester, P. J. (2009). Behavioural plasticity associated with propagule size, resources, and the invasion success of the Argentine ant *Linepithema humile*. *Journal of Applied Ecology*, *46*(1), 19-27.

Salzman, T. C., McLaughlin, A. L., Westneat, D. F., & Crowley, P. H. (2018). Energetic trade-offs and feedbacks between behavior and metabolism influence correlations between pace-of-life attributes. *Behavioral Ecology and Sociobiology*, *72*, 1-18.

Saul, W. C., & Jeschke, J. M. (2015). Eco-evolutionary experience in novel species interactions. *Ecology*, *18*(3), 236-245.

Smith, K., & Wall, R. (1997). The use of carrion as breeding sites by the blowfly *Lucilia sericata* and other Calliphoridae. *Medical and Veterinary Entomology*, *11*(1), 38-44.

Somjee, U. (2021). Positive allometry of sexually selected traits: Do metabolic maintenance costs play an important role? *BioEssays*, *43*(6), 2000183.

Spradbery, J., Mahon, R., Morton, R., & Tozer, R. (1995). Dispersal of the Old World screw-worm fly *Chrysomya bezziana*. *Medical and Veterinary Entomology*, *9*(2), 161-168.

Szabo, B., Damas-Moreira, I., & Whiting, M. J. (2020). Can cognitive ability give invasive species the means to succeed? A review of the evidence. *Frontiers in Ecology and Evolution*, *8*, 530130.

Tanner, C. J., Salalı, G. D., & Jackson, A. L. (2011). Feeding and non-feeding aggression can be induced in invasive shore crabs by altering food distribution. *Behavioral Ecology and Sociobiology*, *65*, 249-256.

Thematic Assessment Report on Invasive Alien Species and their Control of IPBES | *IPBES secretariat*. (2023, April 9). https://www.ipbes.net/ias.

Tsuda, Y., Hayashi, T., Higa, Y., Hoshino, K., Kasai, S., Tomita, T., Kurahashi, H., & Kobayashi, M. (2009). Dispersal of a blow fly, *Calliphora nigribarbis*, in relation to the dissemination of highly pathogenic avian influenza virus. *Japanese of Infectious Diseases*, *62*(4), 294-297.

Yeung, E. J., Klemet-N'Guessan, S., Hossie, T. J., & Fox, M. G. (2023). Boldness, movement and exploration tendency in round goby (*Neogobius melanostomus*) in Southern Ontario. *Journal of Fish Biology*, *103*(4), 790-797.

Zheng, Y., Zeng, Y., Li, J., Gong, J., & Lv, Z. (2023). Boldness behavior and ecological adaptability of stone moroko (*Pseudorasbora parva*) in open-field testing. *Animal Biology*, *1*(aop), 1-18.

Chapter 2

Exploring invasiveness as a predictor of competition outcomes between sympatric blowflies

Images by: Nathan Butterworth, Monash University.

To be submitted for journal publication; title and journal to be decided

2.1 Abstract

The success of invasive species is often attributed to their expression of competitive traits like rapid development, phenotypic plasticity, aggression, and boldness, typically when compared to native competitors in the invaded range. However, less is known about how these traits vary between invasive species, particularly those that are more versus less invasive (e.g., as represented by their geographical spread and/or other factors). To address this, we staged direct competition between invasive blowflies in Aotearoa New Zealand in both laboratory and field contexts. *Calliphora stygia* was used as a less invasive model species (native to Australia, with an Australasian distribution), while *Lucilia sericata* and *Lucilia cuprina* (henceforth *Lucilia spp.*) represented highly invasive species with a near-global extent. Laboratory trials involved manipulation of larval densities and intra/interspecific competition ratios, while field trials were seeded with varying larval mixtures at different times to assess potential succession order effects. In laboratory trials, the less invasive *C. stygia* showed at minimum 2x greater reductions in body size across all competition levels and densities compared to the more invasive *Lucilia spp.*, which itself showed no significant size reductions under balanced (i.e., equal intra/interspecific density) competition. In field trials, the less invasive *C. stygia* were not able to complete development in the presence of multi-species competitors, while the more invasive *Lucilia spp.* were able to develop normally - suggesting competitive advantages in field settings and reinforcing the laboratory results. Collectively, our findings highlight the potential role of species invasiveness in determining competitive outcomes, offering insights into how competitive behaviours may drive invasion success for species with similar life histories and ecological roles.

2.2 Introduction

The interconnectedness of global society, alongside other anthropogenic activities that have changed local abiotic conditions, have collectively facilitated the geographic expansion of countless species (Boivin et al., 2016). Range expansions have progressed to the extent that the average documented count of invasive species (defined here as those that have expanded from their native range through the facilitation of humans and pose adverse effects in their new environment) within any given country is 516 (Pagad et al., 2022). Annual global costs associated with biological invasions exceeded $USD 423 billion in 2024 (IPBES, 2023), and the management costs associated with invasive terrestrial invertebrates alone accounted for ~$USD712 billion as of 2020 (Renault et al., 2022).

From an ecological perspective, invasive species can have a range of measurable localised effects, from the reduction of native species populations to changes in the invaded ecosystem's functioning, stability, and associated long-reaching effects across the entire ecosystem (Ehrenfeld, 2010; Pyšek et al., 2020). Despite the importance of invasive species, we understand little about the mechanistic drivers of invasion success. For example, although we know that traits play an important role in enabling invasive species establishment, the focus is usually on comparing these traits among invasive and native species, or among invasive populations from their home and invaded ranges or range cores and edges. Studies that directly pit one invasive species against another are comparatively rare, limiting our understanding of how differential 'invasiveness' among invaders may play a role in invasion success.

Alongside traits that enable rapid population growth and geographic spread post-invasion (e.g., high fecundity, rapid dispersal), those that facilitate faster and/or more effective use of resources are likely to be important in assisting invasive species to perform better than resident species in the invaded habitat (Fernández & Hamilton, 2015). In particular, behavioural traits, such as aggression and boldness, underpin the competitive interactions between species and are known to be important facets of successful biological invasion (Barry et al., 2020; Gruber et al., 2017a, 2017b; Pettit et al., 2016; Yeung et al., 2023; Zheng et al., 2023). For example, enhanced dispersal and higher aggression has seen the western bluebird (*Siala mexicana*) expand its range and displace its native competitor, the mountain bluebird (*Sialia currucoides*) in the USA (Duckworth & Badyaev, 2007). Similarly, aggressive behaviours during feeding interactions are allowing invasive red-eared slider turtles (*Trachemys scripta elegans*) to outcompete native terrapins (*Mauremys leprosa*) in Spain (Polo-Cavia et al., 2011).

Competition is a pivotal mechanism that enables geographic expansion by invasive species (Paquette & Hargreaves, 2021), with the traits that facilitate competition often context-dependent. For example, the expression of aggressive behaviour is driven by the value of the competed-upon resource (Kilgour et al., 2020; Tanner & Adler, 2009; Tanner et al., 2011) and the balance of competitors, both in terms of their total density and their relative levels of intra- and/or interspecific competition (Kilgour et al., 2020; Manenti et al., 2015; Tanner & Adler, 2009). As it allows species to monopolise resources at a higher rate (Duckworth & Badyaev, 2007; Neumann & Pinter-Wollman, 2022; Tanner et al., 2011), aggressive behaviour is typically a highly repeatable trait during species interactions (Peiman & Robinson, 2010), particularly those between invasive species and their new native competitors (Hudina et al., 2014; Sagata & Lester, 2009). While co-occurring invasive species may both have success if there is limited niche overlap and therefore less competitive interaction (Jackson & Britton, 2014), there is some evidence that inhibitory effects can be exerted upon one invader by another when there is higher niche overlap (Arismendi et al., 2014; Griffen et al., 2008). For example, a recent invasion of the North American east coast by the Asian shore crab (*Hemigrapsus sanguineus*) suppressed the ability of an earlier invader, the European green crab (*Carcinus maenas*), to acquire its preferred food resource (Griffen et al., 2008). Similarly, two invasive salmonids (*Oncorhynchus mykiss* and *Salmo trutta*) in southern Chile prevented the spread of other invasive salmonids in the region through competitive exclusion from an overlapping niche (Arismendi et al., 2014). These observational studies provide intriguing insights into the role of competition in determining invasive outcomes. However, studies that manipulate starting density and intra- and interspecific ratios would further our understanding of competition outcomes between invasive species and of the ways in which invasive communities are structured.

Blowflies (Calliphoridae) are an excellent model system for testing hypotheses about the role of competition in invasion, due to a number of factors. First, they are excellent invaders - they reproduce rapidly, have high fecundity and dispersal rates, and are highly adaptable in new environments (Lagos et al., 2017). Second, they are easy to rear and capture in the lab, with relatively fast developmental cycles (~4-6 weeks), enabling scope for manipulative experiments. Third, competition for carrion at both the larval and adult stage is a key feature of the blowfly life cycle, providing resources for female blowflies to sexually mature and for larvae to progress through development (Wilson & Wolkovich, 2011).

In New Zealand, a matrix of invasive blowfly species live in sympatry and carry out similar functional roles within ecosystems but differ in their global invasive extent. *Calliphora stygia* is native to Australia, with an overall Australasian distribution. In contrast, *Lucilia cuprina* and *Lucilia sericata* (hereafter *Lucilia spp*.) are morphologically very similar, with cosmopolitan distributions encompassing Africa (Williams & Villet, 2014), Europe (Baz et al., 2007; Feddern et al., 2018; Rose & Wall, 2011), North America (Owings & Picard, 2018), South America, and Australasia. These differences in geographic distribution may indicate the potential for divergence in competitive abilities among the more invasive *(Lucilia spp.*) and less invasive *(C. stygia*) species. Here, we use these species to evaluate the role of competitive ability in invasion success, with a particular view towards whether invasiveness is predictive of competitive outcomes. We test this hypothesis by staging larval competition between the two species in a controlled laboratory environment and in a field setting with low levels of manipulation - exploring the effects of total density, food limitation, and both intra- and interspecific competition on overall survival and adult blowfly body mass post-pupation. We hypothesised that our pre-defined more invasive *Lucillia spp*. would outperform our less invasive *C. stygia* in terms of greater survival and smaller effects of larval competition on relative body size.

2.3 Methods

2.3.1 Collection sites

Blowflies were collected from various sites within the Waikato region (Fig. 2.1A) from January to May 2024. *Lucilia spp.* was collected at the University of Waikato campus. *C. stygia* was collected at three sites: the University of Waikato campus, Newstead Gully, and the Edmund Hillary Hope Reserve. The latter two locations are rural sites, possessing dense vegetation. In contrast, the University of Waikato campus is an urban site, with comparatively lower vegetation cover and greater infrastructure. The variation in collection sites for *C. stygia* was due to its inconsistent availability in adequate sample sizes for experimental treatments. However previous genetic analysis has shown an absence of genetic structuring among populations from across the North Island (Croft et al., 2024).

2.3.2 Sample collection and transportation

Bait to catch blowflies was prepared by placing a container filled with beef mince within a self-contained outdoor cage at the University of Waikato glasshouse compound. The cage prevented mammals and birds from interfering with the bait while allowing invertebrates unimpeded access to the meat, encouraging egg-laying by blowflies. Eggs were allowed to hatch and develop on the meat, with larvae then releasing chemicals to break down and odourise the meat to attract more blowflies. Prepared baits were used at field sites to attract target species, with blowflies then caught on the meat and surrounding vegetation using hand nets. Species were preliminarily identified following Dear 1985 and transferred into a mesh cuboid insect cage (W32.5 x D32.5 x H32.5 cm), which was transported by car (up to 45 min) to the invertebrate laboratory at the University of Waikato.

The invertebrate laboratory conditions consisted of a constant temperature (23°C ± 1°C), and 12:12h day:night light cycling. Raw sugar and water were provided to cages in ample quantities and all flies underwent an initial 24-hr acclimation period in their shared cages.

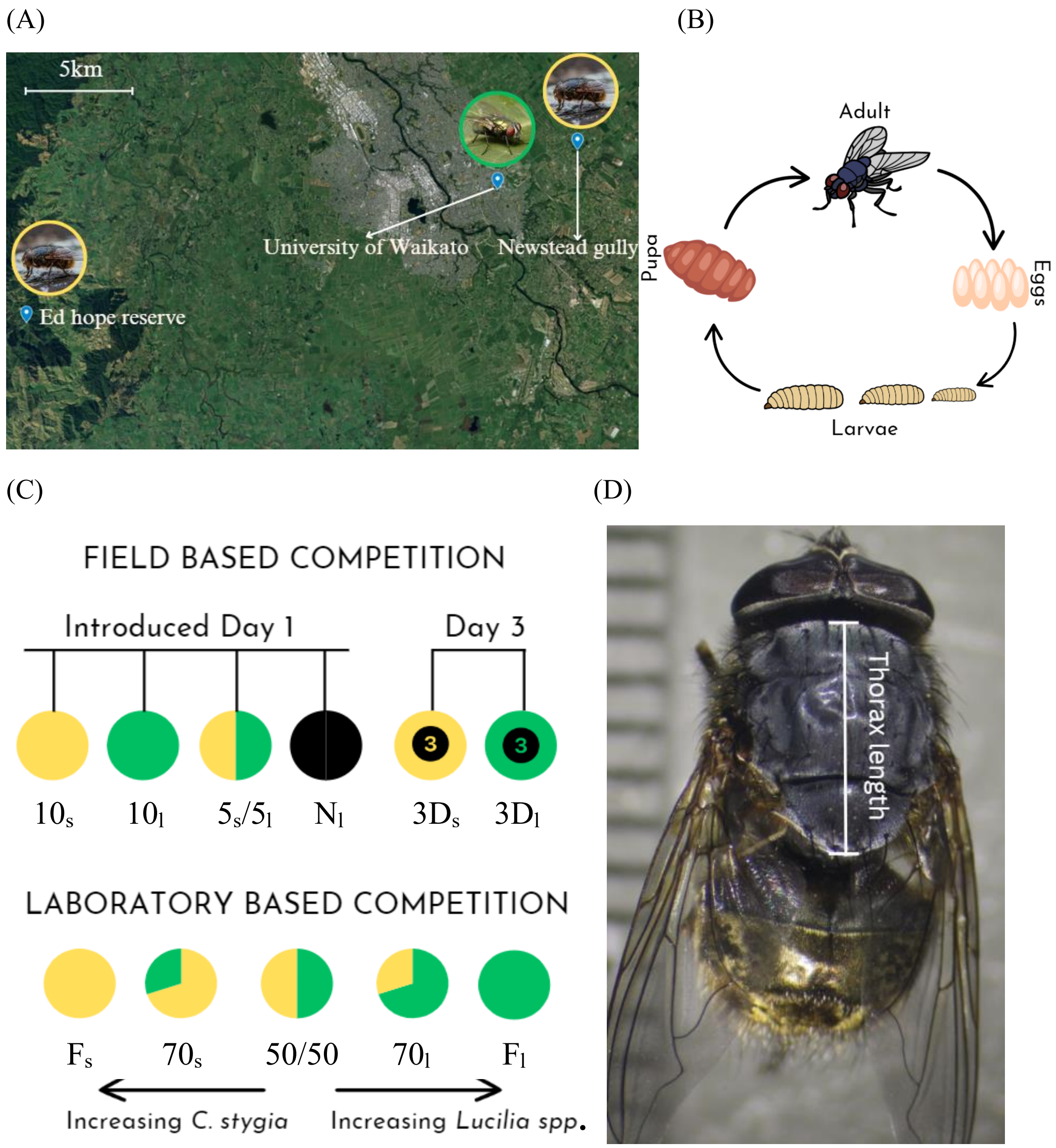

Figure 2.1. Experimental details. (A) The three sites used for blowfly collection; (B) Simplified life cycle of a blowfly: adult blowflies deposit eggs on carrion, once eggs hatch, larvae progress through three developmental instars then enter pupation and emerge as adult blowflies; (C) Experimental design for both field- and laboratory-based competition experiments, with colours depicting the balance of species used (green=*Lucilia spp.*; yellow=*C. stygia)*; (D) Photograph of a *C. stygia* individual depicting the blowfly thorax length measurement region (Photo: D Nunn).

2.3.3 Production of larvae

Following the acclimation period, females were isolated into new mesh cages (L21 x W21 x H6 cm) with sugar, water, and beef mince added to encourage egg-laying. This decision was predicated on the assumption that females had already mated in either the field prior to collection, or during the acclimation period when they were still housed alongside males. *Lucilia spp.* was isolated as single females, but *C. stygia* required other females (n=5) to be present to encourage consistent egg-laying. After approximately 48 hr, all cages were checked for the presence of larvae, at which point the female adult was removed. Second instar larvae were then identified using the key provided in (Dear, 1985) and selected for competition trials (Fig. 2.1B).

2.3.4 Competition assays in the laboratory

The effects of competition on blowfly fitness were examined using four blowfly densities (10, 20, 30, and 50 larvae) and a constant beef mince allotment of 20 ± 0.25 g that resulted in decreasing meat availability as the number of larvae increased (approximate weight of mince per larvae at each density=2.00, 1.00, 0.67, and 0.40 g, respectively) (Fig. 2.1C, Table 2.1A). Treatments also varied in their allocated species ratios, with five levels of intraspecific and interspecific competition: intraspecific treatments of full *C. stygia* (F_s) and full *Lucilia spp.* (F_l); an equal 50% split of both species (50:50); and a 70:30 split favouring *Lucilia spp.* (70_l) or *C. stygia* (70_s) (Fig. 2.1C). Total replications of density and relative species ratios are shown in Table 2.1B.

Table 2.1. Competition assay designs. (A) Total replications conducted using various mixtures of larval density and competition within the laboratory. Blank spaces are trials that were unable to be undertaken due to time constraints; (B) Total replications of field-based competition experiments. Each trial was replicated three times within a four-week period (01/03/24, 08/03/24, 29/03/24).

(A)

Competition assay	**Density**			
	10	20	30	50
F_l	7	7	5	5
F_s	5	5	5	6
50/50	5			5
70_l	5			6
70_s	5			5

(B)

Date	**Competition assay**					
	10_l	10_s	$5_s/5_l$	$3D_l$	$3D_s$	N_l
Number of replications	3	3	3	3	3	3

Individual size-matched larvae were transferred to meat in the previously described densities and species ratios using a fine-tipped paintbrush. Weigh trays containing meat and larvae were then placed inside polypropylene plastic boxes (L21 x W21 x H6 cm) containing chaff as a substrate for pupation, and these were checked daily for blowfly emergence.

2.3.5 Competition assays in the field

Sample collection and larval production for the outdoor competition experiments followed the same methods outlined above. Outside chambers were constructed using plastic containers that allowed blowflies to enter and leave without obstruction. Chambers were

L24.7 x H13.7 x W16 cm and had a double top layer consisting of the container lid plus an inner compartment placed on top for additional protection from any rain. Holes were drilled in both top layers to allow unimpeded access for blowflies, while additional holes were drilled into six points around the bottom of the container to allow any rainwater to drain away. A small plastic container with 75 ± 0.5 g of beef mince in a weigh tray was placed in the centre of each larger container before field deployment.

A total of six treatments were used, with these varying by total larvae and the timing in which larvae were added to the meat: Treatments 1 and 2 had 10 *C. stygia* and 10 *Lucilia spp.*, respectively, with 2nd instar larvae added on Day 1; Treatment 3 was also seeded with larvae from Day 1, but had an equal ratio of *C. stygia* and *Lucilia spp.* (n=5 of each); Treatments 4 and 5 were seeded with 10 larvae (*C. stygia* and *Lucilia spp.*, respectively) after being deployed for 72 hrs; Treatment 6 had no artificial seeding of larvae (Fig. 2.1C). All treatments were left for seven days in the outside area of the glasshouse compound at the University of Waikato (spaced at least 5 m apart), following which the meat trays were placed in individual mesh insect cages with a chaff substrate to allow pupation. Cages were stored in the above-described temperature-controlled room (23°C ± 1°C; 12:12 light:dark cycling) until flies emerged from pupation, at which point they were identified to species or genus level following (Dear, 1985). Each trial was replicated three times within a four-week period (01/03/24, 08/03/24, 29/03/24).

2.3.6 Body sizing and survival rates

Body size was used as a proxy for fitness following competition experiments based on previous research. For example, lower body size has been linked to lower mating success for male blowflies (Stoffolano et al., 2000), and lower fecundity (measured as total egg production) in females (Saunders et al., 2013). Dead blowflies were positioned horizontally and pierced by a small metal pin, allowing a consistent view of the head and thorax. Pinned flies were then placed under a stereo microscope equipped with a Cannon camera and photographed from above. ImageJ v.0.5.8 (Schneider et al., 2012) was used to measure the length of the thorax to obtain a representative body size measurement per individual (Croft et al., 2024) (Fig. 2.1D).

For the laboratory assays, survival rates were measured alongside body size by counting the number of surviving individuals out of the maximum possible survivors (i.e., the total density of a given species within the assay conditions the larvae were subjected to).

2.3.7 Data analysis

All data was analysed in R v.4.3.3 (R Core Team, 2024) and plotted using either Plotly v.4.10.4 (Sievert et al., 2021) or ggplot2 v.3.5.1 (Ginestet, 2011).

A Pearsons's moment correlation test was used to assess the relationship between thorax length and overall body size (assessed by first measuring head length and thorax width and then multiplying the three values together). A correlation was confirmed for both species: *Lucilia spp.*=0.8833, $p<0.001$, *C. stygia*=0.904 , $p<0.001$), hence subsequent analyses used only the thorax length measurement as a proxy for body size.

For the laboratory-based competition experiments, the effects of competition and density on thorax length were assessed using linear models fit with density, competition, collection date, and sex as predictors. To test if the emergence rates of male and female blowflies varied with density and competition, the glm function fit to a binomial distribution in base R was used. Differences in survival rates among treatments were tested in two ways. First, the proportion of surviving larvae per trial was calculated and fit to a beta regression using the betareg v.3.2-0 package (Cribari-Neto & Zeileis, 2010). Second, a glm fit to a binomial distribution was used to test for differences in the failure (count of larvae from the total species density that did not emerge) or success (count of larvae from the total species density that did emerge) response of larvae. Instances of over-dispersion were detected and overcome for both models by fitting to a quasi-binomial distribution. Estimated marginal means was conducted using emmeans v.1.10.0 (Lenth, 2024) to test pairwise comparisons of thorax length and survival rates between density and competition levels.

For the field experiments, a glm was used to examine the relationship between date and total Diptera emergence, fitting to a quasi-Poisson distribution to account for detected over-dispersion. Differences in total emergence and species-specific emergence by treatment were tested using glmmTMB v.1.1.10 (Brooks et al., 2017). The emergence of flies per treatment was fit to a negative binomial model and estimated marginal means were used to assess differences between treatment groups. An ANOVA using the aov function in base R was used to assess differences in emerged *Lucilia spp.* thorax length. Following the ANOVA, a Tukey's HSD test was used to explore pairwise differences between treatment groups using the ghlt function in multcomp (Hothorn et al., 2008). Finally, the probability of occurrence of male and female *Lucilia spp.* was tested using a glm fit to a binomial distribution in base R.

2.4 Results

2.4.1 Laboratory experiments: Intraspecific competition

2..4.1.1 Body size

C. stygia showed significant reductions in thorax length under intraspecific competition as density increased, with estimated marginal means analysis revealing several notable differences between density levels (Fig. 2.2A, Table A2.1). In particular, a significant reduction (0.149 mm) in thorax length was observed at n=10 versus n=30 (p=0.010) and between n=10 and n=50 (0.332 mm shorter at n=10; p<0.001). Additionally, thorax lengths at n=50 were significantly lower than those at n=20 (estimate=0.286, p<0.001) and n=30 (estimate=0.183, p<0.001). Thus, high density intraspecific (F_s) competition caused significant thorax length reductions when compared to all other density levels (Table 2.2).

Lucilia spp. also showed significant thorax length reductions as density increased (Fig. 1A, Table 2). Similar to *C. stygia*, *Lucilia spp*. showed decreases in thorax length between densities of n=10 and n=30 (0.138mm larger at n=10; p<0.010). However, unlike *C. stygia, Lucilia spp*. thorax length reductions did not increase with density. For example, no change was observed between densities of n=30 and n=50 (estimate=-0.019, p=0.999), and differences in thorax length at n=10 vs n=50 (estimate=0.127, p=0.012), were similar to those at n=10 versus n=30 (Table 2.2).

Overall, *C. stygia* experienced both more frequent and stronger effects of density on body size across densities under full intraspecific competition. Exemplifying this, the reduction in thorax length between densities of n=10 versus n=50 was 2.6x greater for *C. stygia* compared to *Lucilia spp*. (Table 2.2).

2.4.1.2 Survival rates

Survival rates for *C. stygia* were higher at densities of n=10 compared to n=30 (estimate=0.340, p=0.037) and n=50 (estimate=0.494, p<0.000) (Fig. 2.2B, Table 2.2). However, this effect was lost under a binomial glm of success and failures for larval survival, where no differences between any density of full intraspecific competition were detected. Comparatively, *Lucilia spp*. displayed no significant changes in larval survival with density under full intraspecific competition in either beta regression or binomial modelling (Fig. 2.2B, Table 2.2).

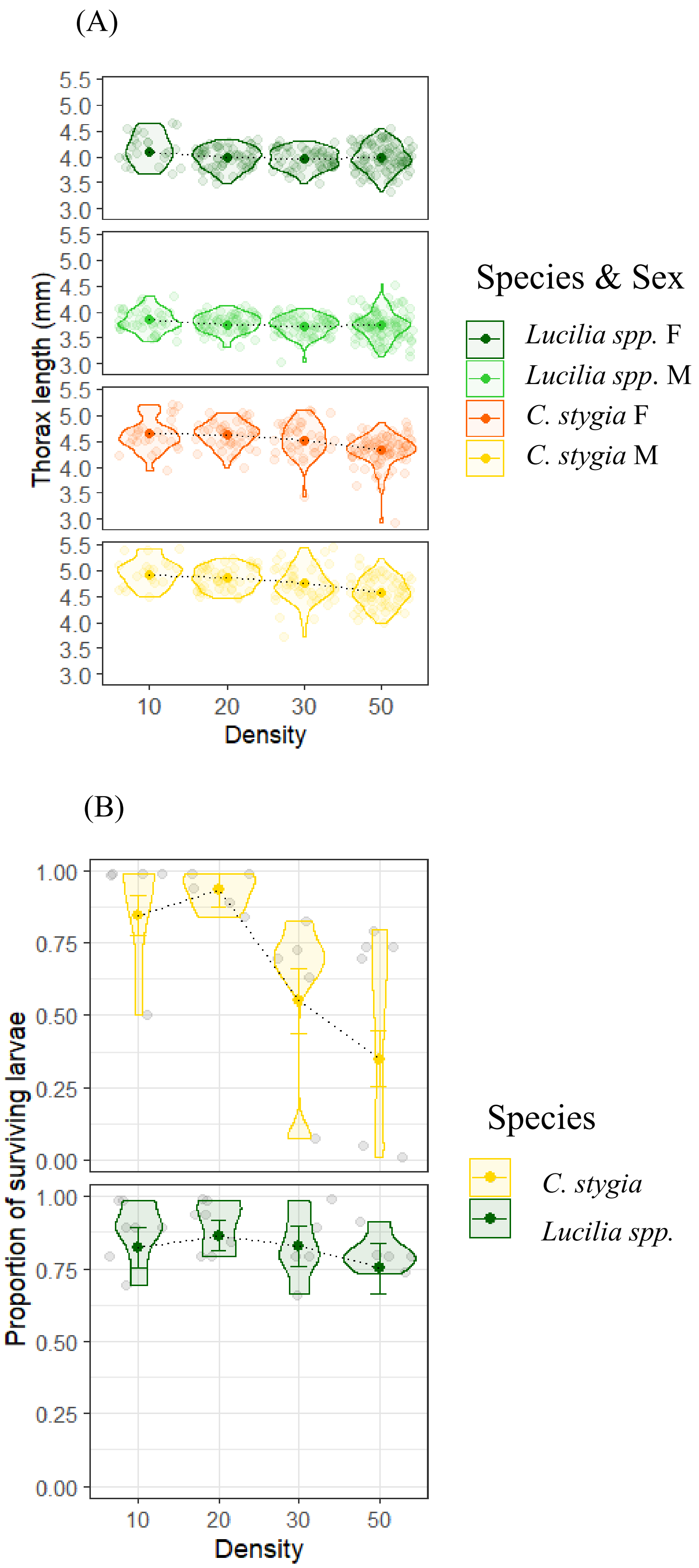

Figure 2.2. The effects of density under full intraspecific competition for *C. stygia* and *Lucilia spp.* on: (A) Thorax length of males and females; (B) Larval survival.

Table 2.2 Pairwise comparisons of thorax length and larval survival from estimated marginal means models. Thorax length estimates are derived from a linear regression testing the effects of density, sex and species on thorax length, while larval survival estimates are derived from a beta regression testing the effects of density on larval survival. Significant p values are indicated in bold.

Comparison	Estimate	Standard error	T Value/Z ratio	P Value
C. stygia **thorax length**				
n=10 vs n=20	0.046	0.046	1.000	0.974
n=10 vs n=30	0.149	0.046	3.234	**0.028**
n=10 vs n=50	0.332	0.043	7.783	**<0.001**
n=20 vs n=30	0.103	0.037	2.779	0.102
n=20 vs n=50	0.286	0.033	8.662	**<0.001**
n=30 vs n=50	0.183	0.034	5.441	**<0.001**
C. stygia **survival rates**				
n=10 vs n=20	0.020	0.066	0.304	1.000
n=10 vs n=30	0.340	0.109	3.134	**0.037**
n=10 vs n=50	0.494	0.100	4.943	**<0.001**
n=20 vs n=30	0.320	0.111	2.876	0.078
n=20 vs n=50	0.475	0.103	4.602	**<0.001**
n=30 vs n=50	0.154	0.133	1.159	0.943
Lucilia spp. **thorax length**				
n=10 vs n=20	0.159	0.389	2.721	0.118
n=10 vs n=30	0.138	0.039	3.549	**<0.010**
n=10 vs n=50	0.127	0.036	3.471	**<0.012**
n=20 vs n=30	0.033	0.032	1.029	0.970
n=20 vs n=50	0.021	0.028	0.725	0.996
n=30 vs n=50	-0.019	0.029	-0.419	0.999
Lucilia spp. **survival rates**				
n=10 vs n=20	-0.013	0.069	-0.183	1.000
n=10 vs n=30	0.039	0.086	0.464	0.999
n=10 vs n=50	0.095	0.095	1.004	0.974
n=20 vs n=30	0.052	0.084	0.625	0.999
n=20 vs n=50	0.108	0.093	1.156	0.944
n=30 vs n=50	0.055	0.106	0.522	0.999

2.4.2. Laboratory experiments: Interspecific competition

2.4.2.1 Body size

Interspecific competition had significant effects on thorax length relative to intraspecific competition for both species when density was low. For *C. stygia*, densities of n=10 and equal balance interspecific competition (50/50) had a positive effect on thorax length compared to F_s (estimate=0.224 mm, p=0.002; Fig. 2.3A). Similarly, *Lucilia spp.* showed significant increases in thorax length at low density competition. For example, when density was low, but interspecific competition was high, thorax length in *Lucilia spp.* was greater when reared under *C. stygia* dominant (70_s) competition relative to rearing under full intraspecific competition (estimate= 0.161, p=0.034; Fig. 2.3B).

Under high density (n=50), neither species displayed changes in thorax length under any balance of interspecific competition relative to intraspecific competition (Fig. 2.3A,B). However, when comparing thorax lengths under the same interspecific competition level between high (n=50) and low (n=10) density, *C. stygia* showed significant reductions under all three levels of interspecific competition (Table 2.3). Comparatively, *Lucilia spp.* only showed significant reductions under *Lucilia spp.* dominant (70_l) competition (Table 2.3). Regardless of density, reductions in thorax length of *C. stygia* under any level of interspecific competition were at least 2x greater than those incurred by *Lucilia spp.* between densities of n=10 and n=50 (Table 2.3).

2.4.2.2 Survival rates

Survival rates were not significantly impacted by any level of interspecific competition relative to full intraspecific competition for either species at densities of n=10 versus n=50 (Fig. 2.3C,D). This trend continued when comparing the survival rates of *C. stygia* and *Lucilia spp.* at the same level of interspecific competition between densities of n=10 and n=50 (Table 2.3). Thus, neither density nor competition level were significant predictors of survival rates for either species, nor did either affect the overall sex ratio for individuals that emerged from pupation (Table. A2.1).

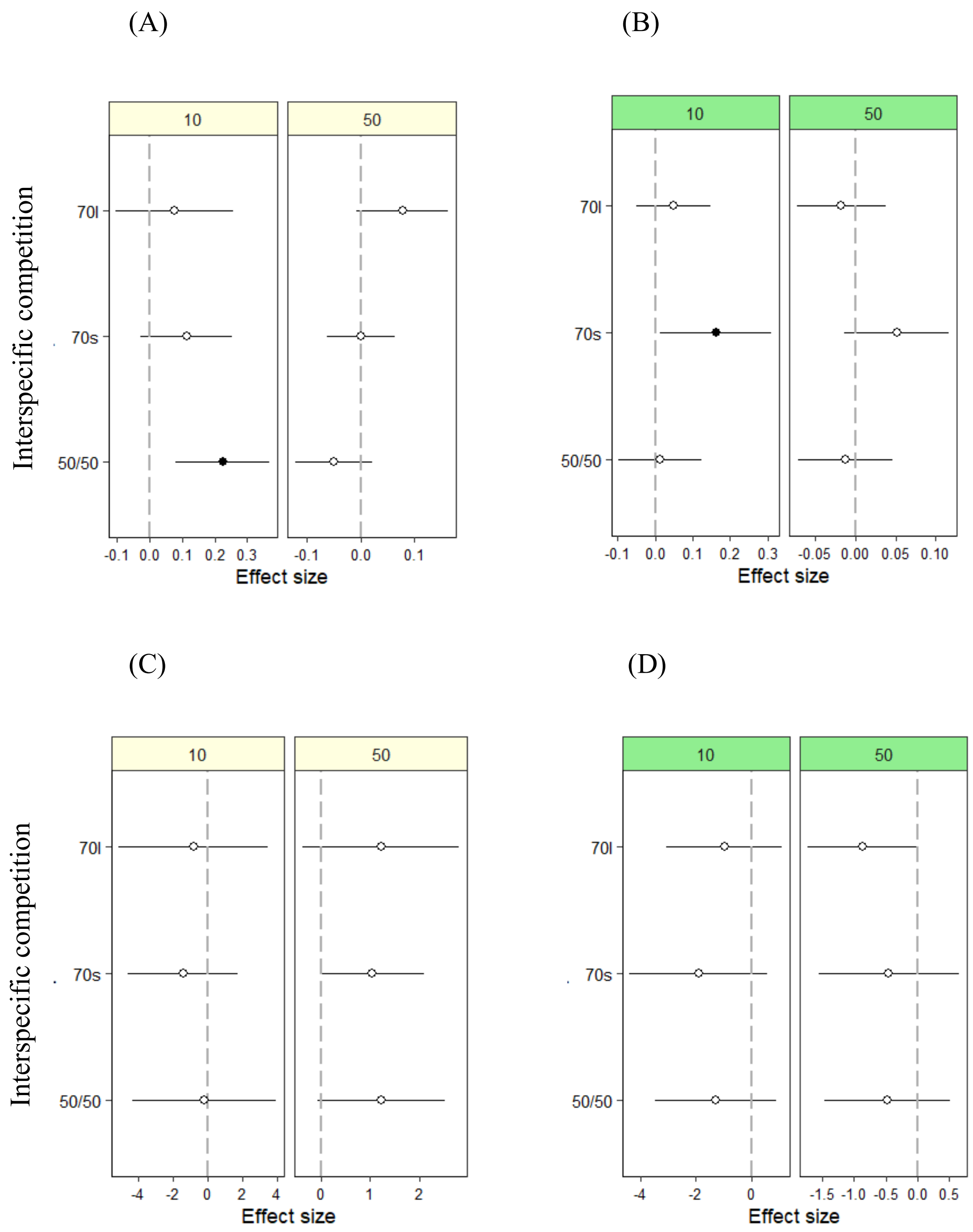

Figure 2.3 Effects of competition on thorax length for: (A) *C. stygia*; (B) *Lucilia spp.*; and on larval survival rates for: (C) *C. stygia* ; and (D) *Lucilia spp.*. All figures feature comparisons within low density (n=10) and high density (n=50) competition and compare the effects of the

noted level of competition relative to full intraspecific competition. The presence of a significant effect is denoted in bold where 95% confidence intervals do not cross zero. Confidence intervals located to the right of zero effect size indicate a positive effect, while those to the left indicate a negative effect.

Table 2.3. Pairwise comparisons of thorax length and larval survival rates under three interspecific competition levels (70_l, 70_s, 50/50) from estimated marginal means modelling for *C. stygia* and *Lucilia spp.*. All comparisons measure the difference between the same competition level at low density (n=10) and high density (n=50). Thorax length comparisons are derived from linear regressions testing the effects of density, competition, sex, and date. Larval survival rates are derived from a GLM fit to a quasi-binomial distribution testing the effects of density and competition. Significant p values are shown in bold type.

Comparison of n=10 vs n=50	Estimate	Standard error	T Value/T ratio	P Value
***C. stygia* thorax length**				
70_l	0.333	0.090	3.690	**0.009**
70_s	0.443	0.062	7.147	**<0.0001**
50/50	0.605	0.067	9.072	**<0.0001**
***C. stygia* survival rates**				
70_l	0.159	1.918	0.083	1.000
70_s	-0.281	1.084	-0.259	1.000
50/50	0.750	1.781	0.421	1.000
***Lucilia spp.* thorax length**				
70_l	0.159	0.043	3.713	**0.008**
70_s	0.202	0.075	2.704	0.174
50/50	0.118	0.053	2.232	0.435
***Lucilia spp.* survival rates**				
70_l	0.4606	0.819	0.562	0.9999
70_s	-0.8781	1.117	-0.786	0.9988
50/50	-0.2307	0.929	-0.248	1.0000

2.4.3 Field competition assays

2.4.3.1 Temporal effects on Diptera emergence

Total species emergence counts from field competition trials can be found in Table 2.4. The total emergence of Diptera species varied significantly with the date of assay establishment, with lower total emergence between replicates 1 (01/03/24) and 2 (08/03/24) (df=15, $p<0.050$) (Fig. 2.4; Table 2.5A). *Sarcophagidae spp.* (flesh flies) were the predominant taxonomic group in the first two replicates (01/03/24, 95.4%) (08/03/24, 73%). The third replicate on the 29/03/24 saw a transition to *C. vicina* as the dominant emergent species (68%) (Fig. 2.4).

C. stygia emerged in low numbers (a total of six individuals emerged across the three trials), while 192 *Lucilia spp.* emerged across all trials, but showed no significant differences in emergence over time (df=15, $p>0.050$) (Table A2.3A).

Table 2.4. Total counts of emerged target blowflies (*C. stygia* and *Lucilia spp.*) and non-target Diptera during field competition trials.

Date	Treatment	*C. stygia*	*Lucilia spp.*	*C. vicina*	*Sarcophagidae spp.*	Total
Replicate 1 (01/03/24)	10_s	0	0	0	312	312
	10_l	0	0	1	245	246
	$5_s/5_l$	0	0	0	44	44
	$3D_s$	0	44	0	217	261
	$3D_l$	0	0	3	91	94
	N_l	0	0	0	92	92
Replicate 2 (08/03/24)	10_s	1	0	0	110	111
	10_l	0	121	0	9	130
	$5_s/5_l$	0	6	0	29	35
	$3D_s$	0	0	0	58	58
	$3D_l$	0	0	0	97	97
	N_l	0	0	0	44	44
Replicate 3 (29/03/24)	10_s	0	0	193	29	222
	10_l	0	4	102	0	106
	$5_s/5_l$	0	0	0	102	102
	$3D_s$	0	0	78	0	78
	$3D_l$	5	0	112	0	117
	N_l	0	17	10	79	106

Table 2.5. Statistical results for the field experiments: (A) Estimated marginal means fit to a quasi-poisson distribution of the effects of date on total Diptera emergence; (B) Estimated marginal means model fit to a negative binomial distribution, investigating the effects of competition treatment on total Diptera emergence; (C) Pairwise comparisons of a negative binomial estimated marginal means model on the effects of competition treatment on total Diptera emergence; (D) Linear regression testing the effect of field-testing treatments on emergent *Lucilia spp.* thorax length (mm); (E) Tukey's HSD post hoc analysis of differences in thorax length (mm) of *Lucilia spp.* between field testing treatments. Significant p values are indicated in bold throughout.

(A) Estimated marginal means model of date on total *Diptera* emergence	**Contrast**	**Log estimate**	**Standard error**	**Z ratio**	**P value**
	Replicate 1 vs 2 (01/03/24 vs 08/03/24)	0.792	0.243	3.260	**0.003**
	Replicate 1 vs 3 (01/03/24 vs 29/03/24	0.361	0.243	0.212	0.203
	Replicate 2 vs 3) (29/03/24 vs 08/03/24)	0.431	0.259	1.665	0.220
(B) Estimated marginal means model of treatment on total *Diptera* emergence	**Treatment**	**Estimate**	**Standard error**	**Z value**	**P value**
	Intercept	5.061	0.251	20.151	< $2e^{-16}$
	10_s	0.252	0.279	0.905	0.365
	$3D_l$	-0.402	0.282	-1.422	0.155
	$3D_s$	-0.270	0.282	-0.959	0.338

	Contrast	Log Estimate	Standard error	Z ratio	P value
	$5_s/5_l$	-0.974	0.289	-3.372	**0.000**
	N_l	-0.707	0.284	-2.487	**0.013**
(C) Pairwise comparisons of *Diptera* emergence between treatments	**Contrast**	**Log Estimate**	**Standard error**	**Z ratio**	**P value**
	10_l vs 10_s	-0.261	0.281	-0.928	0.939
	10_l vs $3D_l$	0.393	0.285	1.381	0.739
	10_l vs $3D_s$	0.262	0.284	0.921	0.941
	10_l vs $5_s/5_l$	0.965	0.292	3.310	**0.012**
	10_l vs N_l	0.698	0.287	2.434	0.145
	10_s vs $3D_l$	0.654	0.285	2.299	0.194
	10_s vs $3D_s$	0.523	0.282	1.851	0.433
	10_s vs $5_s/5_l$	1.226	0.288	4.251	**0.000**
	10_s vs N_l	0.959	0.284	3.375	**0.009**
	$3D_l$ vs $3D_s$	-0.132	0.290	-0.454	0.998
	$3D_l$ vs $5_s/5_l$	0.572	0.291	1.963	0.364
	3Dl vs N_l	0.305	0.289	1.057	0.898
	$3D_s$ vs $5_s/5_l$	0.703	0.295	2.386	0.161
	$3D_s$ vs N_l	0.437	0.289	1.512	0.657

	$5_s/5_l$ vs N_l	-0.267	0.292	-0.914	0.943
(D) Linear regression of treatment on *Lucilia spp.* thorax length (mm)	**Treatment**	**Estimate**	**Standard error**	**T value**	**P value**
	Intercept	4.871	0.768	6.346	$1.66e^{-09}$
	$3D_s$	1.194	0.781	1.529	0.128
	$5_s/5_l$	-0.716	0.570	-1.257	0.210
	N_l	-1.213	0.154	-7.891	**$2.54e^{-13}$**
(E) Pairwise comparison of treatment on *Lucilia spp.* thorax length (mm)	**Contrast**	**Estimate**	**Standard error**	**T value**	**P value**
	$3D_s$ vs10_l	1.194	0.781	1.529	0.205
	$5_s/5_l$ vs 10_l	-0.716	0.570	-1.257	0.323
	N_l vs 10_l	-1.213	0.154	-7.891	**<0.001**
	$5_s/5_l$ vs $3D_s$	-1.910	1.343	-1.423	0.247
	N_l vs $3D_s$	-2.407	0.919	-2.621	**0.018**
	N_l vs $5_s/5_l$	-0.497	0.442	-1.124	0.393

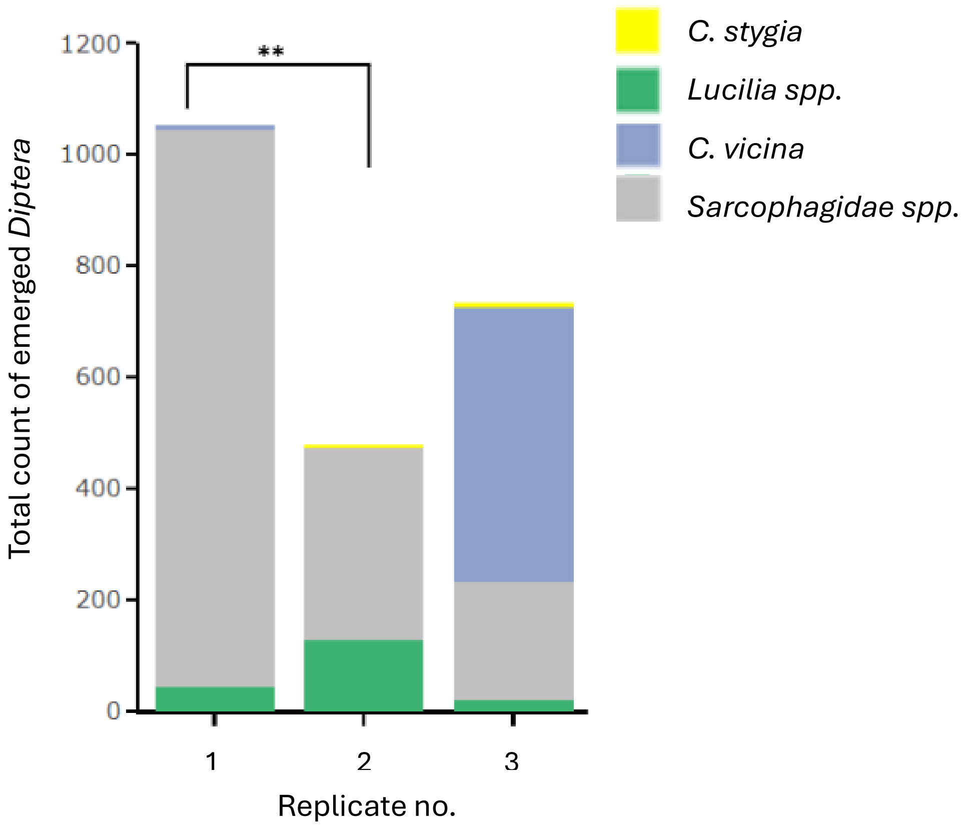

Figure 2.4. Total emergence of flies during replicated field competition assays (replicate 1: 01/03/24; replicate 2: 08/03/24; replicate 3: 29/03/24). Bars are coloured by taxonomic group or species according to the provided key and significant differences are indicated with bars and asterisks at the top of the plot (***=p<0.001; **=p<0.010; *=p<0.050).

2.4.3.2 Within-replicate competition effects

Within a given replicate, significant differences in mean total fly emergence were detected between treatments (Fig.s 2.5, 2.6). Both $5_s/5_l$ (p=0.000) and N_l (p=0.013) treatments saw a reduction in total emergence of flies. In particular, significantly more flies emerged from 10_s than from either 50/50 (p<0.001) or N_l (p<0.010) treatments, and more flies emerged from the 10_l versus $5_s/5_l$ (p<0.010) treatments (Table 2.5C). *Lucilia spp.* only emerged in five out of a total of 18 trials thus, no significant differences in *Luciliia spp.* emergence by treatment could be detected (Table A2.3B).

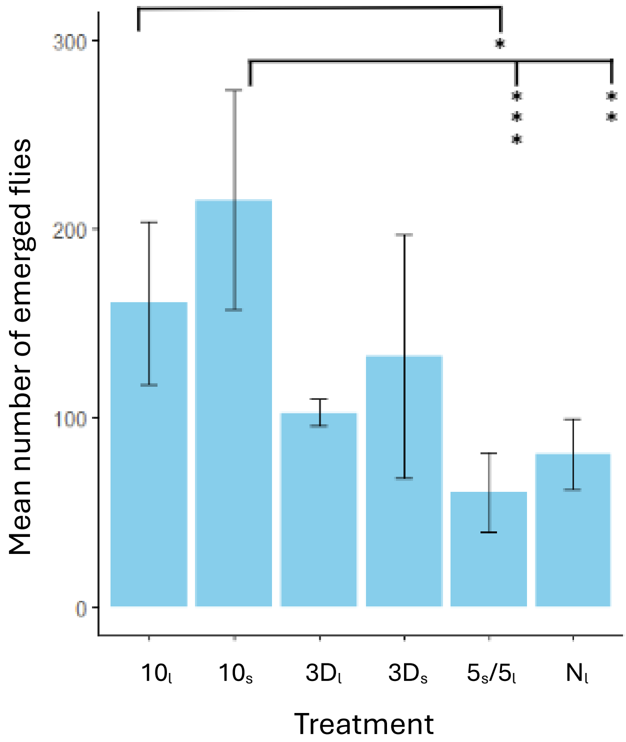

Figure 2.5. Mean number of total emerged flies under different experimental treatments. Refer to Fig. 2.1B for clarification of experimental treatments. Errors bars display standard errors, and significant differences are indicated with bars and asterisks at the top of the plot (***=p<0.001; **=p<0.010; *=p<0.050).

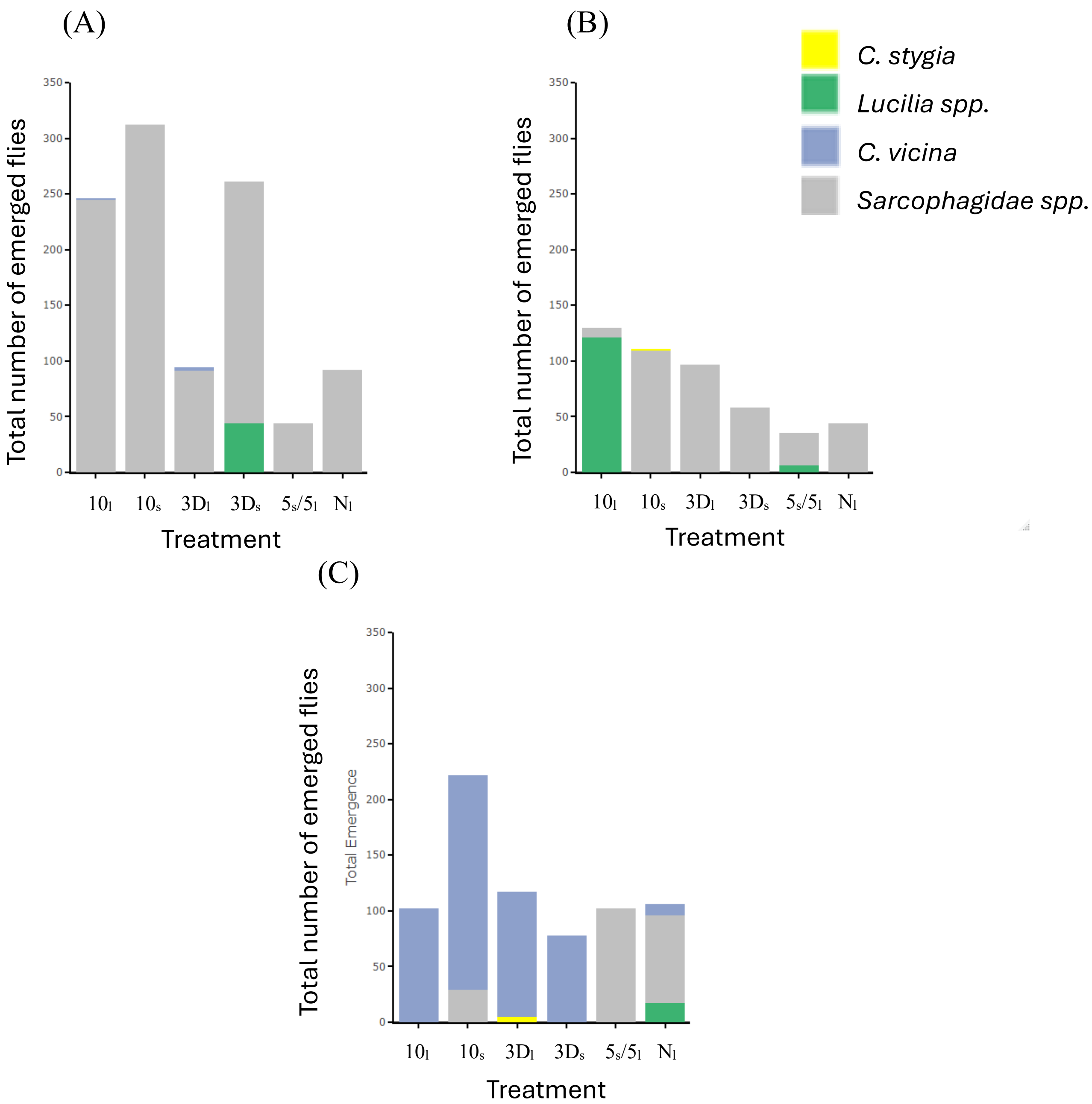

Figure 2.6. Total emergence of flies under different field competition treatments separated by replicate: (A) Replicate 1 (01/03/24); (B) Replicate 2 (08/03/24); (C) Replicate 3 (29/03/24). Bar graphs are stacked and coloured by species as per the provided key.

Examining *Lucilia spp.* emergence data, there was a negative effect of N_l on body size, as measured by thorax length (estimate=-1.213, df=185, p=2.54e^{-13}) (Fig. A2.1A, Table 2.5D). Thorax length was also reduced in the comparisons between N_l versus 10_l (estimate=-1.213, t=-7.891, p<0.001) and N_l versus $3D_s$ (estimate=-2.407, t=-2.621, p<0.050) (Table 2.5E). Treatment did not have a significant effect on the probability of emergence for male or female *Lucilia spp.* (p>0.050). However, sex was a significant predictor of thorax length in the linear model for *Lucilia spp.*, with females being larger (estimate=0.197, t=4.845, df=185, p<0.001). Differences in thorax length between males and females were only detected in the 10_l treatment (estimate=0.189, p<0.01; Fig. A2.1B).

2.5 Discussion

We investigated competition outcomes between sympatric invasive blowfly species under laboratory and field experimental conditions in New Zealand. Our findings indicated that invasive status is a useful predictor of competitive ability, with the more invasive *Lucilia spp.* outcompeting the less invasive *C. stygia* in various metrics under high-density food limitation.

Competition for shared resources and interference dynamics between both conspecifics and heterospecifics can have profound effects on fitness across the animal kingdom, for example lowering reproductive output in Pygmy Owls (*Glaucidium passerinum*) and Spotted hyenas (*Crocuta crocuta*) (Gröning & Hochkirch, 2008; Morosinotto et al., 2017; Watts & Holekamp, 2008), slowing range expansions in the flour beetles *Tribolium castaneum* and *Tribolium confusum* (Legault et al., 2020), and reducing body size and survival rates (Smith & Wall, 1997) for the outcompeted species. In our study, body size reductions between high and low-density treatments were a minimum of ~2x greater for the less invasive *C. stygia* under both intra-and interspecific competition treatments. Moreover, *C. stygia* showed significant thorax length reductions across all levels of competition under high versus low-density conditions, while *Lucilia spp.* exhibited fewer significant reductions in thorax length and no reduction under equal (50/50) competition or *C. stygia*-dominated competition (70_s). Collectively, this suggests that the invasiveness of species may influence the outcomes of competition between sympatric invaders. This hypothesis is supported by previous research. For example, the more invasive signal crayfish (*Pacifastacus leniusculus*; native to North America and invasive in both Europe and Asia; Kouba et al., 2014) is more successful at acquiring shelter, sustains fewer injuries, and better maintains its body size during food competition when directly competed in either isolated or group contexts with the less invasive spiny-cheek crayfish (*Orconectes limosus*; also native to North America, but only invasive in Europe) (Chucholl & Chucholl, 2021; Hudina et al., 2011; Kouba et al., 2014). A similar pattern is observed in invasive mosquitoes, with *Aedes aegypti* (yellow fever mosquito) and *Aedes albopictus* (tiger mosquito) both globally invasive, but the tiger mosquito showing greater predicted range expansion by 2080 (Kraemer et al., 2019; Kraemer et al., 2015); direct competition of these two species results in the tiger mosquito maintaining higher population growth and survivorship in mixed-species cultures (Braks et al., 2004).

Our differential findings in thorax length, emergence, and larval survival between *C. stygia* and *Lucilia spp*. may have other explanations besides invasiveness. For example, the extent to which carrion supports colonisers scales with the mass of the carrion, which in turn affects the diversity and biomass of supported species (Wierer et al., 2024). Current knowledge highlights the importance of the starting density of invaders during the invasion process, as higher density of invaders can increase expression of competitive behaviours (Sagata & Lester, 2009) and mitigate deleterious allele affects associated with low population sizes (Taylor & Hastings, 2005). In our study, density was in fact a more important predictor overall than competition, as indicated by the low number of differences in thorax length and larval survival between competition levels within densities. Moreover, reductions in body size of *Lucilia spp.* at high density were of a smaller magnitude than those experienced by *C.stygia* (minimum of 50% smaller). This suggests that more invasive species may more efficiently use smaller carrion sources than less invasive species when competition is high, and further, that sympatry between invasive species may be in part achievable if interspecific competition effects do not appreciably add to those already exerted by intraspecific competition. Alternatively, we found that *C. stygia* individuals were on average >20% larger than *Lucilia spp.* (depending on the density and treatment) and were also consistently larger under laboratory conditions in the absence of competition and in wild-caught individuals (pers. obs.). Thus, relative body size differences between the two species may influence their responses to food limitation, with larger individuals likely having higher food requirements during larval feeding stages. However, our statistical models remained significant even when accounting for differences in individual body size, suggesting that *Lucilia spp*. may be better at tolerating food limitation.

Food limitation marks a critical point where competitor density directly affects larval development. Reduced food availability has predictable effects on larvae, most notably a decrease in adult blowfly body size and lower larval survival rates (Cook et al., 2024; Kökdener et al., 2020; Rosa et al., 2004). In our laboratory-based competition assays, density and competition only slightly affected survival rates in *C. stygia*, with no measurable effects on *Lucilia spp.* survival. Literature suggests this may result from a trade-off between body size and emergence timing. Some species may shorten feeding or pupation phases in response to competition, where a smaller body size reduces larval mortality under resource limitation (Carmo et al., 2018; Ireland & Turner, 2006). However, responses to food limitation can differ among species. For example, *C. vicina* - an invasive blowfly with a broad global distribution - adjusts time spent in post-feeding larval stages as it reduces feeding time, while post-feeding timing in *L. sericata* remains independent of feeding duration (Komo & Charabidze, 2021). Food limitation also severely impacts the reproductive output of adult blowflies (Williams & Richardson, 1983). Exploring greater levels of food limitation in the future is a potential avenue to further test if *Lucilia spp.* is in fact more tolerant to food limitation.

Larval competition outcomes are often highly nuanced, influenced not just by the competitors themselves, but also by environmental conditions and the quality and abundance of food (Ireland & Turner, 2006; Kökdener et al., 2020). There is evidence of a threshold, where increasing larval density may even facilitate better growth outcomes. For example, *Chrysomya albiceps*, the banded blowfly, performs better in mixed cultures with high interspecific competition, as it preys upon competing larvae (MacInnis & Higley, 2020; Rosa et al., 2004). Additionally, the size of a larval mass can create a microenvironment with a temperature higher than the ambient surroundings (Johnson & Wallman, 2014; Kotzé et al., 2016), enhancing larval development rates to an optimal point (Johnson & Wallman, 2014; Kotzé et al., 2016). Moreover, aggregations of larvae may increase food consumption rates through exodigestion - where larvae excrete enzymes into the carrion to make nutrients more readily available, increasing feeding rates and development (Scanvion et al., 2018). In our study, the lack of body size reductions in *Lucilia spp.* under balanced interspecific competition may indicate an ability to capitalise on density-related benefits. Indeed, *L. sericata* has previously been documented to benefit from interspecific larval cultures under non-food limited conditions, gaining increased body size and survival rates (Komo et al., 2019). However, we were unable to account for potential effects of parental fitness on offspring body mass, as larvae were laid by females in groups. Future research that controls for parental body size effects would help to separate genetic and other drivers of adult body size.

Blowflies are typically among the first groups to colonise carrion, with evidence suggesting that early succession competition dynamics influence colonisation order. In particular, larger blowfly species can predictably arrive first (Brundage et al., 2014; Evans et al., 2020; Rosati, 2014), though this does not guarantee success as other later-arriving insects, such as beetles, can compete with or even prey upon blowfly larvae to significantly reduce their survival rates (Matuszewski & MĄdra-Bielewicz, 2022). The field assays within this study were designed to measure the strength of priority effects that *C. stygia* and *Lucilia spp*. exerted under different balances of larval timing and densities. C. *stygia* was out-competed and failed to emerge in large numbers in our field assay; thus, we were unable to assess differences in emergence counts and body size under different larval seeding treatments. However, *Lucilia spp*. that emerged from meat without prior artificial seeding of larvae (N_l) had lower thorax lengths than those where meat had been seeded with *Lucilia spp*. (from Day 1; 10_l) or *C. stygia* (from Day 3; $3D_s$). This may be in part explained if *Lucilia spp.* is able to exploit different levels of interspecific competition by benefiting from the positive effects of density while mitigating food limitation (Johnson & Wallman, 2014; Komo et al., 2019; Scanvion et al., 2018). However, despite overall Diptera emergence being lower in trials seeded with no larvae and those seeded with a day-one equal species ratio, no significant differences were found in the emergence of *Lucilia* spp. across any level of larval seeding treatments in the current study. This may be because any advantages provided by early succession of *Lucilia spp.* via artificial larval seeding were diminished due to the high abundance of non-target species in the environment (Brundage et al., 2014; Evans et al., 2020; Rosati, 2014). Future work should aim to include higher replication and multiple sites to better elucidate the effects of starting densities and succession order on invasion outcomes - in particular, we expected *C. stygia* to perform well in these assays if body size was a better predicter of colonisation order (Brundage et al., 2014; Evans et al., 2020; Rosati, 2014) than invasiveness; exploring this angle would help to tease apart these potentially competing hypotheses (i.e., when the less invasive species is actually larger).

Overall, our research provides insights into the potential role of invasiveness in predicting competitive outcomes between invasive species with overlapping ecological roles and life histories. While we identified differences in competitive outcomes between the larvae of *C. stygia* and *Lucilia spp.*, the specific mechanisms potentially driving these differences - such as aggression and boldness - remain inferred. Thus, conducting assays that isolate and measure these traits directly is a compelling area of future research that would enhance our understanding of the competitive dynamics at play. Characterising trait performance across a matrix of ecologically similar invasive and native species of varying body size - for both blowflies and other species - would also be invaluable for obtaining further insights into the invasion process.

2.6 Literature cited

Arismendi, I., Penaluna, B. E., Dunham, J. B., García de Leaniz, C., Soto, D., Fleming, I. A., Gomez-Uchida, D., Gajardo, G., Vargas, P. V., & León-Muñoz, J. (2014). Differential invasion success of salmonids in southern Chile: patterns and hypotheses. *Reviews in Fish Biology and Fisheries*, *24*, 919-941.

Barry, T., Gurarie, E., Cheraghi, F., Kojola, I., & Fagan, W. F. (2020). Does dispersal make the heart grow bolder? Avoidance of anthropogenic habitat elements across wolf life history. *Animal Behaviour*, *166*, 219-231.

Baz, A., Cifrián, B., Díaz-äranda, L. M., & Martín-Vega, D. (2007). The distribution of adult blow-flies (Diptera: Calliphoridae) along an altitudinal gradient in Central Spain. *Annales de la Société entomologique de France* (*N.S*), 43(3), 289-296.

Boivin, N. L., Zeder, M. A., Fuller, D. Q., Crowther, A., Larson, G., Erlandson, J. M., Denham, T., & Petraglia, M. D. (2016). Ecological consequences of human niche construction: Examining long-term anthropogenic shaping of global species distributions. *Proceedings of the National Academy of Sciences*, *113*(23), 6388-6396.

Braks, M., Honório, N., Lounibos, L., Lourenço-de-Oliveira, R., & Juliano, S. (2004). Interspecific competition between two invasive species of container mosquitoes, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Brazil. *Annals of the Entomological Society of America*, *97*(1), 130-139.

Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*(2), 378-400.

Brundage, A., Benbow, M. E., & Tomberlin, J. K. (2014). Priority effects on the life-history traits of two carrion. *Ecological Entomology*, *39*(5), 539-547.

Carmo, R. F., Vasconcelos, S. D., Brundage, A. L., & Tomberlin, J. K. (2018). How do invasive species affect native species? Experimental evidence from a carrion blowfly (Diptera: Calliphoridae) system. *Ecological Entomology*, *43*(4), 483-493.

Chucholl, F., & Chucholl, C. (2021). Differences in the functional responses of four invasive and one native crayfish species suggest invader-specific ecological impacts. *Freshwater Biology*, *66*(11), 2051-2063.

Cook, D. F., Tufail, M. S., Howse, E. T., & Voss, S. C. (2024). Manipulating larval rearing media to optimise mass production of the *Calliphora vicina* (Diptera: Calliphoridae). *Austral Entomology*, *63*(1), 95-108.

Cribari-Neto F, Zeileis A (2010). "Beta Regression in R." *Journal of Statistical Software*, 34(2), 1–24.

Croft, L., Matheson, P., Flemming, C., Butterworth, N. J., & McGaughran, A. (2024). Population structure and interspecific hybridisation of two invasive blowflies (Diptera: Calliphoridae) following replicated incursions into New Zealand. *Ecology and Evolution*, *14*(1), e10832.

Dear, J. P. (1985). *Calliphoridae (Insecta: Diptera)*. Fauna of New Zealand, 8.

Duckworth, R. A., & Badyaev, A. V. (2007). Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. *Proceedings of the National Academy of Sciences*, *104*(38), 15017-15022.

Ehrenfeld, J. G. (2010). Ecosystem consequences of biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, *41*(1), 59-80.

Evans, M. J., Wallman, J. F., & Barton, P. S. (2020). Traits reveal ecological strategies driving carrion insect community assembly. *Ecological Entomology*, *45*(5), 966-977.

Feddern, N., Amendt, J., Schyma, C., Jackowski, C., & Tschui, J. (2018). A preliminary study about the spatiotemporal distribution of forensically important (Diptera: Calliphoridae) in the area of Bern, Switzerland. *Forensic*, *289*, 57-66.

Fernández, M., & Hamilton, H. (2015). Ecological niche transferability using invasive species as a case study. *PLoS One*, *10*(3), e0119891.

Ginestet, C. (2011). ggplot2: elegant graphics for data analysis. Oxford University Press.

Griffen, B. D., Guy, T., & Buck, J. C. (2008). Inhibition between invasives: a newly introduced predator moderates the impacts of a previously established invasive predator. *Journal of Animal Ecology*, *77*(1), 32-40.

Gröning, J., & Hochkirch, A. (2008). Reproductive interference between animal species. *The Quarterly Review of Biology*, *83*(3), 257-282.

Gruber, J., Brown, G., Whiting, M. J., & Shine, R. (2017a). Geographic divergence in dispersal-related behaviour in cane toads from range-front versus range-core populations in Australia. *Behavioral Ecology and Sociobiology*, *71*, 1-7.

Gruber, J., Brown, G., Whiting, M. J., & Shine, R. (2017b). Is the behavioural divergence between range-core and range-edge populations of cane toads (*Rhinella marina*) due to evolutionary change or developmental plasticity? *Royal Society Open Science*, *4*(10), 170789.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal: Journal of Mathematical Methods in Biosciences*, *50*(3), 346-363.

Hudina, S., Galić, N., Roessink, I., & Hock, K. (2011). Competitive interactions between co-occurring invaders: identifying asymmetries between two invasive crayfish species. *Biological Invasions*, *13*, 1791-1803.

Hudina, S., Hock, K., & Žganec, K. (2014). The role of aggression in range expansion and biological invasions. *Current Zoology*, *60*(3), 401-409.

Ireland, S., & Turner, B. (2006). The effects of larval crowding and food type on the size and development of the blowfly, *Calliphora vomitoria*. *Forensic Science International*, *159*(2-3), 175-181.

Jackson, M. C., & Britton, J. R. (2014). Divergence in the trophic niche of sympatric freshwater invaders. *Biological Invasions*, *16*, 1095-1103.

Johnson, A. P., & Wallman, J. F. (2014). Effect of massing on larval growth rate. *Forensic*, *241*, 141-149.

Kilgour, R., Norris, D., & McAdam, A. (2020). Carry-over effects of resource competition and social environment on aggression. *Behavioral Ecology*, *31*(1), 140-151.

Kökdener, M., Gündüz, N. E. A., Zeybekoğlu, Ü., & Alakuş, K. (2020). Effects of larval crowding on some biological characteristics of the blowfly, *Calliphora vicina* (Robineau-Desvoidy, 1830 Diptera: Calliphoridae). *Turkish Journal of Entomology*, *44*(1), 101-109.

Komo, L., & Charabidze, D. (2021). Balance between larval and pupal development time in carrion blowflies. *Journal of Insect Physiology*, *133*, 104292.

Komo, L., Scanvion, Q., Hedouin, V., & Charabidze, D. (2019). Facing death together: heterospecific aggregations of blowfly larvae evince mutual benefits. *Behavioral Ecology*, *30*(4), 1113-1122.

Kotzé, Z., Villet, M. H., & Weldon, C. W. (2016). Heat accumulation and development rate of massed maggots of the sheep blowfly, *Lucilia cuprina* (Diptera: Calliphoridae). *Journal of Insect Physiology*, *95*, 98-104.

Kouba, A., Petrusek, A., & Kozák, P. (2014). Continental-wide distribution of crayfish species in Europe: update and maps. *Knowledge and Management of Aquatic Ecosystems*, (413), 05.

Kraemer, M. U., Reiner Jr, R. C., Brady, O. J., Messina, J. P., Gilbert, M., Pigott, D. M., Yi, D., Johnson, K., Earl, L., & Marczak, L. B. (2019). Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nature*, *4*(5), 854-863.

Kraemer, M. U., Sinka, M. E., Duda, K. A., Mylne, A. Q., Shearer, F. M., Barker, C. M., Moore, C. G., Carvalho, R. G., Coelho, G. E., & Van Bortel, W. (2015). The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *Ecology, Epidemiology, and Global Health*, *4*, e08347.

Lagos, M. E., White, C. R., & Marshall, D. J. (2017). Do invasive species live faster? Mass-specific metabolic rate depends on growth form and invasion status. *Functional Ecology*, *31*(11), 2080-2086.

Legault, G., Bitters, M. E., Hastings, A., & Melbourne, B. A. (2020). Interspecific competition slows range expansion and shapes range boundaries. *Proceedings of the National Academy of Sciences*, *117*(43), 26854-26860.

Lenth R (2024). *emmeans: Estimated Marginal Means, aka Least-Squares Means*. R package version 1.10.5-0900003, https://rvlenth.github.io/emmeans/.

MacInnis, A. E., & Higley, L. G. (2020). Competition among three forensically important blow fly species (Diptera: Calliphoridae): *Phormia regina*, *Lucilia sericata*, and *Chrysomya rufifacies*. *Environmental Entomology*, *49*(6), 1473-1479.

Manenti, R., Pennati, R., & Ficetola, G. (2015). Role of density and resource competition in determining aggressive behaviour in salamanders. *Journal of Zoology*, *296*(4), 270-277.

Matuszewski, S., & MĄdra-Bielewicz, A. (2022). Competition of insect decomposers over large vertebrate carrion: Necrodes beetles (Silphidae) vs. blow flies (Calliphoridae). *Current Zoology*, *68*(6), 645-656.

Morosinotto, C., Villers, A., Thomson, R. L., Varjonen, R., & Korpimäki, E. (2017). Competitors and predators alter settlement patterns and reproductive success of an intraguild prey. *Ecological Monographs*, *87*(1), 4-20.

Neumann, K., & Pinter-Wollman, N. (2022). The effect of resource availability on interspecific competition between a native and an invasive ant. *Philosophical Transactions of the Royal Society B*, *377*(1851), 20210146.

Owings, C. G., & Picard, C. J. (2018). New distribution record for *Lucilia cuprina* (Diptera: Calliphoridae) in Indiana, United States. *Journal of Insect Science*, *18*(4), 8.

Pagad, S., Bisset, S., Genovesi, P., Groom, Q., Hirsch, T., Jetz, W., Ranipeta, A., Schigel, D., Sica, Y. V., & McGeoch, M. A. (2022). Country compendium of the global register of introduced and invasive species. *Scientific Data*, *9*(1), 391.

Paquette, A., & Hargreaves, A. L. (2021). Biotic interactions are more often important at species' warm versus cool range edges. *Ecology*, *24*(11), 2427-2438.

Pettit, L. J., Greenlees, M. J., & Shine, R. (2016). Is the enhanced dispersal rate seen at invasion fronts a behaviourally plastic response to encountering novel ecological conditions? *Biology Letters*, *12*(9), 20160539.

Polo-Cavia, N., López, P., & Martín, J. (2011). Aggressive interactions during feeding between native and invasive freshwater turtles. *Biological Invasions*, *13*, 1387-1396.

Pyšek, P., Hulme, P. E., Simberloff, D., Bacher, S., Blackburn, T. M., Carlton, J. T., Dawson, W., Essl, F., Foxcroft, L. C., & Genovesi, P. (2020). Scientists' warning on invasive alien species. *Biological Reviews*, *95*(6), 1511-1534.

R Core Team (2024) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Renault, D., Angulo, E., Cuthbert, R. N., Haubrock, P. J., Capinha, C., Bang, A., Kramer, A. M., & Courchamp, F. (2022). The magnitude, diversity, and distribution of the economic costs of invasive terrestrial invertebrates worldwide. *Science of the Total Environment*, *835*, 155391.

Rosa, G., De Carvalho, L., & Godoy, W. A. C. (2004). Survival rate, body size and food abundance in pure and mixed blowfly cultures. *African Entomology*, *12*(1), 97-105.

Rosati, J. Y. (2014). Spatial and temporal variability in the carrion insect community: using blow flies (Family: Calliphoridae) as a model system to study coexistence mechanisms at multiple scales. *Electronic Theses and Dissertations*. 5193. https://scholar.uwindsor.ca/etd/5193

Rose, H., & Wall, R. (2011). Modelling the impact of climate change on spatial patterns of disease risk: sheep blowfly strike by *Lucilia sericata* in Great Britain. *International for Parasitology*, *41*(7), 739-746.

Sagata, K., & Lester, P. J. (2009). Behavioural plasticity associated with propagule size, resources, and the invasion success of the Argentine ant *Linepithema humile*. *Journal of Applied Ecology*, *46*(1), 19-27.

Saunders, D. S., Wheeler, I., & Kerr, A. (2013). Survival and reproduction of small blow flies (*Calliphora vicina*; Diptera: Calliphoridae) produced in severely overcrowded short-day larval cultures. *European Journal of Entomology*, *96*(1), 19–22.

Scanvion, Q., Hédouin, V., & Charabidzé, D. (2018). Collective exodigestion favours blow fly colonization and development on fresh carcasses. *Animal Behaviour*, *141*, 221-232.

Sievert, C., Parmer, C., Hocking, T., Chamberlain, S., Ram, K., Corvellec, M., Despouy, P., & Sievert, M. C. (2021). Package 'plotly'. R Foundation for Statistical Computing, Vienna. Available in: https://cran. r-project. org/package= plotly (accessed 10 October 2024).

Smith, K., & Wall, R. (1997). Asymmetric competition between larvae of the blowflies *Calliphora vicina* and *Lucilia sericata* in carrion. *Ecological Entomology*, *22*(4), 468-474.

Stoffolano, J. G., Gonzalez, E. Y., Sanchez, M., Kane, J., Velázquez, K., Oquendo, A. L., Sakolsky, G., Schafer, P., & Yin, C.-M. (2000). Relationship between size and mating success in the blowfly *Phormia regina* (Diptera: Calliphoridae). *Annals of the Entomological Society of America*, *93*(3), 673–677.

Tanner, C. J., & Adler, F. R. (2009). To fight or not to fight: context-dependent interspecific aggression in competing ants. *Animal Behaviour*, *77*(2), 297-305.

Tanner, C. J., Salalı, G. D., & Jackson, A. L. (2011). Feeding and non-feeding aggression can be induced in invasive shore crabs by altering food distribution. *Behavioral Ecology and Sociobiology*, *65*, 249-256.

Taylor, C. M., & Hastings, A. (2005). Allee effects in biological invasions. *Ecology Letters*, *8*(8), 895-908.

Thematic Assessment Report on Invasive Alien Species and their Control of IPBES | *IPBES secretariat*. (2023, April 9). https://www.ipbes.net/ias.

Watts, H. E., & Holekamp, K. (2008). Interspecific competition influences reproduction in spotted hyenas. *Journal of Zoology*, *276*(4), 402-410.

Wierer, A., von Hoermann, C., Benbow, M. E., Büchner, C., Feldhaar, H., Fiderer, C., Mitesser, O., Rietz, J., Schlüter, J., & Zeitzler, J. (2024). Mechanisms determining the multi-diversity of carrion visiting species along a gradient of carrion body mass. *Oecologia*, 1-12.

Williams, H., & Richardson, A. (1983). Life history responses to larval food shortages in four species of necrophagous flies (Diptera: Calliphoridae). *Australian Journal of Ecology*, *8*(3), 257-263.

Williams, K., & Villet, M. (2014). Predicting the geographic distribution of *Lucilia sericata* and *Lucilia cuprina* (Diptera: Calliphoridae) in South Africa. *African Invertebrates*, *55*(1), 157-170.

Wilson, E. E., & Wolkovich, E. M. (2011). Scavenging: how carnivores and carrion structure communities. *Trends in Ecology and Evolution*, *26*(3), 129-135.

Yeung, E. J., Klemet-N'Guessan, S., Hossie, T. J., & Fox, M. G. (2023). Boldness, movement and exploration tendency in round goby (*Neogobius melanostomus*) in Southern Ontario. *Journal of Fish Biology*, *103*(4), 790-797.

Zheng, Y., Zeng, Y., Li, J., Gong, J., & Lv, Z. (2023). Boldness behavior and ecological adaptability of stone moroko (*Pseudorasbora parva*) in open-field testing. *Animal Biology*, *1*(aop), 1-18.

Chapter 3

Discussion

Edmund Hillary Hope reserve. Image by: Daniel Nunn

3.1 General overview

In this thesis, I directly competed differentially invasive blowfly species against one another to test the ability of invasive status to predict competition outcomes. I found that the less invasive *C. stygia* was indeed more affected than the more invasive *Lucilia spp.* across all tested levels of intra- and interspecific competition. Thus, my research has provided valuable insights into how we might expect competition dynamics between sympatric invasive species to occur, indicating that invasiveness may be a useful proxy for performance in competition between ecologically similar invasive species.

3.2 Broader implications

A 2015 meta-analysis of invasive species found that, on average, interactions between invasive animals in terrestrial and freshwater ecosystems are found to be neutral, meaning they have no positive or negative effects on the success of other invaders (Jackson, 2015). However, we know that behaviours, such as aggression and boldness, are important in facilitating invasion success (Duckworth & Badyaev, 2007; Hudina et al., 2014; Zheng et al., 2023), and my research suggests that asymmetry in the competitive ability likely plays an important role in determining competitition outcomes between differentially invasive species thereafter. If this is more generally the case in other systems, we might be able to use metrics of 'invasiveness' (e.g., geographic extent, fecundity) to better predict invasion outcomes. In fact, many studies competing differentially invasive species may already exist within the literature without explicitly exploring the predictive ability of invasiveness (e.g., relative levels of establishment and spread are often not quantified). Re-examination of existing literature, with retroactive classification of species by their invasiveness applied, is therefore a potential avenue to rapidly expand on our knowledge in this idea.

Although my primary hypothesis is that invasiveness is the main driver of the observed competitive outcomes, there are other potential explanations that should be considered. For example, differences in larval development times could have allowed one species to capitalise on resources faster than the other. Larval development times for *Lucilia spp.* are highly documented throughout the literature (Bansode et al., 2016; Tarone et al., 2011; Wang et al., 2020), but the same cannot be said for *C. stygia*, perhaps because *Lucilia spp.* is a more prominent global invader. While we did not officially track emergence times in laboratory experiments, observations suggest that *Lucilia spp.* consistently emerged earlier than *C. stygia* during rearing. If *Lucilia spp.* is able to progress through larval instars faster than *C. stygia* during competition, they may have been able to consume resources faster, partly explaining the greater body size reductions for *C. stygia* when competing with *Lucilia spp.*. This would, however, fail to explain the less significant reductions in body size for *Lucilia spp.* under full intraspecific competition. Consideration should also be given to the relative body sizes of *C. stygia* and *Lucilia spp.*: *C stygia* is approximately 20% larger when not food limited, which suggests that its larvae would need to consume comparatively more food than *Lucilia spp*. to reach its maximum body size.

As well as there being other potential explanations for my findings, there are experimental design elements that may have influenced my results. For example, I was unfortunately unable to replicate the interspecific competition levels at middle densities (n=20, n=30), due to limitations in larval production in the laboratory. Completing these additional competition assays may have revealed more nuanced details about how intermediate densities might impact competition outcomes. Previous work has shown that results of competition between mixed cultures of blowfly larvae can vary with density. For example, *Lucilia illustris* is a superior competitor to *Lucilia silvarum* at intermediate, but not high density, competition (Prinkkilá & Hanski, 1995).

In a second example, the lowest ratio of food limitation I used was 0.4 g/larvae, based on previous studies that showed food limitation effects at 0.5 g/larvae (Cook et al., 2024A, 2024B; Ribeiro & Von Zuben, 2010). However, using more extreme food limitation might have revealed stronger, or different, trends in survival rates and body size reductions by forcing a trade-off between investment in body size and earlier emergence times (i.e., longer in the larval stage eating versus earlier pupation and eating cessation), or poorer survival if the critical mass required to complete pupation is unable to be obtained (Ribeiro & Von Zuben, 2010).

A third consideration is the potential influence of parental fitness on larval outcomes. The larvae in my experiments came from wild females for both species, females from *C. stygia* produced larvae only when in groups, and competition experiments were not set up to track individuals through development once larvae were pooled in the treatment ratios. Thus, the parents of any given larvae were not able to be determined, and I was then unable to test for the potential relationship between parental body size and larval size. As a result, I cannot rule out genetic effects as a driver of the measured body size trait and the relatively higher importance of food consumption rate and extent is necessarily implicit.

3.3 Recommendations

Following on from above, some key recommendations were I to repeat these experiments include further exploration of intermediate densities and the potential examination of more extreme food limitation and the relationship between adult and larval body size. In addition, to explore the general transferability of my results, I would suggest including more differentially invasive species into future experiments. Regarding the latter, a key element to consider is that invasive species that are at odds with other invaders are of course, likely also at odds with native species. The consideration of multiple invaders and native species would form an intricate set of intra- and interspecific elements that could be teased apart in carefully designed laboratory studies that vary both density and succession order to better understand the outcome of more realistic competition scenarios.

An interesting anecdote that arose from rearing larvae in the laboratory was that multiple females were required to encourage egg laying for *C. stygia*. In contrast, isolated females of *Lucilia spp.* were able to consistently produce larvae. This provides an intriguing hint that future research into the role of sociality (in the form of cues to encourage egg-laying) may shed further light on the role of sociality versus intraspecific competition in driving range expansion of invasive species (Sexton et al., 2009). Indeed, social structure in the form of larval aggregations can be an important determinant of competition among carrion feeders (Altwegg et al., 2013; Charabidze et al., 2021).

I measured body size, survival rate, and succession order across my experiments. Additional measures to test for fitness outcomes from direct competition that may have provided further insights and could be tested in future include adult fecundity and post-emergence lifespan, both of which have been measured by others when staging competition between blowfly larvae (Prinkkilá & Hanski, 1995). Notably, *L. sericata* exhibits a trade-off in the form of better survival rates, but lower fecundity, under high density competition (Parry et al., 2017).

Finally, as noted in Chapter 2, my data was used to infer the behavioural mechanisms that may drive the observed difference in competitive performance between *C. stygia* and *Lucilia spp.*. Thus, my main recommendation for future work is to more directly examine competitive behaviours, such as boldness and aggression, in the target species. These could be examined in isolation to see if more invasive species are bolder and more aggressive, but also in concert within a single species to test for behavioural syndromes (i.e., traits that are highly correlated with one another, such that more invasive species are superior in suites of correlated traits) (Tamin et al., 2023).

3.4 Conclusions

My thesis results suggest that invasiveness may act in a predictable manner to determine the outcome of competitive interactions between differentially invasive species. Our more invasive model species, *Lucilia spp.,* incurred smaller reductions in body size under food limitation, and the presence of significant reductions was dependent on the intra-interspecific species balance. Comparatively, body size reductions for the less invasive *C. stygia* were greater and occurred under all levels of intra-interspecific competition.

This knowledge is consistent with, but expands upon, the premise that invasive species are more competitive than native species, by showing differences in competitiveness and the expression of key behavioural traits along a continuum - not just as we move from native species to invaders, but also when we compare less and more invasive species. Future work in this area has high potential to further advance our understanding of invasion dynamics using the described model blowfly system.

3.5 Literature cited

Altwegg, R., Collingham, Y. C., Erni, B., & Huntley, B. (2013). Density-dependent dispersal and the speed of range expansions. *Diversity and Distributions*, *19*(1), 60-68.

Bansode, S., More, V., & Zambare, S. (2016). Effect of different constant temperature on the life cycle of a fly of forensic importance *Lucilia cuprina*. *Entomology, Ornithology & Herpetology*; *Current Research*, *5*(3), 2161-0983.

Charabidze, D., Trumbo, S., Grzywacz, A., Costa, J. T., Benbow, M. E., Barton, P. S., & Matuszewski, S. (2021). Convergence of social strategies in carrion breeding insects. *BioScience*, *71*(10), 1028-1037.

Cook, D. F., Tufail, M. S., Howse, E. T., & Voss, S. C. (2024). Manipulating larval rearing media to optimise mass production of the blow fly *Calliphora vicina* (Diptera: Calliphoridae). *Austral Entomology*, *63*(1), 95-108.

Cook, D. F., Tufail, M. S., Voss, S. C., Howse, E. T., & Rogers, E. K. (2024). Maggots cannot live on meat meal alone: production parameters for mass rearing of the ovoviviparous blowfly, *Calliphora dubia* (Diptera: Calliphoridae). *Journal of Economic Entomology*, *117*(3), 750-761.

Duckworth, R. A., & Badyaev, A. V. (2007). Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. *Proceedings of the National Academy of Sciences*, *104*(38), 15017-15022.

Hudina, S., Hock, K., & Žganec, K. (2014). The role of aggression in range expansion and biological invasions. *Current Zoology*, *60*(3), 401-409.

Jackson, M. C. (2015). Interactions among multiple invasive animals. *Ecology*, *96*(8), 2035-2041.

Parry, N. J., Pieterse, E., & Weldon, C. W. (2017). Longevity, fertility and fecundity of adult blowflies (Diptera: Calliphoridae) held at varying densities: implications for use in bioconversion of waste. *Journal of Economic Entomology*, *110*(6), 2388-2396.

Prinkkilá, M. L., & Hanski, I. (1995). Complex competitive interactions in four species of *Lucilia* blowflies. *Ecological Entomology*, *20*(3), 261-272.

Ribeiro, C. d. S., & Von Zuben, C. J. (2010). Nutritional ecology of blowflies (Diptera, Calliphoridae): estimates of critical larval weight for pupation on two different. *Revista Brasileira de Entomologia*, *54*, 661-664.

Sexton, J. P., McIntyre, P. J., Angert, A. L., & Rice, K. J. (2009). Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics*, *40*, 415-436.

Tamin, T., Morinay, J., Germain, M., Récapet, C., & Doligez, B. (2023). Behavioural syndrome between boldness and aggressiveness and link with reproductive success in a wild bird population. *Animal Behaviour*, *197*, 27-41.

Tarone, A., Picard, C., Spiegelman, C., & Foran, D. (2011). Population and temperature effects on *Lucilia sericata* (Diptera: Calliphoridae) body size and minimum development time. *Journal of Medical Entomology*, *48*(5), 1062-1068.

Wang, M., Wang, Y., Hu, G., Wang, Y., Xu, W., Wu, M., & Wang, J. (2020). Development of *Lucilia sericata* (Diptera: Calliphoridae) under constant temperatures and its significance for the estimation of time of death. *Journal of Medical Entomology*, *57*(5), 1373-1381.

Zheng, Y., Zeng, Y., Li, J., Gong, J., & Lv, Z. (2023). Boldness behavior and ecological adaptability of stone moroko (*Pseudorasbora parva*) in open-field testing. *Animal Biology*, *1*(aop), 1-18.

Appendix A2

Table A2.1. Type 3 ANOVA analysis of the effects of various metrics on thorax length for both *C. stygia* and *Lucilia spp.* under full intraspecific competition (F_s and F_l). Significant p values are indicated in bold.

	Sum of squares	Df	F value	P value
Intercept	882.25	25	114	**< 2.2e-^{16}**
Density	0.91	31	4.80	**0.003**
Species	7.26	1	115.397	**< 2.2e-^{16}**
Sex	6.95	1	110.6	**<2.2e-^{16}**
Density: Species	2.80	3	14.853	**1.927e-^{09}**
Species: Sex	12.31	1	195.726	**< 2.2e-^{16}**

Table A2.2. Binomial model of emergence probability for male and female *C. stygia* and *Lucilia spp.,* as predicted by competition and density.

Competition/Density	Estimate	Standard error	Z value	P value
C. stygia				
Intercept	0.081	0.257	0.316	0.752
70_l	0.294	0.302	0.970	0.332
70_s	0.108	0.237	0.457	0.648
F_s	-0.098	0.234	-0.420	0.674
n=20	0.145	0.303	0.478	0.633
n=30	0.220	0.308	0.715	0.474
n=50	-0.244	0.220	-1.111	0.267
Lucilia spp.				
Intercept	0.135	0.263	0.514	0.607
70_l	-0.369	0.259	-1.426	0.154
70_s	-0.320	0.305	-1.049	0.294
F_l	0.177	0.229	0.773	0.440
n=20	-0.297	0.271	-1.095	0.274
n=30	-0.200	0.272	-0.736	0.462
n=50	-0.161	0.212	-0.756	0.450

Table A2.3. Statistical results for field experiments. (A) Generalised linear model fit to a quasi-poisson distribution, testing the effects of replicate number on *Lucillia spp.* emergence counts in field competition testing; (B) Negative binomial model of *Lucilia spp.* emergence under various field-testing treatments.

(A) Emergence of *Lucilia spp.* by replicate	**Replicate**	**Estimate**	**Standard error**	**T value**	**P value**
	Intercept	1.992	1.137	1.753	0.100
	2 (8/03/24)	1.060	1.319	0.804	0.434
	3 (29/03/24)	-0.740	2.000	-0.370	0.717
(B) Emergence of *Lucilia spp.* by treatment	**Treatment**	**Estimate**	**Standard error**	**Z value**	**P value**
	Intercept	3.730	1.497	2.491	0.012
	$3D_s$	-1.044	2.121	-0.492	0.623
	$5_s/5_l$	-3.037	2.154	-1.410	0.159
	N_l	-1.995	2.129	-0.937	0.349

(A) (B)

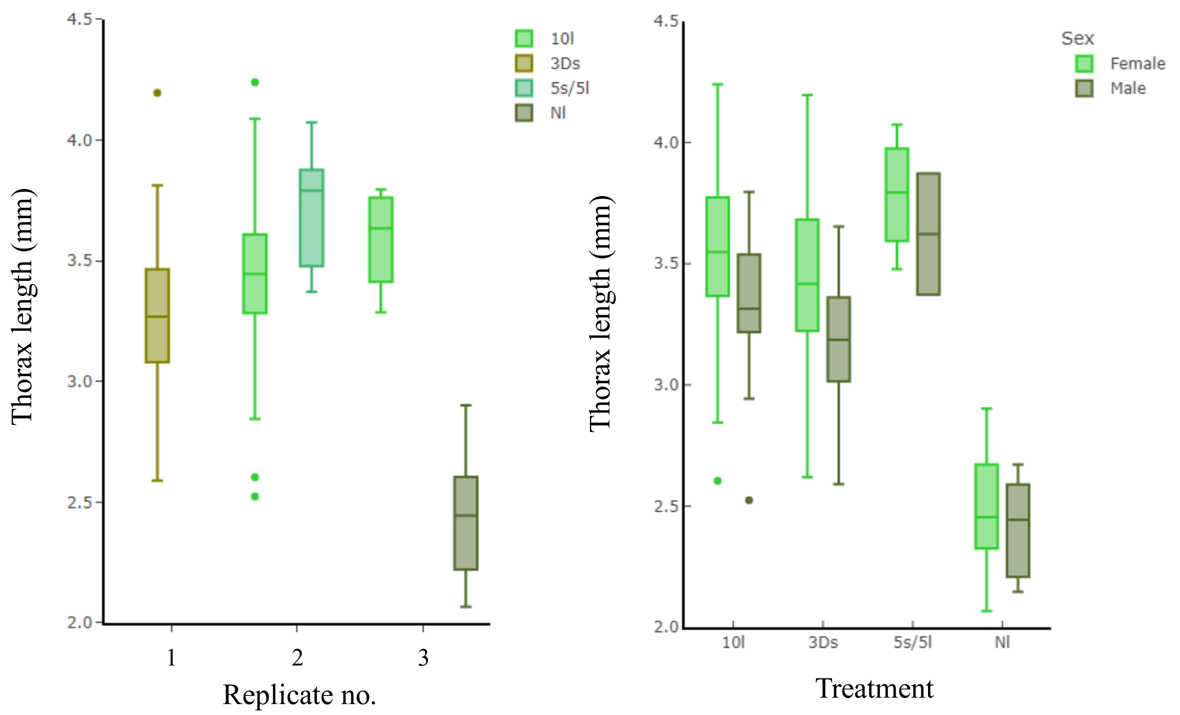

Figure A2.1. Field competition assays: (A) The effects of date and treatment on emerged *Lucilia spp.* thorax length (replicate 1: 01/03/24; replicate 2: 08/03/24; replicate 3: 29/03/24); (B) Thorax length of emerged male and female *Lucilia spp.*.