Disruptive houseguests: effects of social parasitism on colony threat response in an urban social wasp

by

Brianna M. Kaldor-Mair

A Thesis submitted to the Faculty of Graduate and Postdoctoral Studies

In partial fulfillment of the requirements of the degree of

Master of Science

Department of Biological Sciences

University of Manitoba

Winnipeg

Contents

Acknowledgements

I would like to acknowledge the University of Manitoba and NSERC for funding the research presented in this thesis. I would like to thank my supervisor, Dr. Byron Van Nest, for providing excellent guidance and mentorship to me throughout this project. I would like to thank my committee members, Dr. Patricia Ramey-Balci and Dr. Kenneth Jeffries for their advice and support throughout this project, and my labmates in the Van Nest laboratory for supporting me as colleagues and friends. I would like to thank Cameron Ruml for arranging research permits with the Winnipeg municipal government, and Dr. Robert Currie and his family for allowing me access to do research on private property. I would like to thank Dr. Levi Newediuk for his advice on statistical methods, and Kate Kvich for her assistance with my fieldwork.

In addition, I would like to acknowledge that all of the research presented in this thesis was conducted on Treaty 1 territory, which is the home of the Anishinaabeg, Dene, Ininiwak, Dakota-Oyate, Inuit, and Swampy Cree First Nations, as well as the homeland of the Red River Métis Nation. I am grateful to the Indigenous peoples listed here for their stewardship of this land, and I appreciate the opportunity to do research on their traditional, unceded territory.

Contributions of Authors

Brianna Kaldor-Mair carried out both field and microscopy experiments, as well as statistical analysis and generation of figures. Brianna Kaldor-Mair wrote the manuscript with support from Dr. Byron Van Nest. Assembly and testing of field equipment was done by Dr. Byron Van Nest. Project idea conceived by Brianna Kaldor-Mair and Dr. Byron Van Nest.

Abstract

Social parasitism is a life history strategy by which an animal takes advantage of another animal's parental care behaviours to further its own reproductive success. Social parasitism is relatively common among social insects, though its ecological and evolutionary implications are understudied in many groups, such as the social wasps. Here, I present research on the effects of a socially parasitic yellowjacket (*Vespula flavopilosa.*) on the threat response behaviour and neurobiology of two free-living yellowjacket species (*Vespula pensylvanica* and *Vespula maculifrons*) over the colony cycle. Behavioural tests were done via a disturbance assay, in which a target was placed at the nest entrance, and strikes after disturbance were recorded. Colony senescence played an important role in aggression, with threat responses decreasing in intensity and duration over the colony cycle. Fluctuations in parasite proportion affected the colony threat responses of both species, including increasing aggression in one and decreasing aggression in another. Confocal microscopy was done on specimen brains collected during the field season, with immunolabeling of serotonin for analysis due to its relevance in regulating insect aggression. Distribution of serotonin in the mushroom body calyces, a region of the brain previously implicated in aggression, was positively correlated to how quickly colonies responded to disturbance in collected specimens. These findings suggest a role of serotonergic neurons in this region in colony-level aggression.

Figures

Tables

Chapter 1: Introduction and Literature Review

Eusociality and colony behaviour

Eusociality is a life history strategy that involves colonial living, reproductive division of labour, and coordinated behaviours (Sherman et al. 1995). While eusociality has been observed in vertebrates and crustaceans, it most commonly occurs in insects (Sherman et al. 1995; Tóth and Duffy 2005). Within the insects, eusociality has evolved independently several times, particularly in several lineages of the order Hymenoptera (DeSilva 2021). The colony structure among eusocial insects commonly assumed to be 'typical' is comprised of a single reproductive member or 'queen,' also often called a foundress, a sterile 'worker' caste, and short-lived males or 'drones' (DeSilva 2021). In some species, workers may be specialized for certain tasks, behaviourally or morphologically (Giraldo et al. 2013; Pahlke et al. 2019). Eusocial insect colonies may deviate from the typical colony structure by having multiple queens (polygyny), multiple co-operating colonies (polydomy), or circumstantial reproduction by workers (gamergamy) (Borowiec et al. 2021).

The ability to respond to stimuli such as threats in a co-ordinated way as a colony is considered a defining feature of eusociality (Tóth and Duffy 2005). Colony-level behaviours like defensiveness are influenced by circumstances and environmental factors—Brito et al. (2018) found that colonies of the social wasp *Parachartergus pseudoapicalis* engaged in more intense defensive behaviour in colonies with higher proportions of brood. Environmental factors may also play a role in colony behaviour, with weather conditions affecting the foraging intensity and defensiveness of temperate ant colonies (Pinter-Wollman et al. 2012). However, despite these influences, different behavioural phenotypes do exist consistently between colonies in eusocial insects independent of context (Bengston and Dornhaus 2014; Jandt et al. 2020). In honey bees (*Apis mellifera*), certain colony phenotypes were associated with colony fitness: colonies with higher foraging intensity and defensiveness were more likely to survive the winter (Wray et al. 2011). Behavioural syndromes, ie. some behavioural phenotypes commonly occurring together, have also been observed at the colony level, with more defensive colonies of the ant *Temnothorax rugatulus* also being more likely to seek out and forage at novel food resources (Bengston and Dornhaus 2014).

Another key component of eusocial behaviour is communication between colony members, through visual, acoustic, or olfactory cues (Hunt and Richard 2013; Cini et al. 2021). Acoustic signaling via gastric drumming has been observed in social wasps and ants, often in relation to assessment of food quality and quantity, and larval provisioning (Ishay and Brown 1975; Hunt and Richard 2013). Olfactory cues such as cuticular hydrocarbons are used among many social insects for identification of nestmates, and alarm pheromone for targeting of threats during a defensive response (Reed and Landolt 2000; Cini et al. 2021). Mimicry of these cues is one strategy employed by social parasites invade the nests of their hosts (Lorenzi 2003; Barbero et al. 2012).

Eusociality and social parasitism

While most parasites take advantage of their host's physiology, social parasites, also known as brood parasites, take advantage of host parental care behaviours (Pollock et al. 2021). Socially parasitic behaviour is thus constrained to animals that display alloparental care, ie. parental care by non-parent members of the social group (Pollock et al. 2021). Social parasitism has been observed in some fish species, but appears commonly in two main groups of animals: nesting birds, and eusocial insects (Pollock et al. 2021). Within eusocial insects, there are three defined modes of social parasitism: 'temporary' parasitism, or usurpation, inquiline or 'permanent' social parasitism, and dulosis, or 'slave-making.' Each of these modes have both obligate and facultative members observed (Borowiec et al. 2021).

Temporary social parasites attack an already established insect colony, killing the resident queen and using the remaining host workers to raise the first clutch of brood laid by the invading queen (Borowski et al. 2021). Over time, the socially parasitic workers will replace the original inhabitants of the nest, leading to a colony entirely made up of the socially parasitic species (Borowski et al. 2021). Inquilines, or permanent social parasites, will invade an established colony without killing the resident queen, and use camouflage or mimicry to use the existing and replenishing worker caste to raise the socially parasitic brood (Casacci et al. 2021). Some social parasites are capable of temporary or inquiline parasitism, depending on the circumstances (Sledge et al. 2001). Dulosis, or 'slave-making,' is a rare but well-studied form of social parasitism observed in ants (Czechowski and Godzińska 2015). Dulotic ants will raid nests of another species for brood, raising the stolen pupae as their own worker caste (Czechowski and Godzińska 2015).

When discussing the evolutionary origins of social parasitism, Emery's rule is often invoked: the rule states that social parasites are often closely related to their preferred hosts, particularly if the parasitism is facultative (Saga et al. 2017; Borowiec et al. 2021). Temporary and inquiline social parasitism are often observed between conspecifics, in some cases with lineages specialized for a socially parasitic lifestyle (Cervo et al. 1990; Nakamura and Dobata 2024). Closely related species have also been observed to facultatively usurp and parasitize each other interchangeably (Saga et al. 2017). However, while phylogenetic evidence supports Emery's rule in some clades, such as ants, it does not hold for others, such as social wasps in the genus *Vespula* (Lopez-Osorio et al. 2015; Borowiec et al. 2021). Even within ants, cases of social parasitism by members of the order Lepidoptera have been observed (Barbero et al. 2012).

Socially parasitic animals, particularly inquilines, have numerous strategies to invade and exploit host colonies, including the aforementioned mimicry of chemical and acoustic signalling between colony members (Casacci et al. 2021). In paper wasps (*Polistes spp.*), invading social parasites will coat nest materials with hydrocarbons similar to those on their cuticle, causing resident workers to associate their smell with the overall olfactory environment of the colony (Elia et al. 2017). In other socially parasitic species, there is evidence that social parasites will impair nestmate recognition in host workers, as overall nestmate discriminatory behaviour decreases in colonies where social parasites are present (Sledge et al. 2001; Lorenzi 2003).

Social parasitism also affects host worker behaviour and physiology. Workers of the ant *Formica fusca* displayed a significant increase in grooming behaviour of pupae when both obligate and facultative social parasites were present, with obligate social parasites having the most dramatic effect (Tragust et al. 2025). Social parasite invasion has also been observed to increase colony cohesiveness and dominance interactions between workers in a species of social wasp (Cini et al. 2020). Changes in brain and antennal gene expression have also been observed in host workers when social parasites are present (Cini et al. 2020; Stoldt et al. 2023). While some of these behaviours can be indicative of social parasite manipulation of hosts, other behavioural changes may be representative of preparation for worker rebellion (Czechowski and Godzińska 2015; Cini et al. 2020). Worker rebellion against social parasites has been most commonly observed in a laboratory setting, though it is occasionally observed in the field (Czechowski and Godzińska 2015). Worker rebellion can take a variety of forms, including attacking social parasites, killing socially parasitic brood and newly eclosed workers, reproduction by workers, or exodus of workers from the parasitized colony (Czechowski and Godzińska 2015).

There are several life history traits that affect how social parasitism may function in a eusocial insect colony. Colonies with higher discriminatory behaviour toward non-nestmates are overall less susceptible to takeover by social parasites (Nakamura and Dobata 2024). Polygyny has a positive correlation with social parasite invasion, suggesting that acceptance of cohabiting queens can also make a species more vulnerable to invasion by social parasites (Dahan and Rabeling 2022). However, association between queens in some polygynous species have been shown to be useful in fighting off potential invaders (Gamboa 1978; Tibbetts and Reeve 2003). In many social wasps, invasion of a social parasite is usually violent, though inquilinism and non-aggressive invasion have been observed (Cervo et al. 1990; Tibbetts and Reeve 2003).

Modulation of insect aggression via serotonin

Biogenic amines are molecules with important roles as neurotransmitters and neuromodulators in the animal nervous system (Barbero and Casacci 2025). Within the insect nervous system, the three biogenic amines that play the most prominent roles in behavioural regulation are octopamine, dopamine, and serotonin (Barbero and Casacci 2025). Dopamine and serotonin in particular play major roles in regulating aggressive behaviour including competition for mates, establishment of social hierarchies, and response to external threats (Aonuma and Benelli 2023).

Serotonin (5-HT) is widely distributed in the insect brain, though anatomical studies in the model fly *Drosophila melanogaster* have characterized prominent serotonergic neural groups linking areas such as the central complex, the mushroom bodies, and the lateral protocerebrum (Alekseyenko et al. 2019). Serotonergic circuits are highly conserved in the insect brain across orders, though behavioural functions of these circuits vary across taxa (Kamhi et al. 2017).

Serotonin's role in aggression toward individuals, however, has been demonstrated in a variety of taxa, across a variety of circumstances (Kamhi et al. 2017). Newsom et al. (2020) found that dietary supplementation with 5-HTP, a serotonin precursor, increased aggression toward conspecifics in both male morphs of the beetle *Onthophagus taurus,* when behavioural phenotypes regarding aggression usually differ between the two morphs. Oral application of serotonin directly also increased the incidence of aggressive responses to tactile stimuli in the trap jaw ant *Odontomachus kurowiae* (Aonuma 2020). In *D. melanogaster,* both direct injection of serotonin into the haemolymph and upregulation of serotonin via genetic manipulation resulted in flies more prone to starting and escalating aggressive interactions with conspecifics (Dierick and Greenspan 2007). Activation of serotonergic circuits using temperature-sensitive expression in transgenic *D. melanogaster* produced similar results, while blocking these circuits resulted in flies that would initiate aggressive encounters, but failed to escalate them (Alekseyenko et al. 2010).

While there is strong evidence for the role of serotonin in modulating aggression, its role likely varies depending on the serotonin receptor subtype (Bubak et al. 2020). Blocking the 5-HT$_1$ receptor in male crickets abolished courtship-related aggression between males, while blocking the 5-HT$_2$ receptor resulted in no change in these interactions (Rillich et al. 2019). The 5-HT$_2$ receptor has been speculated to have a role in aggression inhibition in some insect species, though investigation of this role is ongoing (Bubak et al. 2020). It is also likely that the role of serotonin as a promoter of aggression is not conserved across arthropod taxa—Dong et al. (2024) found that dietary supplementation with serotonin was an effective inhibitor of conspecific-focused territorial aggression in the spider *Pardosa pseudoannulata.*

In addition to individual-focused aggression, serotonin also plays an important role in eusocial insect behaviour (Aonuma and Benelli 2023). Serotonin has roles in regulating interpretation of olfactory cues related to nestmate recognition in the ant *Tetramorium caespitum*, and differences in the morphology of serotonergic neurons in the mushroom bodies, an area of the brain associated with both aggression and olfactory processing, underlie the 'soldier' caste phenotype (a specialized worker caste for nest defense) in the ant *Pheidole dentata* (Giraldo et al. 2013; Bubak et al. 2016). High serotonin levels in several brain regions have also been associated with increased threat responsiveness in honey bee colonies *(Apis mellifera)*, as well as a noticeable increase in serotonin demonstrated in the brains of individuals exposed to alarm pheromone (Nouvian et al. 2018). The roles of serotonin in eusocial insects remains under-explored relative to studies on courtship-related or territorial interactions.

A brief word on wasps as study organisms

As most entomologists are often reminded, insects tend to be perceived negatively by the general public, evoking feelings of fear and disgust in many people (Fukano and Soga 2021). These attitudes have led to a lack of interest in insect conservation, and misinformation surrounding the existence and degree of insect biodiversity loss, despite a consensus among experts that insect biodiversity is declining at a concerning rate (Saunders et al. 2025). Wasps in particular are viewed negatively by the public, and negative opinion has also led to a lack of research interest into their biology outside of a pest control context (Sumner et al. 2018). Wasps represent a large portion of hymenopteran species, and provide numerous ecosystem services as predators, decomposers, pollinators, and bioindicators (Brock et al. 2021). I chose wasps as the system in which to investigate my study questions, in part, due to the factors listed above. I would urge my fellow researchers to do the same.

My study

The research presented in this thesis consists of two experiments. The first, presented in chapter two, is a behavioural field study on urban nests of two social wasp species, *Vespula pensylvanica* and *Vespula maculifrons*, testing how colony-level defense responses differ over the colony cycle and with increasing intensity of the social parasite *Vespula flavopilosa.* The second experiment, presented in chapter 3, consists of immunohistochemistry and microscopy on the brains of specimens collected during the aforementioned field experiments. This experiment examines the amount and distribution of serotonin in three regions of the brain: the optic lobe, lateral protocerebrum, and the mushroom body calyces. Serotonin measurements for these three regions were compared by species, colony *V. flavopilosa* intensity, and behaviour data collected during the field season. Specimens from all three species were used. With these two experiments I investigate how social parasitism influences colony behaviour, and how colony behaviour is influenced by serotonin.

References

Alekseyenko, O.V., Lee, C., Kravitz, E.A. 2010. Targeted Manipulation of Serotonergic Neurotransmission Affects the Escalation of Aggression in Adult Male *Drosophila melanogaster*. *PLoS One,* **5**(5): e10806. DOI: 10.1371/journal.pone.0010806.

Alekseyenko, O.V., Chan, Y., Okaty, B.W., Chang, Y., Dymecki, S.M., Kravitz, E.A. 2019. Serotonergic modulation of aggression in *Drosophila* involves GABAergic and cholinergic opposing pathways. *Curr. Biol.* **29**(13): 2145-2156. DOI: 10.1016/j.cub.2019.05.070

Aonuma, H. 2020. Serotonergic control in initiating defensive responses to unexpected tactile stimuli in the trap-jaw ant *Odontomachus kuroiwae. J. Exp. Biol.* **223:** jeb.228874. DOI: 10.1242/jeb.228874.

Aonuma, H., Benelli, G. 2023. Aminergic control of aggressive behavior in social insects. *Entomologia* **43**(5): 927-937. DOI: 10.1127/entomologia/2023/1994

Barbero, F., Casacci, L.P. 2025. The effect of biogenic amines in the neuromodulation of insect social behavior. *Curr. Opin. Insect Sci.* **71:** 101390. DOI: 10.1016/j.cois.2025.101390.

Barbero, F., Patricelli, D., Witek, M., Balletto, E., Casacci, L.P., Sala, M., Bonelli, S. 2012. *Myrmica* Ants and Their Butterfly Parasites with Special Focus on the Acoustic Communication. *Psyche (Camb. Mass.)* **2012:** 1-11. DOI: 10.1155/2012/725237.

Bengston, S., Dornhaus, A. 2014. Be meek or be bold? A colony-level behavioural syndrome in ants. *Proc. R. Soc. B* **281:** 20140518. DOI: 20140518.

Borowiec, M.L., Cover, S.P., Rabeling, C. 2021. The evolution of social parasitism in *Formica* ants revealed by a global phylogeny. *Proc. Natl. Acad. Sci.* **118**(38): e2026029118. DOI: 10.1073/pnas.2026029118.

Borowski, C.P., Daglio, A., Jacobson, R.S., Loope, K.J., Walker, C.E. 2021. Interspecific Usurpation in the Downy Yellowjacket, *Vespula flavopilosa (Hymenoptera: Vespidae). Entomol. News* **130**(1): 101-107. DOI: 10.3157/021.130.0110.

Brito, E.L.S., Aragao, M., Santos, G.M.M. 2018. Colony defensive behavior by the swarm-founding wasp *Parachartergus pseudoapicalis*: increase on investment predicts the intensity of nest defense. *Insectes Soc.* **65:** 411-417. DOI: 10.1007/s00040-018-0627-6.

Brock, R.E., Cini, A., Sumner, S. 2021. Ecosystem services provided by aculeate wasps. *Biol. Rev.* **96**(4): 1645-1675. DOI: 10.1111/brv.12719

Bubak, A.N., Yeager, J.D.W., Renner, K.J., Swallow, J.G., Greene, M.J., Roman, G. 2016. Neuromodulation of nestmate recognition decisions by pavement ants. *PLoS One,* **11**(11): e0166417. DOI: 10.1371/journal.pone.0166417.

Bubak, A.N., Watt, M.J., Yeager, J.D.W., Renner, K.J., Swallow, J.G. 2020. The stalk-eyed fly as a model for aggression – is there a conserved role for 5-HT between vertebrates and invertebrates? *J. Exp. Biol.* **223**(1): jeb132159. DOI: 10.1242/jeb.132159.

Casacci, L.P., Barbero, F., Ślipiński, P., Witek, M. 2021. The inquiline ant *Myrmica karavajevi* uses both chemical and vibroacoustic deception mechanisms to integrate into its host colonies. *Biology,* **10**(7): 654. DOI: 10.3390/biology10070654

Cini, A., Branconi, R., Patalano, S., Cervo, R., Sumner, S. 2020. Behavioural and neurogenomic responses of host workers to social parasite invasion in a social insect. *Insectes soc*. **67:** 295-308. DOI: 10.1007/s00040-020-00765-6.

Cini, A., Casacci, L.P., Nehring, V. 2021. Uncovering variation in social insect communication. *Curr. Zool.* **67**(5): 515-518. DOI: 10.1093/cz/zoab065

Cervo, R., Lorenzi, M.C., Turilazzi, S. 1990. Nonaggressive usurpation of the nest of *Polistes Biglumis bimaculatus* by the social parasite *sulcopolistes atrimandibularis (Hymenoptera: Vespidae). Insectes Soc.* **37**(4): 333-347.

Czechowski, W., Godzińska, E.J. 2015. Enslaved ants: not as helpless as they were thought to be. *Insectes Soc.* **62**(1): 9-22. DOI: 10.1007/s00040-014-0377-z

Dahan, R.A., Rabeling, C. 2022. Multi-queen breeding is associated with the origin of inquiline social parasitism in ants. *Sci. Rep.* **12**(1): 14680. DOI: 10.1038/s41598-022-17595-0.

DeSilva, J. 2021. Life History and the Transitions to Eusociality in the Hymenoptera. *Front. Ecol. Evol.* **9:** 727124. DOI: 10.3389/fevo.2021.727124.

Dierick, H.A., Greenspan, R.J. 2007. Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. *Nat.Genet.* **39**(5): 679-682.

Dong, S., Chen, T., Chen, Y., Wang, Y., Yan, Y., Liu, X., Liu, Z., Yu, N. 2024. Serotonin suppresses intraspecific aggression in an agrobiont spider, *Pardosa pseudoannulata*, without affecting predation on insects. *Insect Sci.* **32**(4): 1427-1438. DOI: 10.1111/1744-7917.13456.

Fukano, Y., Soga, M. 2021. Why do so many modern people hate insects? The urbanization–disgust hypothesis. *Sci. Total Environ.* **777:** 146229. DOI: 10.1016/j.scitotenv.2021.146229.

Gamboa, G.J. 1978. Intraspecific defense: advantage of social cooperation among paper wasp foundresses. *Science,* **199**(4336): 1463-1465.

Giraldo, I.M., Patel, E., Gronenberg, W., Trainello, J.F.A. 2013. Division of labor and structural plasticity in an extrinsic serotonergic mushroom body neuron in the ant *Pheidole dentata. Neurosci. Lett.* **534:** 107-111. DOI: 10.1016/j.neulet.2012.11.057.

Hunt, J.H., Richard, F.J. 2013. Intracolony vibroacoustic communication in social insects. *Insectes Soc.* **60**(4): 403-417. DOI: 10.1007/s00040-013-0311-9.

Ishay, J., Brown, M.B. 1975. Patterns in the sounds produced by *Paravespula germanica* wasps. *J. Acoust. Soc. Am.* **57**(6): 1521-1525. DOI: 10.1121/1.380594.

Jandt, J.M., Detoni, M., Loope, K.J., Santoro, D. 2020. *Vespula* wasps show differences in colony-level aggression over time and across contexts. *Insectes Soc.* **67:** 367-381. DOI: 10.1007/s00040-020-00768-3.

Kamhi, J.F., Arganda, S., Moreau, C.S., Trainello, J.F.A. 2017. Origins of aminergic regulation of behavior in complex insect social systems. *Front. Syst. Neurosci.* **11:** 74. DOI: 10.3389/fnsys.2017.00074

Lopez-Osorio, F., Perrard, A., Pickett, K., Carpenter, J.M., Agnarsson, I. 2015. Phylogenetic tests reject Emery's rule in the evolution of social parasitism in yellowjackets and hornets *(Hymenoptera: Vespidae, Vespinae). R. Soc. Open Sci.* **2**(9): 150159. DOI: 10.1098/rsos.150159.

Lorenzi, M.C. 2003. Social wasp parasites affect the nestmate recognition abilities of their hosts *(Polistes atrimandibularis and P. biglumis , Hymenoptera, Vespidae). Insectes Soc.* **50**(1): 82-87. DOI: 10.1007/s000400300013

Nakamura, T.P., Dobata, S. 2024. Discrimination against non-nestmates functions to exclude socially parasitic conspecifics in an ant. *Ethology,* **131**(3): e13533. DOI: 10.1111/eth.13533.

Newsom, K.D., Moczek, A.P., Schwab, D. B., Dingemanse, N. 2020. Serotonin differentially affects morph-specific behavior in divergent populations of a horned beetle. *Behav. Ecol.* **31**(2): 352-360. DOI: 10.1093/beheco/arz192.

Nouvian, M., Mandal, S., Jamme, C., Claudianos, C., d'Ettore, P., Reinhard, J., Barron, A., Giurfa, M. 2018. Cooperative defence operates by social modulation of biogenic amine levels in the honey bee brain. *Proc. R. Soc. B* **285**(1871): 20172653. DOI: 10.1098/rspb.2017.2653.

Pinter-Wollman, N., Gordon, D.M., Holmes, S. 2012. Nest site and weather affect the personality of harvester ant colonies. *Behav. Ecol.* **23**(5): 1022-1029. DOI: 10.1093/beheco/ars066.

Pollock, H.S., Hoover, J.P., Uy, F.M.K., Hauber, M. E. 2021. Brood parasites are a heterogeneous and functionally distinct class of natural enemies. *Trends. Parasitol.* **37**(7): 588-596. DOI: 10.1016/j.pt.2021.02.005.

Reed, H.C., Landolt, P.J. 2000. Application of Alarm Pheromone to Targets by Southern Yellowjackets *(Hymenoptera: Vespidae). Fla. Entomol.* **83**(2): 193. DOI: 10.2307/3496156.

Rillich, J., Rillich, R., Stevenson, P.A. 2019. Differential modulation of courtship behavior and subsequent aggression by octopamine, dopamine and serotonin in male crickets. *Horm. Behav.* **114:** 104542. DOI: 10.1016/j.yhbeh.2019.06.006

Saga, T., Kanai, M., Shimada, M., Okada, Y. 2017. Mutual intra- and interspecific social parasitism between parapatric sister species of *Vespula* wasps. *Insectes Soc*. **64**(1): 95-101. DOI: 10.1007/s00040-016-0519-6.

Saunders, M.E., Lees, A.C., Grames, E.M. 2025. Understanding and counteracting the denial of insect biodiversity loss. *Curr. Opin. Insect Sci.* **68:** 101338. DOI: 10.1016/j.cois.2025.101338.

Sherman, P.W., Lacey, E.A., Reeve, H.K., Keller, L. 1995. Forum: The eusociality continuum. *Behav. Ecol.* **6**(1): 102-108. DOI: 10.1093/beheco/6.1.102

Sledge, M.F., Dani, F.R., Cervo, R., Dapporto, L., Turilazzi, S. 2001. Recognition of social parasites as nest-mates: adoption of colony-specific host cuticular odours by the paper wasp parasite *Polistes sulcifer. Proc. R. Soc. B*. **268**(1462): 2253-2260. DOI: 10.1098/rspb.2001.1799.

Stoldt, M., Collin, E., Macit, M.N., Foitzik, S. 2023. Brain and antennal transcriptomes of host ants reveal potential links between behaviour and the functioning of socially parasitic colonies. *Mol. Ecol.* **32**(18): 5170-5185. DOI: 10.1111/mec.17092

Sumner, S., Law, G., Cini, A. 2018. Why we love bees and hate wasps. *Ecol. Entomol.* **43:** 836-845. DOI: 10.1111/een.12676.

Tibbetts, E.A., Reeve, H.K. 2003. Benefits of foundress associations in the paper wasp Polistes dominulus: increased productivity and survival, but no assurance of fitness returns. *Behav. Ecol.* **14**(4): 510-514. DOI: 10.1093/beheco/arg037.

Tóth, E., Duffy, J.E. 2005. Coordinated group response to nest intruders in social shrimp. *Biol. Lett.* **1**(1): 49-52. DOI: 10.1098/rsbl.2004.0237.

Tragust, S., Brinker, P., Włodarczyk, T. 2025. The transition to a social parasitic lifestyle influences maintenance and expression of essential immune defence traits. *Behav. Ecol. Sociobiol.* **79**(11): 113. DOI: 10.1007/s00265-025-03639-8.

Wray, M.K., Mattila, H.R., Seeley, T.D. 2011. Collective personalities in honeybee colonies are linked to colony fitness. *Anim. Behav.* **81**(3): 559-568. DOI: 10.1016/j.anbehav.2010.11.027.

Chapter 2: Effects of Social Parasitism on Colony Defensive Behaviour

Introduction

The eusocial insects that my study focuses on are social wasps in the genus *Vespula*, known commonly as yellowjackets. Yellowjackets are ubiquitous across North America and Europe, as both native and invasive species, and members of the genus are also invasive in Australia and New Zealand (Lester et al. 2017). Yellowjackets are most commonly studied in the ranges and contexts in which they are invasive (Lester et al. 2017). Though they provide ecosystem services as decomposers and predators in their native range, public opinion of yellowjackets as pests has led to a lack of interest in their biology or conservation (Sumner et al. 2018; Maclean et al. 2021). Social parasitism is relatively common within this genus, as well as intra-species takeover of nests (Saga et al. 2017). Polygyny has also been observed in yellowjacket colonies (Vetter and Visscher 1997).

There are three yellowjacket species included in my study, all native to North America: *Vespula pensylvanica, Vespula maculifrons,* and *Vespula flavopilosa.* All three species are members of the *Vespula vulgaris* species complex, a monophyletic group within the genus *Vespula* (Lopez-Osorio et al. 2014). *Vespula pensylvanica* and *V. maculifrons* are free-living, while *V. flavopilosa* is a facultative social parasite (Stein and Fell 1994).

V. pensylvanica, commonly known as the western yellowjacket, is native to temperate North America and invasive in Australia, New Zealand, and Hawaii (Wilson-Rankin 2014; Loope and Wilson-Rankin 2021). *Vespula pensylvanica* has been noted to have a high degree of behavioural plasticity and has been noted to acclimate to a variety of social and environmental contexts (Wilson-Rankin 2014). Though Maclean et al. (2021) noted some reduction in *V. pensylvanica* populations with loss of forest cover, *V. pensylvanica* appears to be one of the more resilient yellowjacket species to the challenges introduced by urbanization.

V. maculifrons, also known as the eastern yellowjacket, is relatively common in North America but considerably less well-studied than *V. pensylvanica,* having not been observed to be invasive in other regions (Ross et al. 1981). *V. maculifrons* has been observed to have lower foraging rates than most North American yellowjacket species, possibly indicating a slower rate of colony growth (Ross et al. 1981). However, given enough time, *V. maculifrons* can be on the larger range of colony size for yellowjackets, with a mean of 6,558 total nest cells in observational studies (Stein and Fell 1994). *Vespula maculifrons* alarm pheromone has been characterized in laboratory studies, and is the first yellowjacket species for which these studies have been done (Landolt et al. 1995). *Vespula maculifrons* has been well-documented as a host species for *V. flavopilosa,* with several instances of social parasitism by *V. flavopilosa* recorded in literature (MacDonald et al. 1980; Borowski et al. 2021).

Vespula flavopilosa is presumed to be a facultative social parasite, with instances of small, seemingly free-living colonies observed (MacDonald et al. 1980; Stein and Fell 1994). However, Borowski et al. (2021) argues that *V. flavopilosa* should be considered an obligate social parasite until more conclusive evidence of the contrary is provided, as all the colonies investigated in their study showed evidence of usurpation. Though *V. maculifrons* is its most widely observed host, *V. flavopilosa* has been recorded parasitizing various members of the *V. vulgaris* species group, including *Vespula germanica,* an invasive species in North America (Borowski et al. 2021). *Vespula germanica* presence has been documented in central Canada since the 1970s, though the harsh winter climates of central Canada have led to low overwintering success in this temperate European species, and thus low overall population levels (Galloway and Preston 2012).

The ability of a eusocial insect colony to mount a collective defense and protect its investment in brood is one of the major ecological advantages of eusociality (Brito et al. 2017). Eusocial insect colonies have been noted to have distinct behavioural phenotypes in the frequency and intensity of their colony defensive behaviour (Jandt et al. 2020). Colony defensive behaviour has also been found to be influenced by environmental factors, such as habituation to repeated disturbances over short periods of time, ie. multiple disturbances per day (Jernigan et al. 2018). It is unclear how the invasion of a social parasite affects the colony's ability to respond to external disturbances, though studies on paper wasps (*Polistes sp.)* have shown behavioural changes associated with the invasion of a social parasite, as well as changes in brain gene expression (Cini et al. 2020). Parasitized workers displayed a higher level of centrality, and more dominance interactions occurred among individuals (Cini et al. 2020). These behaviours, postulated to be the beginnings of a worker rebellion, have been observed in the ants as well (Cini et al. 2020; Czechowski and Godsińska 2015). While the effects of these behaviours on other colony processes have not been well-studied, it is possible that the increase to internal dominance interactions may decrease worker vigilance to external threats.

In this portion of my study, I tested how parasite intensity affects the colony level defensive responses of *V. pensylvanica* and *V. maculifrons* colonies. My hypotheses were as follows:

(I) Social parasitism will disrupt colony response to external threats. Colony aggression will decrease with increasing parasite proportion.

(II) Response to *V. flavopilosa* will be the same for both host species. The presence of *V. flavopilosa* will affect colony response in the same direction and extent for both *V. pensylvanica* and *V. maculifrons*. The behavioural differences between these species have not been previously investigated, therefore a statement of no change is provided as the hypothesis in this case.

Research for this study was conducted in the city of Winnipeg, Manitoba, Canada. Winnipeg is a city in central Canada with a land area of 5,285km^2 and population of 834,678 per most recent census data, making it the eighth largest city in the country (Statistics Canada 2022). Winnipeg has low population density, with an average of 157.9 people per square kilometre (Statistics Canada 2022). The city of Winnipeg contains 35km^2 of parkland, and 17% of the city's area is covered with tree canopy (City of Winnipeg 2021; Park People 2023). Winnipeg is located at the bottom of the Red River valley, a topographically flat flood plain landscape, and has historically been prone to flooding prior to construction of diversion infrastructure (Natural Resources Canada 2012). There are a total of four rivers running through the city, with the two largest converging in the center (Natural Resources Canada 2012). Winnipeg's climate is strongly seasonal, with warm, humid summers and long, harsh winters (Environment and Climate Change Canada 2020). Summer months in Winnipeg (June-August) have an average daily high of 24.8°C, with extremes of up to 37.8°C recorded, while winter months (December-February) have average daily lows of -19.3°C, with extreme lows of -45.0°C recorded (Environment and Climate Change Canada 2020).

Methods

Study sites and nest locations

Four urban green spaces were selected with permission of the municipal government: Osborne Village Green, Munson Park, Bonnycastle Park, and Assiniboine Forest (Fig. 1; Fig.2). Sites were selected for the 2024 field season based on observed yellowjacket activity during the 2023 field season (see 'Methods Testing' below). Searches for yellowjacket nests were conducted by placing protein-rich bait at a location near the center of the site (sliced Bologna sausage), and observing yellowjacket activity to and from the bait for a period of fifteen minutes. Temperature, humidity, and wasp activity were recorded for each interval. Online calls were also put out for any private property owners willing to allow research on wasp nests in privately owned areas.

Over the course of the field season, six yellowjacket colonies were located: four *V. pennsylvanica* colonies and two *V. maculifrons* colonies (Table 1). Of these colonies, two were located at the Bonnycastle Park site, two at the Assiniboine Forest site, and two on private property (Fig. 2; Table 1). A colony of undetermined species was traced from the Osborne Village Green site to a nearby private property, but due to the hazardous location of the colony, it was not testable and was destroyed by city insect control at the request of the property owner. Though yellowjacket activity was observed at the Munson Park site, no colonies were located at this site. All six testable colonies contained *V. flavopilosa*; cumulative proportions of *V. flavopilosa* are listed in Table 1. In addition to the six testable colonies, two other non-testable colonies were located: an apparent free-living *V. flavopilosa* colony, which weakened and died shortly after discovery, and a *V. maculifrons* colony that was inaccessible due to its location, a crevice in a concrete wall that could not be effectively disturbed. Both colonies were sampled and identified, but assay performance was not successful. Both colonies were located on private property in Winnipeg's downtown area. A *V. germanica* colony was also observed on private property in Winnipeg's southern suburbs. Once the colony was identified as an invasive species, property owners were advised to have it destroyed by city insect control.

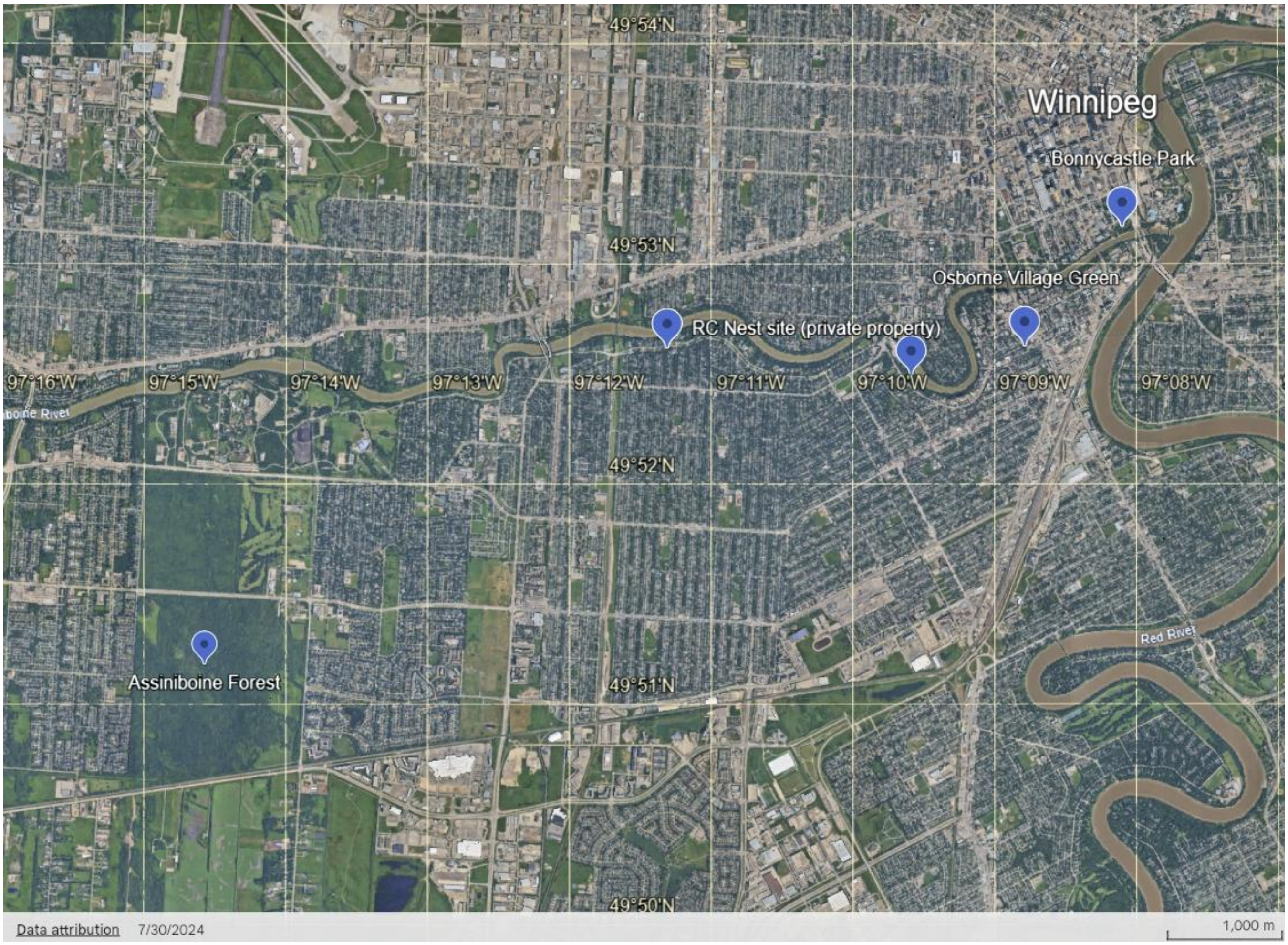

Figure 1: Aerial photo of Winnipeg, with study sites marked. Image taken with Google Earth (earth.google.com/web/)

Figure 2: Aerial views of the four sites used in the study. From smallest to largest, sites are: Osborne Village green (top left)- area 1231m^2, Bonnycastle park (top right)- area 13,403m^2, Munson park (Bottom left)- area 27,844m^2, and Assiniboine Forest (bottom right)- area 2.01km^2. Images taken with Google Earth (earth.google.com/web/)

Behavioural assay

Trials for the disturbance assay were performed once every seven days until the colony was deemed to have collapsed due to the onset of fall and the end of the colony cycle. Times of day when assays were performed were kept as consistent as possible for each nest, and always took place between 11:00 and 16:00. Temperature and humidity were recorded directly prior to conducting each assay. A hollow black decoy (30 cm diameter) was mounted on a tripod standing at a height of 80 cm, and placed as closely as possible to the nest entrance (Fig.3). Wasp strikes to the decoy created short, high-amplitude pressure transients to its internal structure, similar to striking a drum membrane. An electret microphone mounted on the inner surface of the decoy detected these pressure transients; its output was amplified and adjusted so that individual strikes could be identified. Timestamps of identified strikes were then recorded to an SD card for offline analysis. Microphone sensitivity was adjusted manually to record strikes without picking up background noise (Fig.3).

In order to provoke a defensive response, a ‘scratch’ stimulus was performed consisting of three scratches at the nest entrance with a metal fork. Three variables were measured in this assay: latency- defined as time from initiation of the scratch stimulus to the first strike, duration- defined as time from the first strike to the decoy to the last, and total strikes to the decoy. Trials were ended after a fifteen second period of inactivity (ie. no strikes to the decoy). As the counter only records timestamps for strikes, this fifteen second period was not recorded in the data. Rate of strikes per second was also calculated for each trial. These methods were adapted from Jandt et al. (2020).

Sampling and species identification

Upon locating a yellowjacket colony, and prior to each behavioural trial, five specimens were sampled from the nest entrance using glass scintillation vials. Collected specimens were immediately anaesthetized on ice, and identified via dichotomous key upon return to the lab and prior to dissection (Kimsey and Carpenter 2015). Social parasite intensity was calculated and recorded for each sampling event, both as the proportion of *V. flavopilosa* in the five specimens taken that day (instantaneous load), and as an updated proportion of all specimens sampled from that colony so far (cumulative). Instantaneous parasite intensity was used as a measure of short-term (ie. week-to-week) fluctuations in *V. flavopilosa* presence, while cumulative parasite intensity was used as a measure of the presence of *V. flavopilosa* over the colony cycle.

Figure 3: Apparatus for behavioural assay, seen here at a nest entrance in Bonnycastle park. Strikes from the decoy (black) are recorded to the counter (box). Nest entrance location is indicated by the blue arrow.

Statistical analysis of field data

Field data were analyzed using generalized linear mixed models in R (version 4.5.2) using the car (version 3.1.3), lme4 (version 1.1.38), and lmerTest (version 3.1.3) packages. R-squared for mixed models was calculated using the MuMIn package (version 1.48.11). Because repeated measurements were taken from each colony, colony ID was added to the models as a random effect. In order to represent the impact of environmental variables on the behavioural data collected, candidate models were made with various combinations of date, temperature, and humidity. Akakieke's information criteria (AIC) were calculated for each model using the AICcmodavg package (version 2.3.4), and for each variable the model with the lowest AIC was selected and incorporated into the full model. Variables measured were log transformed to better meet model assumptions prior to modeling.

Each model tested the impact of parasite load on either latency, duration, or total strikes. Parasite intensity was calculated from samples of five specimens each time a trial was performed. To measure short-term fluctuations, *V. flavopilosa* proportion was calculated out of those five per trial. To measure longer term fluctuations in parasite intensity, *V. flavopilosa* proportion was calculated cumulatively, with the number of host and parasite specimens added to previous counts, and parasite intensity re-calculated after each sampling event. Linear models were constructed for both methods of calculating parasite intensity.

Results

Searches and colony location

Colony location during the 2024 field season was difficult due to excessively rainy weather and cool spring conditions, both of which have been documented to result in reduced yellowjacket populations (Akre and Reed 1981). *V. flavopilosa* proportions were highly variable within testable colonies in both the short-term and cumulative measurements for both host species (Fig. 4). Of the two species tested, *V. maculifrons* nests had higher proportions of *V. flavopilosa* overall (Fig.4; Table 1). Proportions of parasites calculated cumulatively from all samples taken ranged from 0.23 to 0.91, with an average parasite proportion of 0.53 (Table 1). The nest designated 'BS' was assumed to be a free-living *V. flavopilosa* colony until the last sampling event, when individuals of *V. maculifrons* were identified. It is assumed that *V. maculifrons* workers had been present at low levels in the colony throughout the testing period, and that the low number of samples taken per trial had not reflected their presence.

Table 1: Overview of nest locations and species demographics. Cumulative V. flavopilosa proportions are calculated as the proportion of V. flavopilosa specimens out of all specimens collected from the respective nest.

Nest ID	**Host species**	**Location**	**Cumulative *V. flavopilosa* proportion (%)**
BR	*V. pensylvanica*	Bonnycastle park	23
BC	*V. pensylvanica*	Bonnycastle park	44
AB	*V. pensylvanica*	Assiniboine forest	45
AS	*V. pensylvanica*	Assiniboine forest	60
RC	*V. maculifrons*	Private property	52
BS	*V. maculifrons*	Private property	91

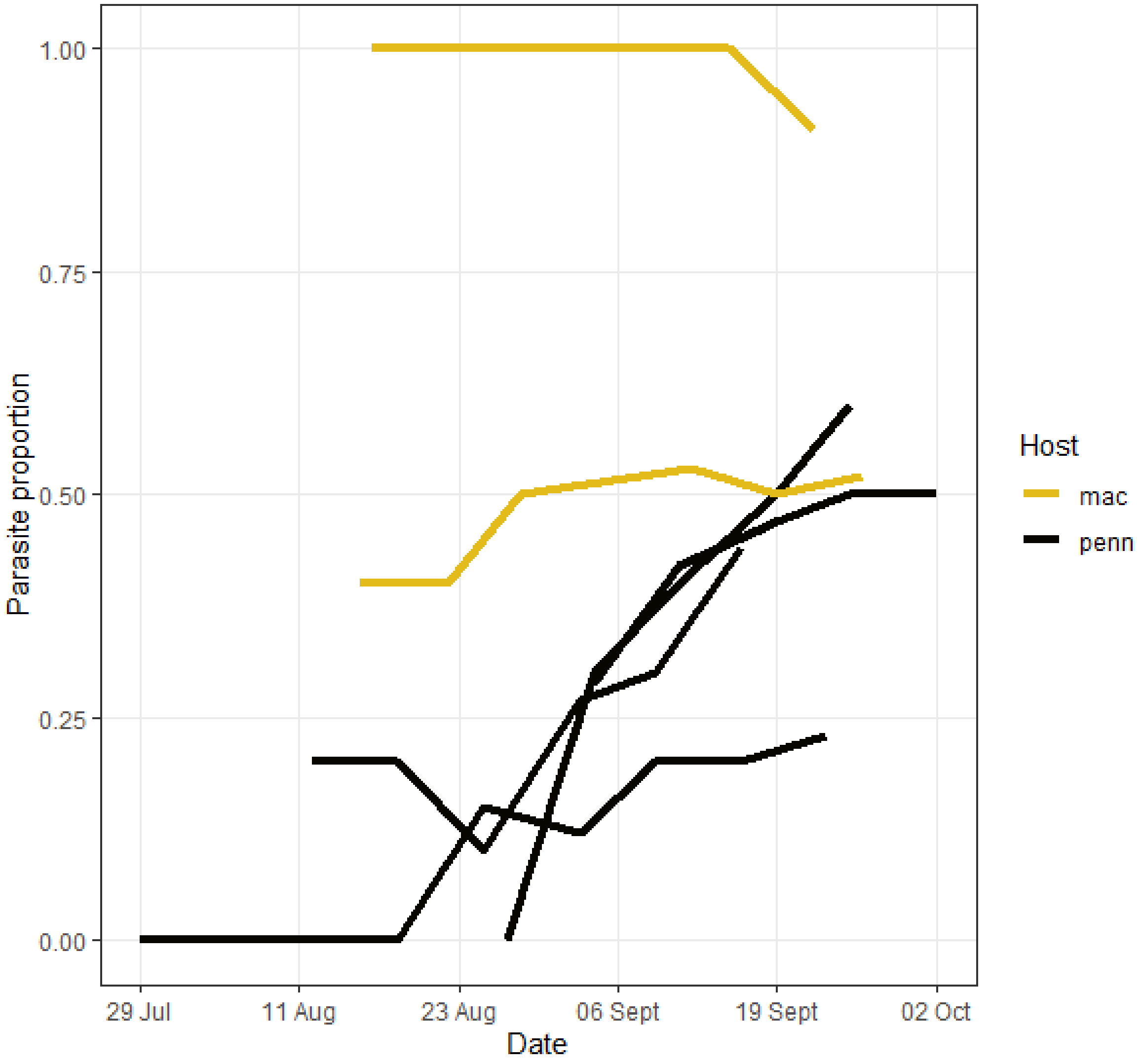

Figure 4: Timeline of *V. flavopilosa* intensity in identified nests. 'Penn' denotes V. pensylvanica, while 'Mac' denotes *V. maculifrons*. *V. flavopilosa* proportion was calculated using the cumulative method.

Disturbance assay results

Between six and eight assays were performed on the six testable colonies in the period from July 29 to October 02, 2024. Colony averages for each variable are listed in Table 2. Six candidate models for each variable were tested, with the selected environmental variable added to the model. Linear modeling results for both instantaneous and cumulative modes of calculating parasite intensity are reported below.

Table 2: Cumulative behaviour variable averages and sampling information by colony. Standard errors are shown for each average. Averages for latency and duration are measured in seconds.

Nest ID	**Date identified**	**Trials**	**Specimens collected**	**Average latency**	**Average duration**	**Average strikes**
BR	22-Jul-24	8	40	5.38 ± 2.27	390.34±104.27	295.88±151.64
BC	29-Jul-24	8	25	6.24 ± 2.61	215.69 ± 53.4	125.83 ± 46.36
AB	24-Jul-24	6	22	14.48 ± 7.58	224.78 ± 57.14	99.20 ± 42.97
AS	21-Aug24	6	25	8.48 ± 2.5	222.76 ± 65.83	39.00 ± 15.07
RC	13-Aug-24	6	25	0.449 ± 0.17	163.83 ± 62.94	55.83 ± 20.3
BS	15-Aug-24	6	23	1.25 ± 0.37	467.10 ± 240.72	149.40 ± 120.1

Latency

Large fluctuations in colony latency (ie. time from disturbance to first strike) occurred throughout the colony cycle, and there was no apparent correlation between latency and proportion of *V. flavopilosa* with either calculation method (Fig.5). Temperature was selected to represent environmental variables from the candidate models present. Full results and Akieke's information criteria for latency candidate models can be found in Table 3. One assay's latency data was lost due to a recording error.

When using the instantaneous measure of parasite intensity, temperature had a marginal effect on latency (p=0.08). No other significant effects were observed. When using the cumulative method of calculating parasite load, host species was significant, with *V. maculifrons* having significantly lower latency (ie. being quicker to respond to disturbance) than *V. pensylvanica* (p=0.03). No effects were observed for temperature or parasite load in this model. Full summary of linear modeling for latency can be found in tables 4 and 5.

Table 3: Candidate model selection results for latency. All models have log-transformed latency as the response variable. ΔAICc represents the difference in AIC score between the best-fit model and the model being compared, while AICc weight represents the proportion of predictive power represented by the model in question when compared to the other models listed.

Model Terms	**AIC**	**ΔAICc**	**AICc Weight**
Temperature	135.64	0	0.44
Date + Temperature	137.03	1.39	0.22
Temperature + humidity	137.63	1.99	0.16
Humidity	138.72	3.08	0.09
Temperature * humidity	139.33	3.69	0.07
Date	141.92	6.28	0.02

Table 4: Generalized linear model results for latency using the instantaneous mode of calculation ($R^2 = 0.459$). 'Interaction' denotes results for the interaction between the factors of host species and parasite load. Model formula: Log(latency) ~ parasites*host + temperature + (1|nest ID)

Variable	**Degrees of Freedom**	**t-value**	**p-value**
Parasites	29	0.926	0.362
Host species	29	1.65	0.109
Temperature	29	-1.81	0.080·
Interaction	29	0.157	0.876

Table 5: Generalized linear model results for latency using the cumulative measure of parasite intensity ($R^2 = 0.441$). 'Interaction' denotes results for the interaction between host species and parasite load. Model formula: Log(latency) ~ parasites*host + temperature + (1|nest ID)

Variable	**Degrees of Freedom**	**t-value**	**p-value**
Parasites	29	1.38	0.17
Host species	29	2.27	0.03*
Temperature	29	-1.64	0.11
Interaction	29	-0.25	0.80

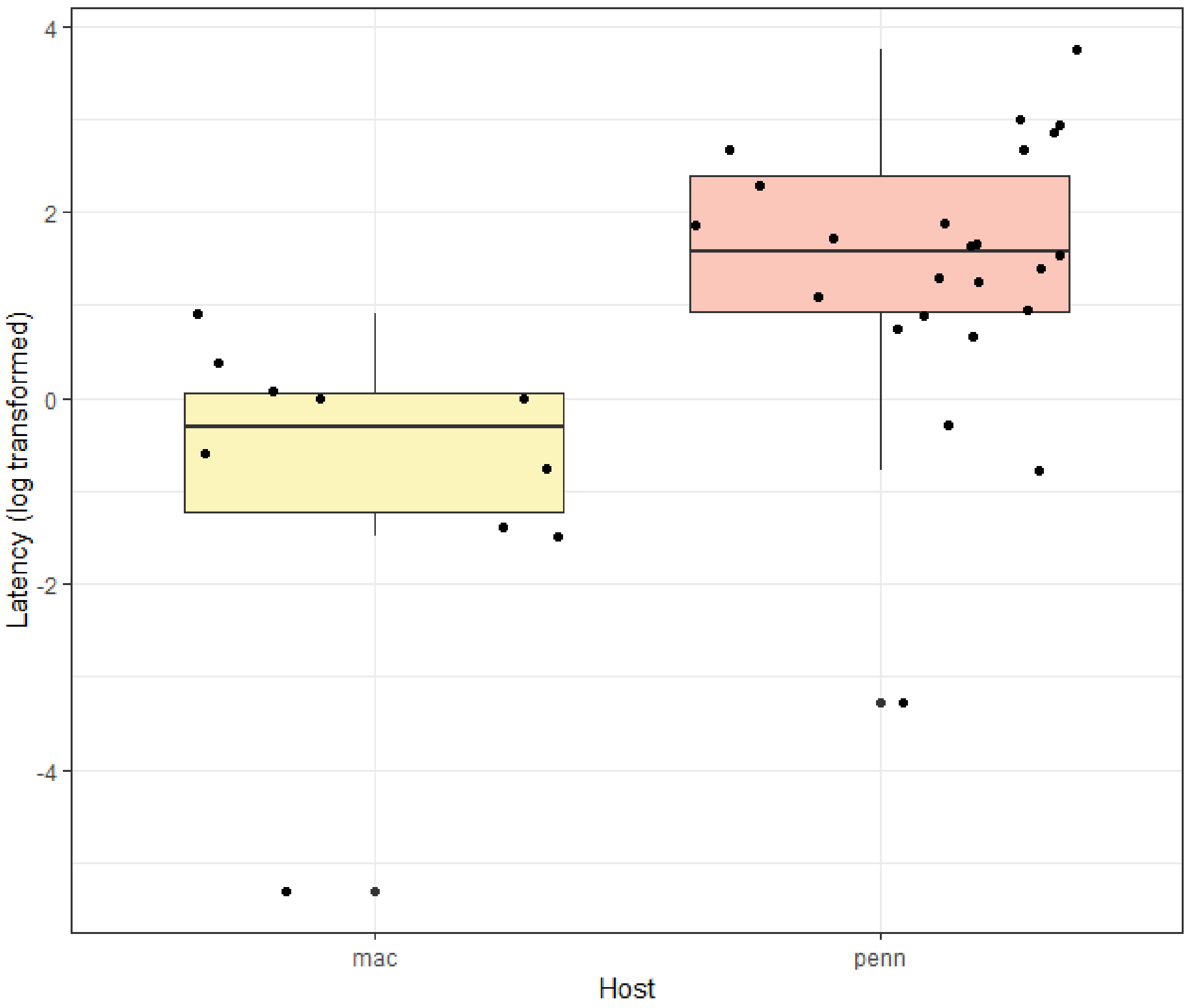

Figure 5: Species differences in latency. 'mac' denotes *V. maculifrons* , while 'penn' denotes *V. pennsylvanica*.

Duration

Date was selected from candidate models as the best environmental variable to represent in linear models for attack duration (Table 6). Effects of instantaneous parasite load differed by species, with attack duration appearing to increase with growing parasite proportion in *V. maculifrons* and decrease with parasite proportion in *V. pensylvanica* (Fig. 6). Date also had a significant effect in this model. Cumulative parasite intensity did not have a significant effect on attack duration for either host species. However, date maintained its significance in this linear model, with attack duration decreasing in both host species over the colony cycle (Fig. 7). Full summary of linear modeling for attack duration can be found in tables 7 and 8.

Table 6: Candidate model selection results for duration. All models have log-transformed duration as the response variable.

Model formula	AIC	ΔAICc	AICc Weight
Date	94.80	0	0.63
Date + Temperature	97.35	2.56	0.80
Temperature	98.30	3.50	0.91
Temperature + humidity	100.06	5.26	0.96
Humidity	100.80	6.01	0.99
Temperature * humidity	102.78	7.99	1.00

Table 7: Model results for duration using the instantaneous mode of calculation (R2 = 0.317). 'Interaction' denotes results for the interaction between the factors of host species and parasite load. Model formula: Log(duration) ~ parasites*host + date + (1|nest ID)

Variable	Degrees of Freedom	t-value	p-value
Parasites	30	2.75	0.010*
Host species	30	2.47	0.019*
Date	30	-2.15	0.040*
Interaction	30	-2.04	0.050·

Table 8: Model results for duration using the cumulative measure of parasite intensity (R2 = 0.222). 'Interaction' denotes results for the interaction between host species and parasite load. Model formula: Log(duration) ~ parasites*host + date + (1|nest ID)

Variable	Degrees of Freedom	t-value	p-value
Parasites	30	1.562	0.129
Host species	30	1.338	0.191
Date	30	-2.245	0.032*
Interaction	30	-0.452	0.655

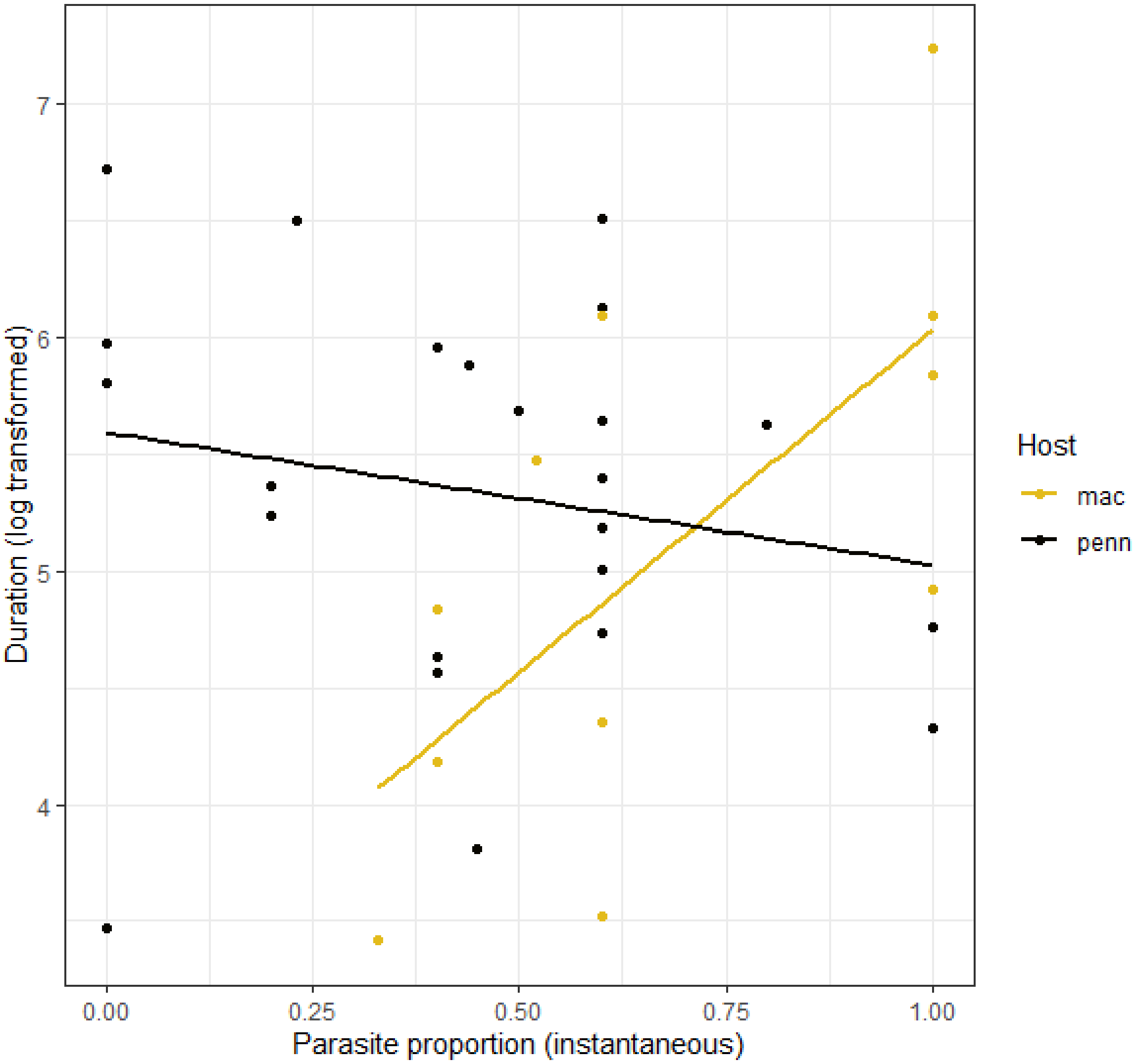

Figure 6: Duration in relation to instantaneous parasite proportion. 'Mac' denotes *V. maculifrons*, 'penn' denotes *V. pensylvanica*.

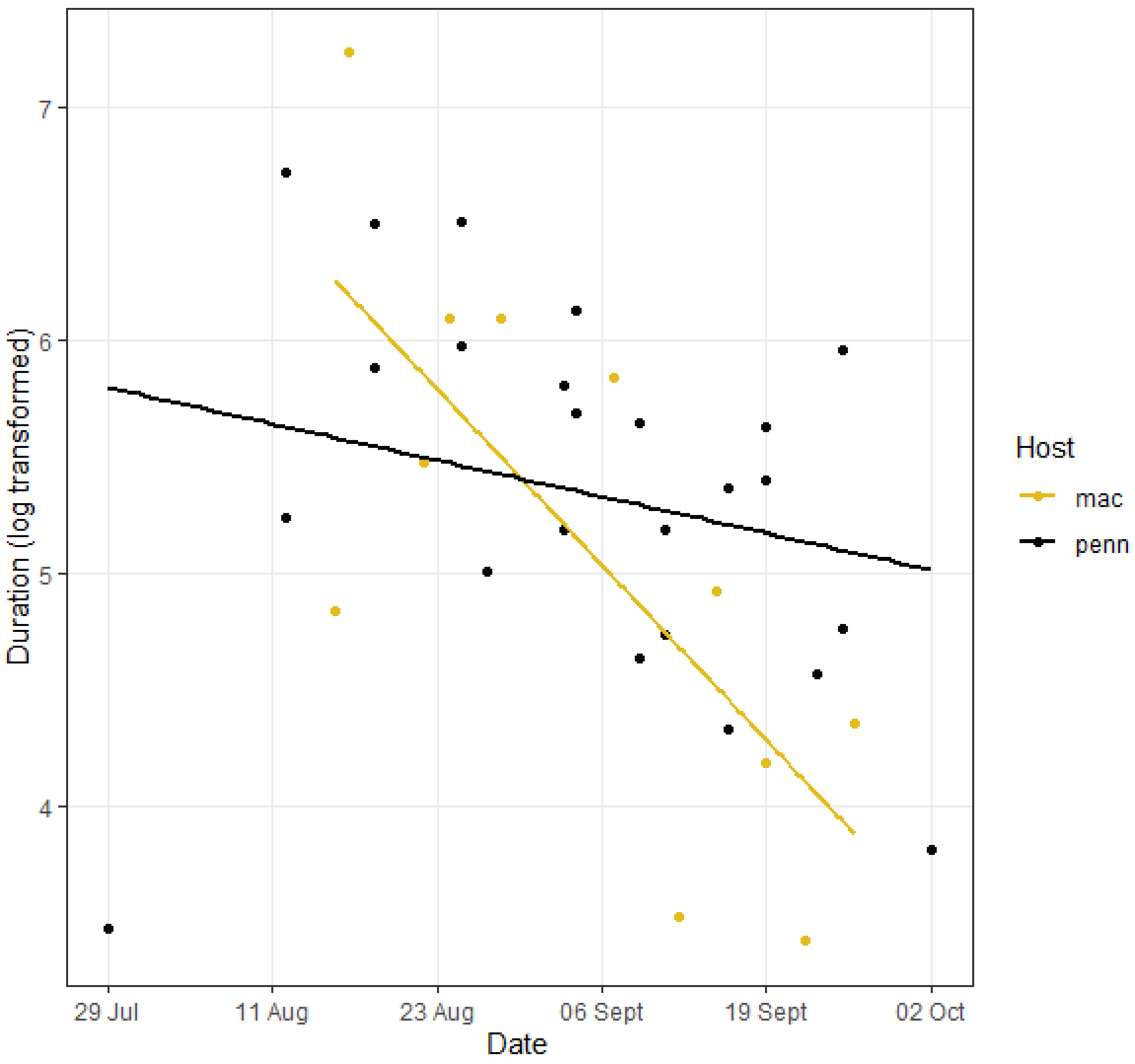

Figure 7: Duration over the colony cycle. 'mac' denotes V. maculifrons, while 'penn' denotes V. pensylvanica.

Total Strikes

Date was selected as the environmental variable to be included in the linear modeling for total strikes. Though interaction between temperature and humidity yielded a slightly stronger AIC in the candidate models, the difference was negligible and overfitting was a concern for these data. Thus, the more parsimonious model option was selected instead. Full results for candidate modeling can be found in Table 9. Date held significance in the linear models for both instantaneous and cumulative parasite proportion, with total strikes decreasing over the colony cycle in both host species (Fig. 9). Though total strikes appeared to decrease with instantaneous parasite proportion in *V. pensylvanica* (Fig.8)*,* linear modeling results do not support a significant difference in total strikes between host species, or in relation to *V. flavopilosa* proportion. Full results for linear modeling can be found in Tables 10 and 11.

Table 9: Candidate model selection results for total strikes. All models have log-transformed duration as the response variable.

Model formula	**AIC**	**ΔAICc**	**AICc Weight**
Temperature*humidity	479.62	0	0.32
Date	480.44	0.82	0.21
Humidity	481.50	1.88	0.13
Date + temperature	481.54	1.92	0.12
Temperature	481.64	2.02	
Temperature + humidity	482.00	2.38	0.10

Table 10: Model results for total strikes using the instantaneous mode of calculation (R2 = 0.341). 'Interaction' denotes results for the interaction between the factors of host species and parasite load. Model formula: Log(total strikes) ~ parasites*host + date

Variable	Degrees of Freedom	t-value	p-value
Parasites	30	0.150	0.881
Host species	30	0.927	0.361
Date	30	-2.98	0.006*
Interaction	30	-0.500	0.621

Table 11: Model results for total strikes using the cumulative measure of parasite intensity (R2 = 0.348). 'Interaction' denotes results for the interaction between host species and parasite load. Model formula: Log(total strikes) ~ parasites*host + date + (1|nest)

Variable	Degrees of Freedom	t-value	p-value
Parasites	3.23	1.562	0.129
Host species	3.62	1.338	0.191
Date	29.4	-2.245	0.032*
Interaction	8.89	-0.452	0.655

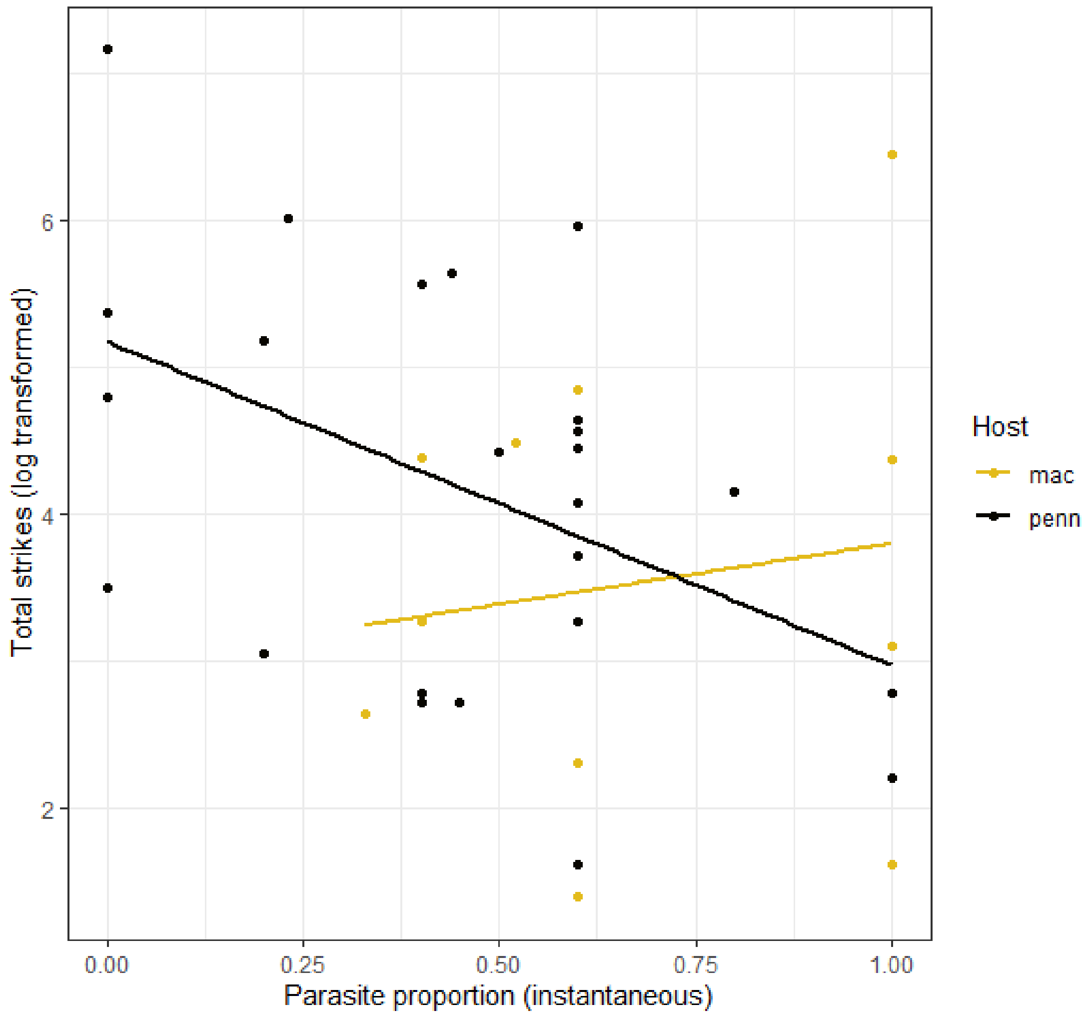

Figure 8: Total strikes in relation to instantaneous parasite proportion. 'Mac' denotes *V. maculifrons*, while 'penn' denotes *V. pensylvanica*.

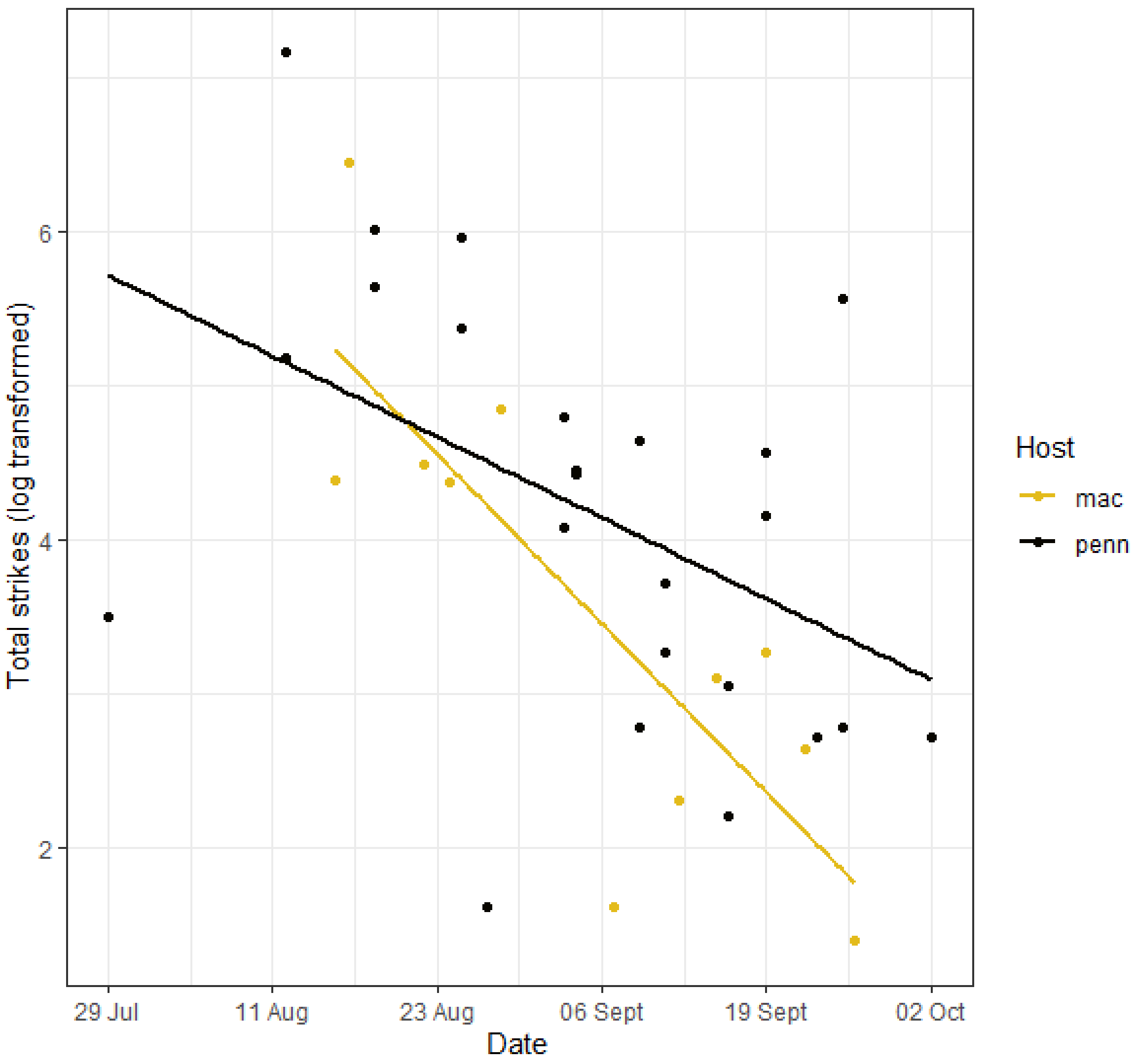

Figure 9: Trends in total strikes across the colony cycle. 'mac' denotes V. maculifrons while 'penn' denotes V. pensylvanica.

Discussion

The small sample size, high intra and inter-colony variability, and overall ubiquity of *V. flavopilosa* in located colonies present challenges in interpreting these data. In addition, the speed at which the responses happened made it impossible to distinguish if the host workers or *V. flavopilosa* workers were doing the majority of the attacks on the decoy. Based on comparison of reported yellowjacket observations in the Winnipeg area and recorded environmental data from 2018-2022, the high degree of precipitation that occurred in the 2024 field season likely presented a challenge for the establishment of yellowjacket colonies, and may have contributed to the difficulty we encountered in locating yellowjacket colonies (iNaturalist 2024; Environment and Climate Change Canada 2020). These observations are supported by patterns observed in the literature, with yellowjacket populations being noticeably lower in years with rainy springs and summers (Akre and Reed 1981).

Host species was the only predictive variable for latency out of those tested in our models, with *V. maculifrons* colonies being quicker to respond to disturbances than *V. pensylvanica* colonies (Table 5; Fig. 5). These findings provide some support for species differences in responsiveness within the genus, though the low sample size of our study warrants further investigation into how responsiveness differs between species. It is also ambiguous whether the more responsive species in question is *V. maculifrons* or *V. flavopilosa,* as both testable *V. maculifrons* colonies included in this study had relatively high proportions of *V. flavopilosa.* The defensive behaviour of socially parasitic wasps has not been thoroughly studied, and it is possible that facultative social parasites are more sensitive to external threats than their free-living counterparts.

Duration was the only behavioural variable that had an apparent connection to *V. flavopilosa* intensity, and that effect appeared to be dependent on host species (Table 7). It appears that duration of the colony defense increased with instantaneous parasite proportion in *V. maculifrons*, and appeared to have decreased in *V. pensylvanica* (Fig. 6). This effect did not appear when using the cumulative measure of *V. flavopilosa* presence, indicating that it was likely short-term fluctuations in *V. flavopilosa* presence leading to this effect (Table 7; Table 8). These findings suggest that effects of social parasite invasion on overall colony behaviour are species-specific, even when responding to external stimuli. An additional area of study for these differences may be whether *V. pensylvanica* is more prone to worker rebellion than *V. maculifrons*, or vise versa.

One apparently free-living colony of *V. flavopilosa* was found during the 2024 field season. As was noted in the results, the colony weakened and appeared to die shortly after its discovery in early September, approximately one month before the deaths of the *V. pensylvanica* and *V. maculifrons* colonies observed in this study. It has been noted in the literature that when they occur, free-living *V. flavopilosa* colonies are typically smaller than the colonies of other temperate yellowjacket species (MacDonald et al. 1980). Though further research into the ecological dynamics shaping these behaviours is needed, it is possible that a higher proportion of *V. flavopilosa* foundresses invaded other colonies rather than trying to form their own nests in suboptimal conditions.

While in some colonies, *V. flavopilosa* presence either steadily rose or remained high, the proportion of *V. flavopilosa* in other colonies plateaued at low or moderate levels (Fig. 4). While there appears to be some level of species difference in parasitism trajectory, the low sample size of *V. maculifrons* colonies makes it difficult to determine whether this difference is the norm (Fig. 4). While we cannot confirm the status of the foundresses in any of these nests, these results suggest that the original foundress of the host nest remained alive in some of the studied colonies. While colony invasion by a social parasite often results in the death of the host foundress, nonviolent invasion has been observed in socially parasitic wasps (Cervo et al. 1990). Mimicry of host olfactory cues by social parasites has been observed in other wasp taxa (Sledge et al. 2001; Lorenzi 2003). Yellowjackets in particular have been observed to have relatively poor nestmate discrimination abilities, and facultative polygyny is relatively common in this taxon (Vetter and Visscher 1997; Loope et al. 2018). Taking these factors into account, invasion by a social parasite into the host nest without killing the host foundress is a possible explanation for the low intensity of *V. flavopilosa* in some nests.

The colony cycle appeared to be the largest determining factor in the threat responses of yellowjacket colonies in this study, with two of the measures of colony aggression (strike duration and total strikes) decreasing over the study period (Tables 7-9; Fig.7; Fig.9). These results show the opposite trend previously observed in paper wasp colonies (*Polistes sp.*) where colony aggression increased with colony senescence (Judd 1998). Possible explanations for this decrease are lower investment in brood as the season declines, or worker die-off. Though a proportion of yellowjacket workers will sometimes overwinter, this phenomenon is rare in temperate environments, and typically only foundresses overwinter with workers dying off in the autumn (Vetter and Visscher 1997). Jandt et al. (2020) measured colony investment in brood and did not find a correlation with aggression. However, this study took place over a shorter timescale in an invasive context. Invasive colonies of yellowjackets in New Zealand, where the aforementioned study took place, are not subject to the intense seasonality that temperate yellowjackets experience in their native range, and do not experience seasonal colony senescence to the same degree (Jandt et al. 2020).

While incomplete, the findings of my behavioural experiments indicate a role of colony senescence in colony behaviour, and show some social parasitism dynamics that bear further study. Further research directions include the study of foundress behaviour and interactions, measurement of factors such as colony size, and further research into how environmental factors influence the prevalence and intensity of social parasitism.

References

Akre, R.D., Reed, H.C. 1981. Population cycles of yellowjackets in the Pacific Northwest. *Env. Entomol.* **10**(3): 267-274.

Borowiec, M.L., Cover, S., Rabeling, C. 2021. The evolution of social parasitism in *Formica* ants revealed by a global phylogeny. *Proc. Natl. Acad. Sci*. **118**(38): e2026029118. DOI: 10.1073/pnas.2026029118

Brito, E.L.S., Aragao, M., Santos, G.M.M. 2018. Colony defensive behavior by the swarm-founding wasp *Parachartergus pseudoapicalis*: increase on investment predicts the intensity of nest defense. *Insectes Soc*. **65:** 411-417. DOI: 10.1007/s00040-018-0627-6.

Carpenter, J.M., Glare, T.R. 2010. Misidentification of *Vespula alascensis* as *V. vulgaris* in North America *(Hymenoptera: Vespidae; Vespinae). American Museum Novitates* **3690:** 1-7. DOI: 10.1206/706.1.

Cervo, R., Lorenzi, M.C., Turilazzi, S. 1990. Nonaggressive usurpation of the nest of *Polistes Biglumis bimaculatus* by the social parasite *sulcopolistes atrimandibularis (Hymenoptera: Vespidae). Insectes Soc.* **37**(4): 333-347.

Cini, A., Branconi, R., Patalano, S., Cervo, R., Sumner, S. 2020. Behavioural and neurogenomic responses of host workers to social parasite invasion in a social insect. *Insectes soc*. **67:** 295-308. DOI: 10.1007/s00040-020-00765-6.

City of Winnipeg. 2021. Winnipeg comprehensive urban forest strategy: state of the urban forest. Retrieved from: https://engage.winnipeg.ca/urbanforest

Czechowski, W., Godzińska, E.J. 2015. Enslaved ants: not as helpless as they were thought to be. *Insectes Soc.* **62**(1): 9-22. DOI: 10.1007/s00040-014-0377-z

Environment and Climate Change Canada. 2020. Canadian climate normals: 1991-2020 data. Retrieved from: https://climate.weather.gc.ca/climate_normals/results_1991_2020_e.html?searchType=stnName_1991&txtStationName_1991=Winnipeg&searchMethod=contains&txtCentralLatMin=0&txtCentralLatSec=0&txtCentralLongMin=0&txtCentralLongSec=0&stnID=242000000&dispBack=1

Galloway, T.D., Preston, W.B. 2012. Distribution and occurrence of yellowjackets *(Hymenoptera: vespidae: Vespula spp)* in Manitoba. *Can. Ent*. **114**(1): 79-80. DOI: 10.4039/Ent11479-1.

iNaturalist community. Occurrences of *Vespula spp.* from Canada, observed between 1990 and 2024. Exported from: GBIF.org (30 January 2026) GBIF Occurrence Download https://doi.org/10.15468/dl.c6k78t

Jandt, J.M., Detoni, M., Loope, K.J., Santoro, D. 2020. *Vespula* wasps show differences in colony-level aggression over time and across contexts. *Insectes Soc.* **67:** 367-381. DOI: 10.1007/s00040-020-00768-3.

Jernigan, C.M., Birgiolas, J., McHugh, C., Roubik, D.W., Wcislo, W.T., Smith, B.H. 2018. Colony-level non-associative plasticity of alarm responses in the stingless honey bee, *Tetragonisca angustula. Behav. Ecol. Sociobiol.* **72**(3): 58. DOI: 10.1007/s00265-018-2471-0.

Judd, T.M. 1998. Defensive behavior of colonies of the paper wasp, *Polistes fuscatus,* against vertebrate predators over the colony cycle. *Insectes Soc.* **45:** 197-208. DOI: 10.1007/s000400050080.

Kimsey, L.S., Carpenter, J.M. 2012. Vespinae of North America *(Vespidae, Hymenoptera). J. Hymenopt. Res.* **28:** 37-65. DOI: 10.3897/JHR.28.3514

Lester, P.J., Haywood, J., Archer, M.E., Shortall, C.R., Sanders, N. 2017. The long-term population dynamics of common wasps in their native and invaded range*. J. Anim. Ecol.* **86**(2): 337-347. DOI: 10.1111/1365-2656.12622.

Loope, K.J., Millar, J.G., Wilson-Rankin, E.E. 2018. Weak nestmate discrimination behavior in native and invasive populations of a yellowjacket wasp *(Vespula pensylvanica). Biol. Invasions* **20**(12): 3431-3444. DOI: 10.1007/s10530-018-1783-3.

Loope, K.J. and Wilson-Rankin, E.E. 2021. Viral load, not food availability or temperature, predicts colony longevity in an invasive eusocial wasp with plastic life history. *Sci. Rep.* **11**(1): 10087. DOI: 10.1038/s41598-021-89607-4.

Lopez-Osorio, F., Pickett, K.M., Carpenter, J.M., Ballif, B.A., Agnarsson, I. 2014. Phylogenetic relationships of yellowjackets inferred from nine loci (*Hymenoptera: Vespidae, Vespinae, Vespula* and *Dolichovespula*). *Mol. Phylogenet. Evol.* **73:** 190-201. DOI: 10.1016/j.ympev.2014.01.007.

Lopez-Osorio, F., Perrard, A., Pickett, K., Carpenter, J.M., Agnarsson, I. 2015. Phylogenetic tests reject Emery's rule in the evolution of social parasitism in yellowjackets and hornets *(Hymenoptera: Vespidae, Vespinae). R. Soc. Open Sci.* **2**(9): 150159. DOI: 10.1098/rsos.150159.

Lorenzi, M.C. 2003. Social wasp parasites affect the nestmate recognition abilities of their hosts *Polistes atrimandibularis* and *P. biglumis, (Hymenoptera, Vespidae). Insectes Soc.* **50**(1): 82-87. DOI: 10.1007/s000400300013.

MacDonald, J.F., Matthews, R.W., Jacobson, R.S. 1980. Nesting Biology of the Yellowjacket, *Vespula flavopilosa (Hymenoptera: Vespidae). J. Kans. Entomol. Soc.* **53**(2): 448-458.

MacLean, J.L., Van Akker, L., Van Hezewijk, B.H. 2021. The structure of temperate yellowjacket communities is affected by land development and loss of forest cover. *Agri and Forest Entomology* **23**(3): 269-275. DOI: 10.1111/afe.12429.

Natural Resources Canada. 2012. Geological survey of Canada: geoscientific insights into the Red River and its flood problem in Manitoba- geomorphology of the Red River. Retrieved from: https://web.archive.org/web/20110604201210/http://gsc.nrcan.gc.ca/floods/redriver/geomorphology_e.php

Park People. 2023. Canadian city parks report 2023- City Profile: Winnipeg. Retrieved from: https://ccpr.parkpeople.ca/2023/cities/winnipeg-2022

Saga, T., Kanai, M., Shimada, M., Okada, Y. 2017. Mutual intra- and interspecific social parasitism between parapatric sister species of *Vespula* wasps. *Insectes Soc*. **64**(1): 95-101. DOI: 10.1007/s00040-016-0519-6.

Sledge, M.F., Dani, F.R., Cervo, R., Dapporto, L., Turilazzi, S. 2001. Recognition of social parasites as nest-mates: adoption of colony-specific host cuticular odours by the paper wasp parasite *Polistes sulcifer. Proc. R. Soc. B*. **268**(1462): 2253-2260. DOI: 10.1098/rspb.2001.1799.

Statistics Canada. 2022. Focus on geography series, 2021 census of population: Winnipeg, census metropolitan area. Retrieved from: https://www12.statcan.gc.ca/census-recensement/2021/as-sa/fogs-spg/Page.cfm?lang=E&topic=1&dguid=2021S0503602

Stein, K.J., Fell, R.D. 1994. Correlation of queen sperm content with colony size in yellowjackets *(Hymenoptera: Vespidae)*. *Popul. Ecol.* **23(6):** 1497-1500.

Sumner, S., Law, G., Cini, A. 2018. Why we love bees and hate wasps. *Ecol. Entomol.* **43:** 836-845. DOI: 10.1111/een.12676.

Ross, K.G., Matthews, R.W., Morse, R.A. 1981. Laboratory culture of four species of yellowjackets*, Vespida spp*., foundress nest initiation. *Ann. Entomol. Soc. Am.* **74:** 247-254.

Ross, K.G. 1983. Studies of the Foraging and Feeding Behavior of Yellowjacket Foundresses, *Vespula (Paravespula) (Hymenoptera: Vespidae),* in the Laboratory. *Ann. Entomol. Soc. Am.* **76**(5): 903-912. DOI: 10.1093/aesa/76.5.903.

Vetter, R.S., Visscher, P.K. 1997. Plasticity of annual cycle in *Vespula pensylvanica* shown by a third year polygynous nest and overwintering of queens inside nests. *Insectes soc*. **44:** 353-364.

Wilson-Rankin, E.E. 2014. Social context influences cue-mediated recruitment in an invasive social wasp. *Behav. Ecol. Sociobiol.* **68**(7): 1151-1161. DOI: 10.1007/s00265-014-1726-7.

Chapter 3: Relationships between environment, behaviour, and serotonin in the yellowjacket brain

Introduction

As in vertebrates, serotonin is a key molecule for regulation of a variety of physiological and behavioural processes in arthropods, including regulation of aggressive behaviour (Blenau and Thamm 2011). However, unlike in vertebrates, increased serotonin in the arthropod brain is often associated with increased aggressive behaviour (Blenau and Thamm 2011). Dierick and Greenspan (2007) found that both direct injection of serotonin into the brain and upregulation of serotonin production via genetic manipulation in *Drosophila melanogaster* resulted in flies with increased tendency to start and escalate aggressive interactions. Aleksyenko et al. (2010) also found that direct activation of serotonergic circuits in *D. melanogaster* increased aggressive interactions, while disrupting serotonergic circuits resulted in flies who would initiate aggressive interactions, but failed to escalate them in the typical way. Despite this evidence, the roles of serotonin in the arthropod nervous system are likely nuanced, as activation of some receptor subtypes appears to attenuate aggression rather than increase it (Bubak et al. 2020).

Serotonin is also associated with regulation of complex social behaviour and phenotypic change in adult insects, such as the transition from the solitary phase to the gregarious or swarming phase of the desert locust *Schistocerca gregaria* (Rogers et al. 2004). The role of serotonin in behavioural and phenotypic change can also be seen in hymenopterans, with differences in serotonergic branching patterns being noted between the 'major' and 'minor' worker castes in the ant *Pheidole dentata* (Giraldo et al. 2013).

In this study, I investigated whether changes in the amount and distribution of serotonin can be associated with social parasitism in three species of yellowjacket: *Vespula pensylvanica, Vespula maculifrons,* and the facultative social parasite *Vespula flavopilosa.* There exists some evidence that social parasitism in hymenopterans changes behaviour and brain gene expression of parasitized workers, but the neurological effects of social parasitism on both host and parasite has not been well studied thus far (Cini et al. 2020). In addition, I tested the correlation between the amount and distribution of serotonin in the brain to aggressive behaviour at the colony level. I investigated three regions of the brain for these purposes: the optic lobe, the lateral protocerebrum, and the mushroom body (MB) calyces.

The optic lobe has serotonergic neurons arranged in distinct band-like patterns that appear to be highly conserved across insect taxa (Van der Woude and Smid 2017; Aonuma and Benelli 2023). Its primary role is in visual processing and attention, and changes with dominance interactions or aggression have not been observed in neurological studies (Molina and O'Donnell 2008; Paulk et al. 2014; Uy et al. 2021). However, anatomical studies comparing the brains of socially parasitic wasps to their free-living relatives have observed significantly larger optic lobes in socially parasitic species (Rozanski et al. 2022). In my images of the optic lobe, I expected to observe species differences between *V. flavopilosa* and the two free-living species, as well as a relationship to colony-level aggression.

The lateral protocerebrum is a large and multifaceted region in the pancrustacean brain with dense groupings of serotonergic neurons (Namiki and Kanzaki 2019). The lateral protocerebrum has demonstrated roles in processing and responding to olfactory cues and acoustic signaling (Namiki et al. 2014; Pfulh et al. 2014). While not well studied in hymenopterans specifically, the lateral protocerebrum appears to have structure and function that, with some exceptions, are highly conserved across arthropod taxa (Sullivan and Beltz 2001). Due to the demonstrated presence of alarm pheromone in the yellowjackets, I expected an increase in serotonin in this region to correlate with colony level aggression, but not with species, as the response behaviour to alarm pheromone is highly conserved across taxa (Landolt et al. 1995; Reed and Landolt 2000).

The calyces (input neuropils) of the MBs are an extremely well-studied area of the arthropod brain with multiple characterized behavioural roles, including in aggressive interactions (Blenau and Thamm 2011). In particular, MB calycal volume has been demonstrated to increase with social dominance in multiple hymenopteran species, including bees and social wasps (Molina and O'Donnell 2008; Pahlke et al. 2019). Neuropils in this region have been known to form microglomeruli, which increase in volume and decrease in density as synaptic organization increases (Groh and Rössler 2019). In this region, I expected to see species differences, with *V. flavopilosa* having larger serotonergic particles than *V. pensylvanica* or *V. maculifrons,* due to socially parasitic insects often being specialized for more dominant behaviour (Rozanski et al. 2022). In addition, I expected to observe a correlation between particle size and colony-level aggression.

Methods

Dissections and histology

Specimens for this study were collected from colonies located during the 2024 field season (See chapter 2). After collection, anaesthetization, and identification, specimens were decapitated, and their head capsules were opened using microdissection tools under a dissecting microscope. Opened head capsules were fixed overnight in a 4% formaldehyde solution at 4℃. After fixing, brains were removed from the head capsules and stored in phosphate-buffered saline (PBS) at 4ºC until the end of the field season. Following the field season, brains were washed 3x in PBS and embedded in 7% agarose gel for sectioning. Brains were then sectioned along the coronal plane with a Compresstome vibrating microtome (Precisionary instruments VF-210-0Z) at a thickness of 100µm. Two sections from the middle part of the brain were selected for each specimen. Brains embedded in agarose typically yielded 8-12 sections, unless tilting occurred during the embedding process. Slices were washed 3x in PBS with added 5% Triton X detergent (PBST) and blocked with 5% bovine serum albumin for 1 hour at room temperature. The bovine serum was then removed, and a primary polyclonal rabbit anti-serotonin antibody (Sigma-Aldrich S5545) was added at a concentration of 1:267. Slices with the primary antibody were incubated at 4˚C for 48 hours. All washes for this step were done on a Corning LSE platform rocker (C6703).

Following incubation, slices were washed 6x in PBST, then labeled with a secondary goat anti-rabbit Alexa Fluor 488 antibody (Invitrogen A11034) and Alexa Fluor 555 Phalloidin (Invitrogen A34055), both at a concentration of 1:667 and once again incubated at 4˚C for 48 hours. After labeling with the secondary antibody, slices were washed a final 6x in plain PBS (plates wrapped in foil to prevent photobleaching of the secondary antibody), and mounted under a dissecting microscope using Vectashield mounting medium with DAPI (Vector Laboratories VECTH1500), on slides with #1.5 coverslips, and sealed with clear nail polish. Slides were blinded to prevent bias, with slide number and date mounted as the only information provided on each slide (brain IDs, species, and date collected for each section were stored in a separate notebook and not consulted until all slides had been imaged and analyzed). Mounted slides were then imaged with a Zeiss LSM 700 confocal laser scanning microscope (Zeiss Microscopy) using a 10x 0.45 NA objective and a Plan-Apochromat 40x 1.4 NA oil-immersion objective. As with the previous step, all washes in preparation for secondary labeling were done on a Corning LSE platform rocker (C6703).

Imaging and image analysis

An initial image was taken of each brain slice at 10x magnification for orientation purposes (Fig. 10). Three laser channels were used: 405 nm (5 mW) for DAPI, 488 nm (10 mW) for anti-serotonin, and 555 nm (10 mW) for phalloidin. Within the 10x image, the MB calyces can be identified as a cup-shaped structure at the dorsal end of the brain (top of image, Fig. 10; Fig.11). The optic lobes are rounded structures on each lateral end of the brain (leftmost end of image, Fig.10; Fig.12). The lateral protocerebrum is a large region near the midsection of the brain below the MB calyces, which displayed high levels of fluorescence (labelled in green) when labeled with anti-serotonin (center of image, Fig. 10; Fig.13).

Following the 10x imaging, z-stacked images at 40x magnification were taken of the lobula (the innermost neuropil of the optic lobe), lateral protocerebrum, and MB calyces if they appeared in the slice (Figs.11-13). Each stacked image contained 30 slices, with a Z-step distance of 0.4µm. From each Z-stacked image, a rectangular region of interest (ROI) was selected for further processing and particle analysis. ROI size and placement were kept as consistent as possible within each region, with ROIs of 50µm x 100µm for the optic lobe and lateral protocerebrum, and 70µm x 70µm for the MB calycal lips (Fig.10). Intensity of particles (minimum, maximum, and average) for each ROI was measured using ImageJ's ROI manager and its intensity function (Schindelin et al. 2012). ROIs were then processed using the Deconvolutionlab2 plugin for ImageJ (Schindelin et al. 2012; Sage et al. 2017).

Deconvoluted images were prepared for particle analysis by having their background subtracted, leaving only the particles of interest (Fig.14). Background subtraction was done using a Renyi entropy based thresholding method, as demonstrated by Jirakitipuwapat et al. (2020). Entropy-based thresholding methods are commonly used in a variety of image analysis protocols to separate particles of interest from the image background (Sahoo et al. 1997). This thresholding method uses equations designed for calculating entropy to create a bimodal probability distribution of grayscale values within the image (Sahoo et al. 1997). The valley of this bimodal distribution represents the threshold, wherein all values below this point (ie. the half of the probability distribution with the lower grayscale values) are eliminated as background (Sahoo et al. 1997). The threshold must be adjusted manually for a given image so that the calculation of grayscale values is appropriate (Jirakitpuwapat et al. 2020). Renyi entropy is a method of calculating entropy that combines several other entropy equations, including Shannon entropy (Jirakitpuwapat et al. 2020). The method of Renyi entropy based thresholding for images was demonstrated by Jirakitpuwapat et al. (2020), with a threshold placement of 1% for each image (Fig.14).

Once the threshold had been set, the grayscale distribution described above was used to subtract the background, leaving only the serotonin particles left in the image (Fig.14, panel 3).

After background subtraction, the particle analysis function in ImageJ was used to calculate particles per stack, percent area coverage of each slice, and average particle size for each slice (Schindelin et al. 2012). Raw data for each ROI was saved to a CSV file.

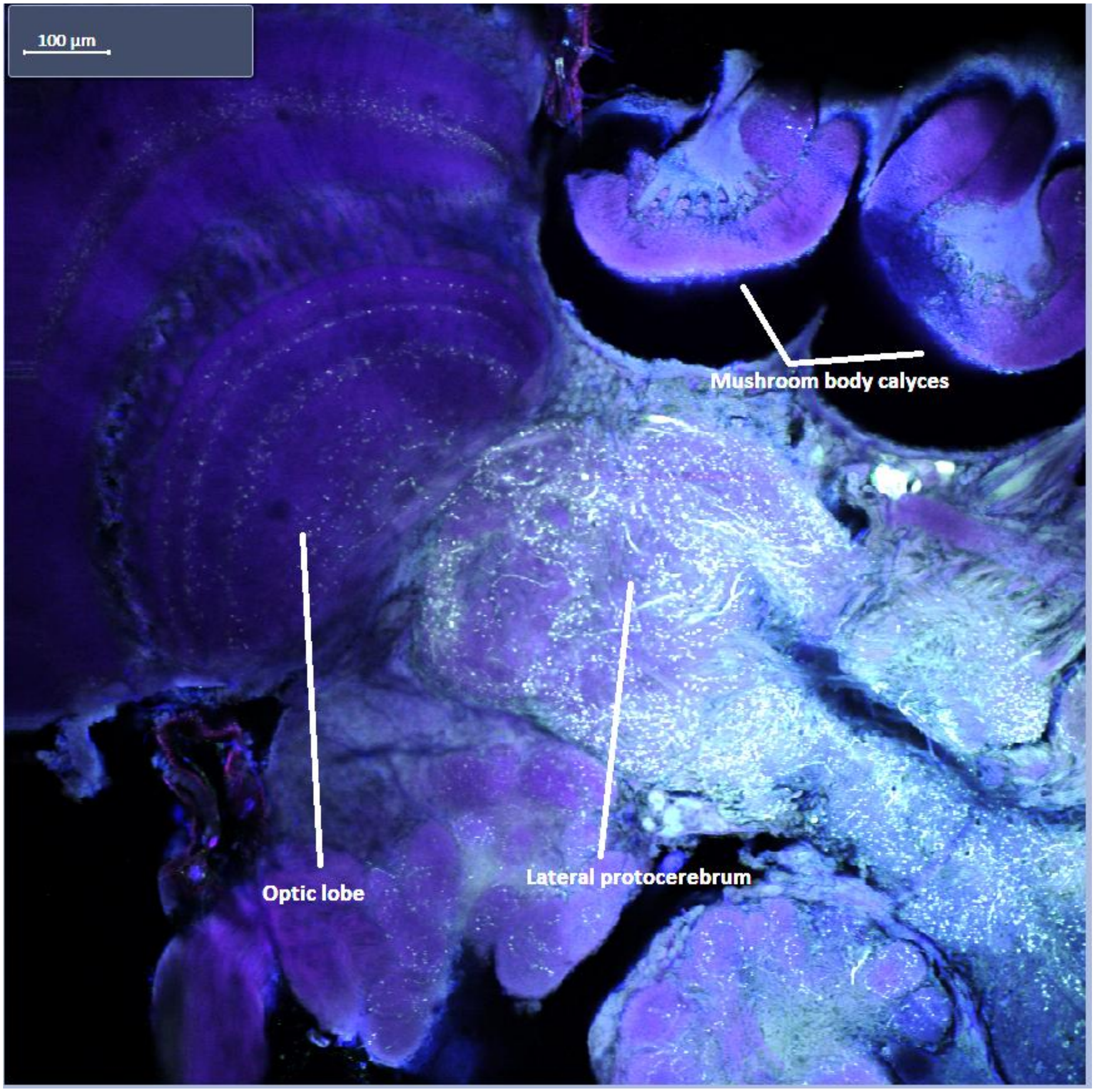

Figure 10: Orientation image at 10x magnification with all three regions present and all three labels merged. DAPI (indicating cell bodies) is labelled blue, serotonin is labelled green, and phalloidin labeled in magenta.

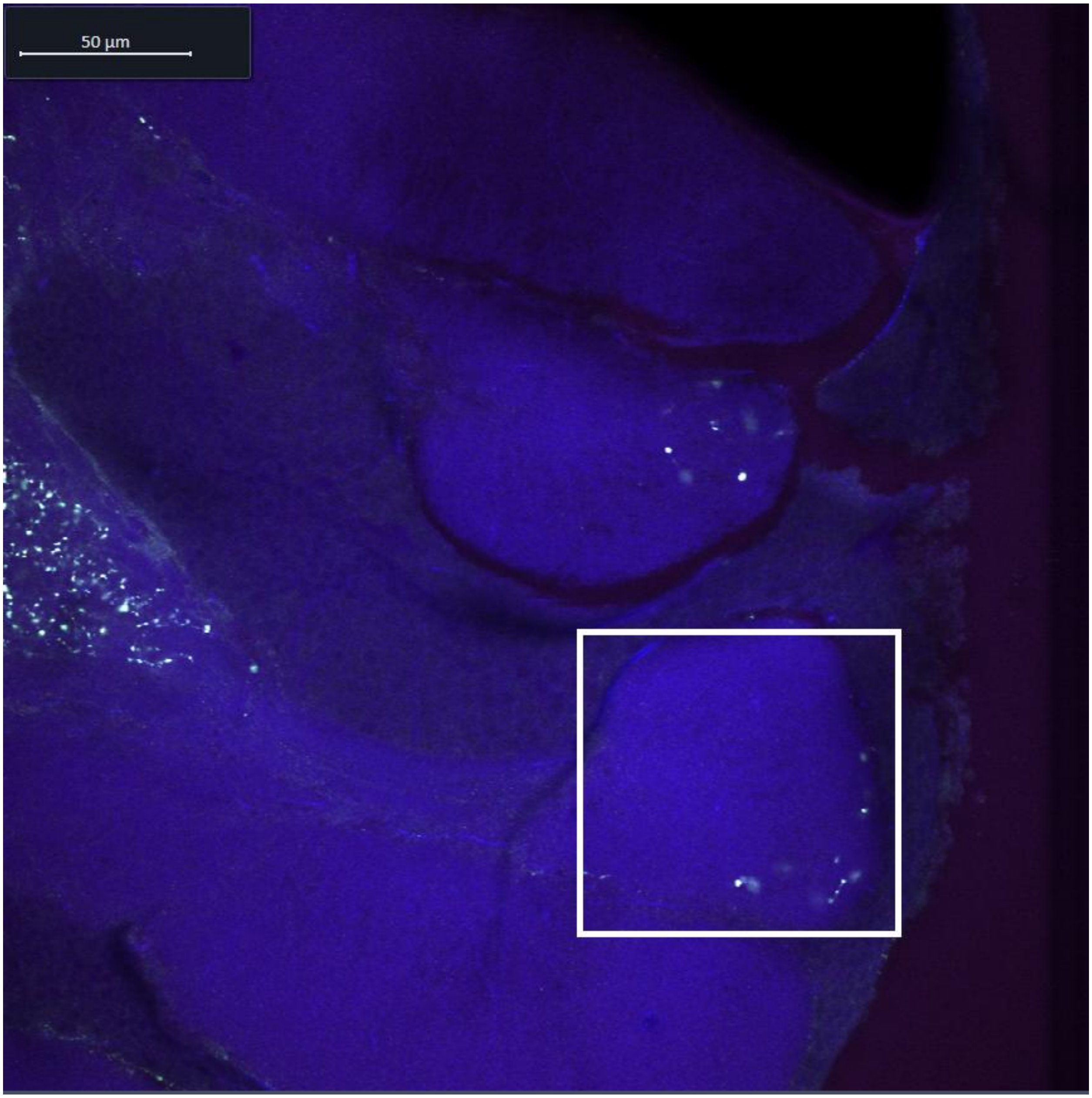

Figure 11: 40x z-stacked image of a mushroom body calyx with puncta in the calycal lips. One of the two calycal lips has been outlined in white for ease of identification. All labels shown merged (see Fig. 10).

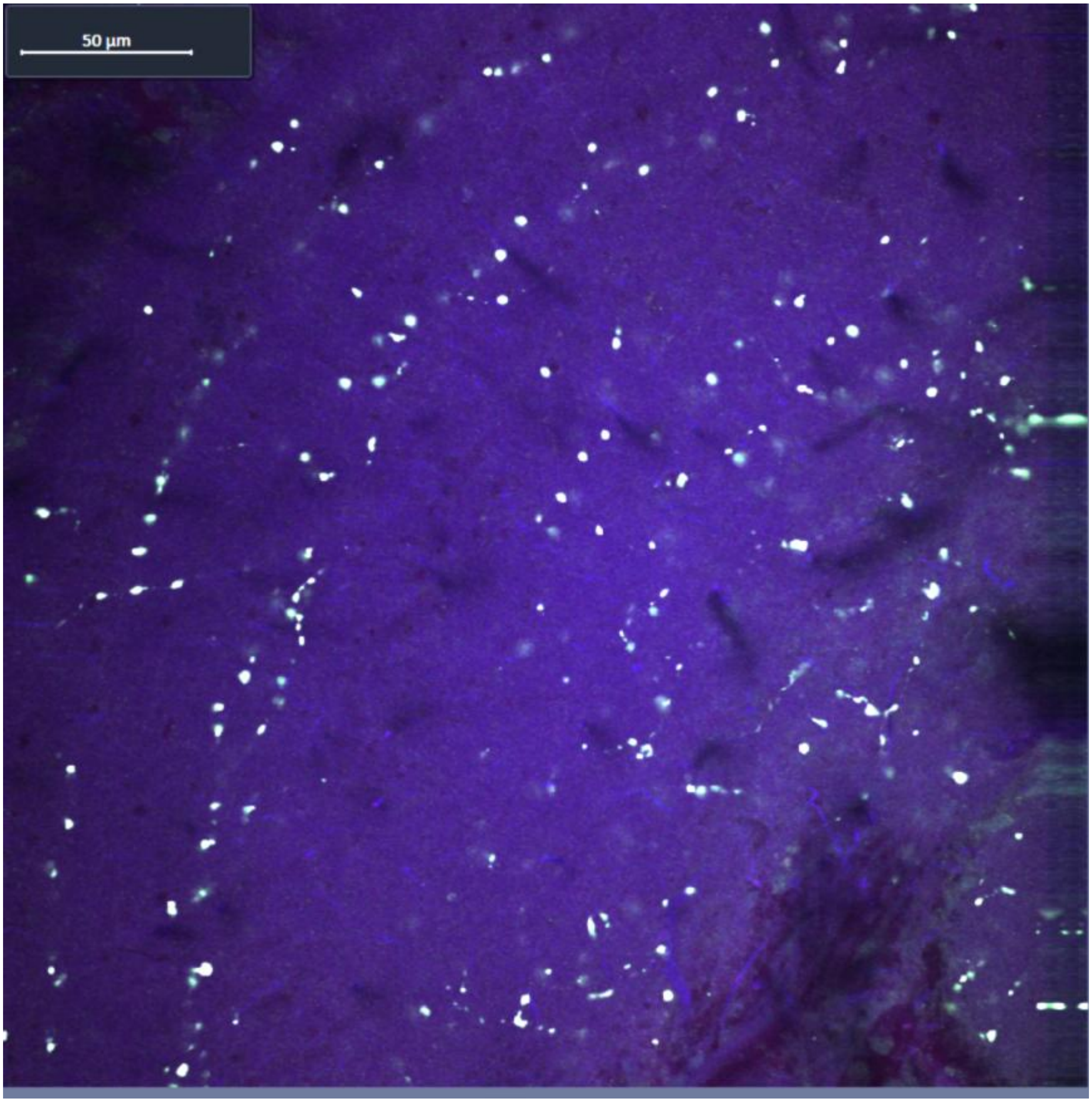

Figure 12: 40x z-stacked image of the inner portion of an optic lobe. All three labels shown (see Fig. 10).

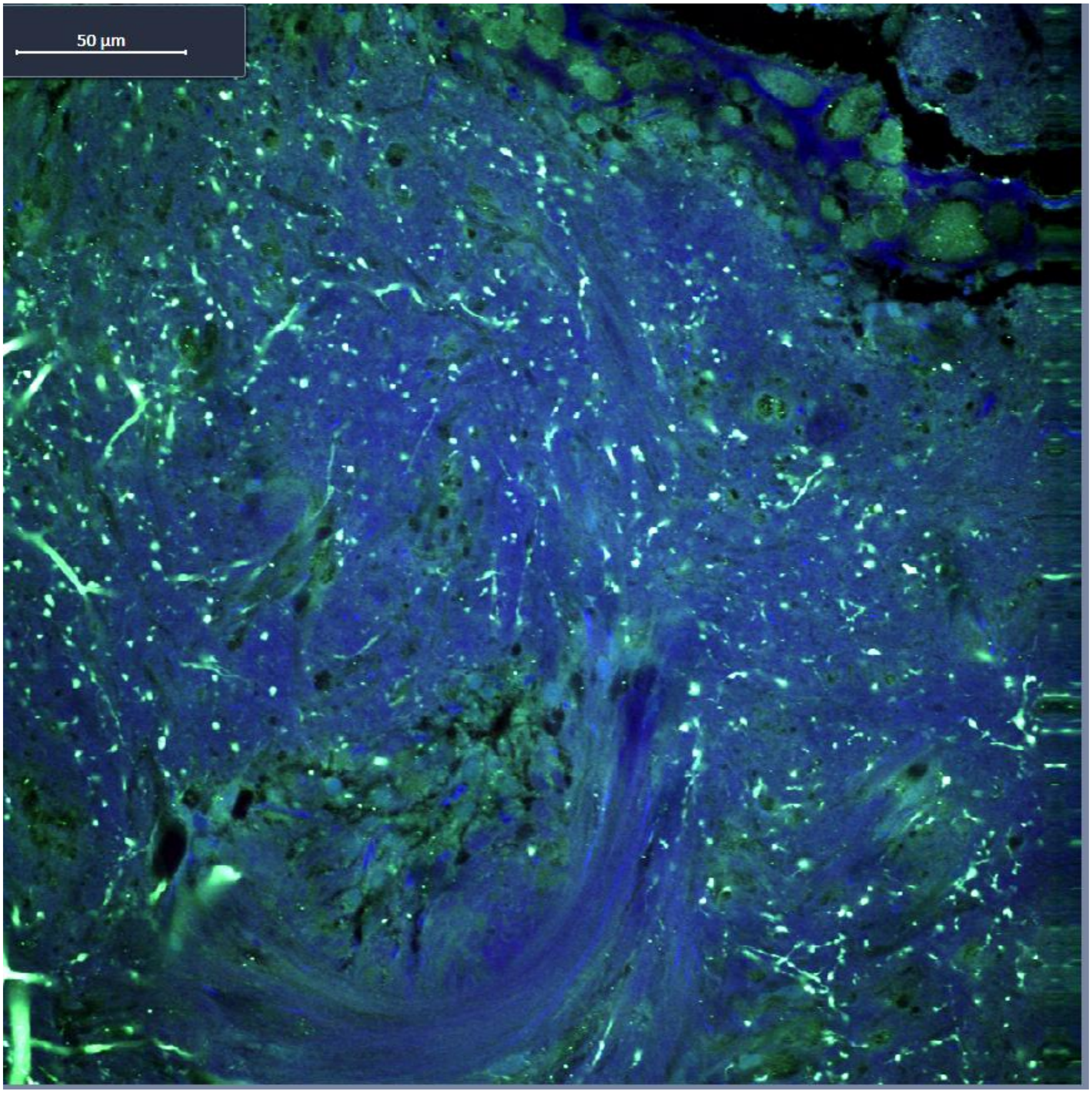

Figure 13: 40x z-stacked image of the lateral protocerebrum. All three labels shown (see Fig. 10).

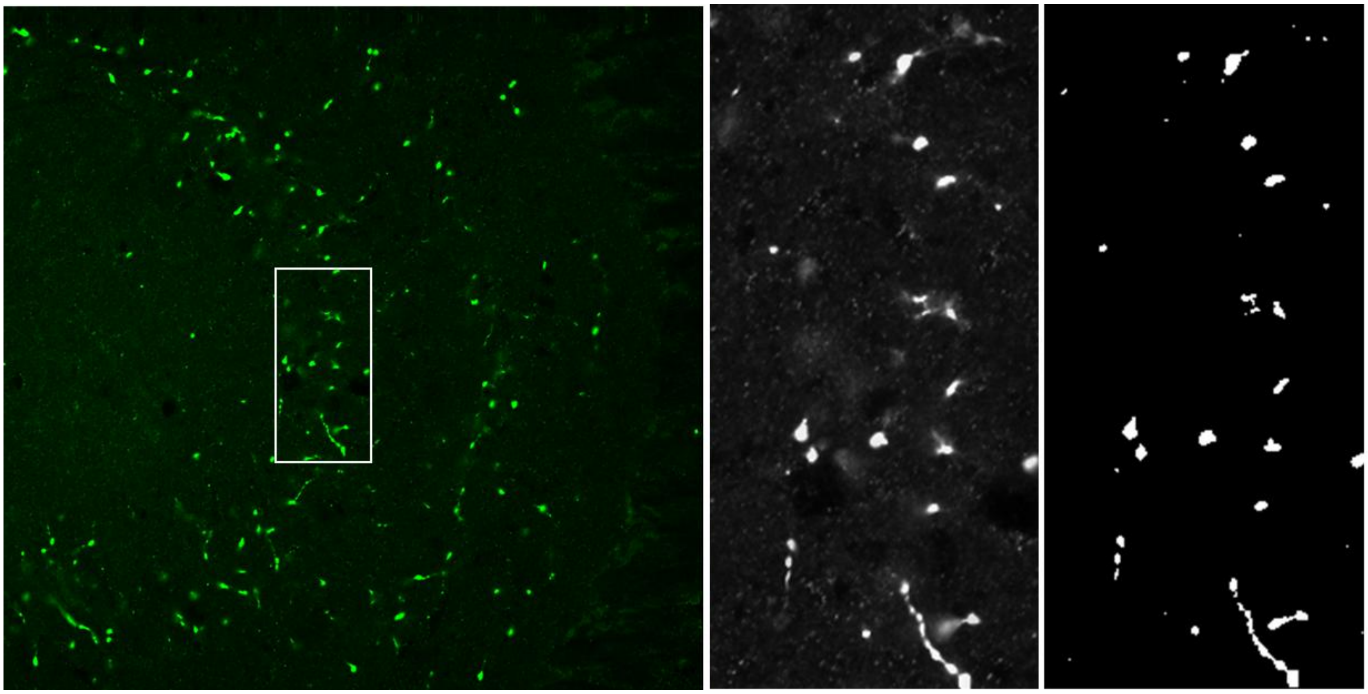

Figure 14: From left to right: stacked image of the optic lobe at 40x with ROI indicated (serotonin channel only); Deconvoluted ROI from stacked image; ROI after background removal in preparation for particle analysis.

Data processing and statistical analysis

Data from image analysis for particles per stack, particle size, and percent area coverage were averaged for each 30-part image stack for ease of handling, and entered into a CSV file for each separate brain region. Included in each entry were also values for colony latency, duration, and total strikes (see chapter 2). Behaviour values for each entry were recorded as the values from the individual's nest of origin for the trial on the day the individual was collected. Brain ID, species, and colony ID were also recorded. After image data was unblinded, duplicate entries for the same brain ID were removed in order to mitigate challenges with repeated measures. Images that the data were taken from were assessed for clarity, absence of background noise, and presence of puncta in all levels of the stack. The clearest image for each region of each brain was selected, and information from the selected image was used for analysis.

Statistical analysis of imaging data was conducted using generalized linear mixed models in R (version 4.5.2) using the car (version 3.1.3), lme4 (version 1.1.38), and lmerTest (version 3.1.3) packages. R-squared for mixed models was calculated using the MuMIn package (version 1.48.11). Nest ID was added to the models as a random effect to control for repeated measures. All anatomical variables were log transformed to compensate for non-normal distributions and heteroscedasticity. Models were constructed and tested for relationships of anatomical variables to species and colony parasite (*V. flavopilosa*) proportion, as well as to behavioural variables measured from testable colonies during the field season (see chapter 2). Latency, used as a measure of colony responsiveness, is defined as the time in seconds between a disruptive stimulus to the colony, and the first strike to a target placed outside the colony entrance. Similarly, duration of strikes to the target and total strikes to the target were also measured (see chapter 2).

Results

General description and challenges to histology and imaging

Contamination of the DI water used to make phosphate-buffered saline for storage of collected brains led to a mold problem among the storage plate, and subsequently to significant specimen loss. Of the data collected, the majority is from late-season specimens, and some sampling events were not represented in imaging data. Overall, a total of 46 images of the optic lobe, 44 images of the mushroom body calyces, and 38 images of the lateral protocerebrum were collected and suitable for analysis.

The lateral protocerebrum had the highest mean for all three tested variables, ie. average particles per z-stack, serotonin particle size (converted from pixels into μm^2), and percent area coverage by particles (table 12). The mushroom body calyces had the lowest averages for all three variables measured. Full summary statistics for all three regions can be found in table 12.

Table 12: Summary statistics for brain image analysis by region. All summary statistics presented with both mean and standard error.

Region	**Average particles**	**Particle size (μm^2)**	**Percent area coverage**
Optic lobe	51.8 ± 5.59	1.52 ± 0.877	1.15 ± 0.08
Lateral protocerebrum	78.2 ± 8.63	1.62 ± 1.53	2.59 ± 0.36
Mushroom body calyces	45.6 ± 7.26	1.20 ± 1.23	0.71 ± 0.07

Optic Lobe

The optic lobe was the only brain region examined in which species differences were found; model results indicated that *V. pensylvanica* (one of the host species) differed significantly from the other two species in terms of particle size (p = 0.0345; Table 13). Particle size in *V. pensylvanica* was lower overall, though all three species displayed considerable variation (Fig. 15). Full model results for species differences can be found in table 13. No significant results were observed for *V. flavopilosa* (the facultative parasite) proportion in any anatomical measurement of the optic lobe in either host species.

Table 13: Model results for species differences in the optic lobe. Model formula: Log[anatomy variable] ~ Species + (1|Nest)

Variable	**Cumulative R^2**	**p-value (*V. pensylvanica)***	**p-value (*V. maculifrons)***
Average particles	0.165	0.981	0.695
Particle size	0.165	0.035*	0.945
Percent area coverage	0.117	0.201	0.312

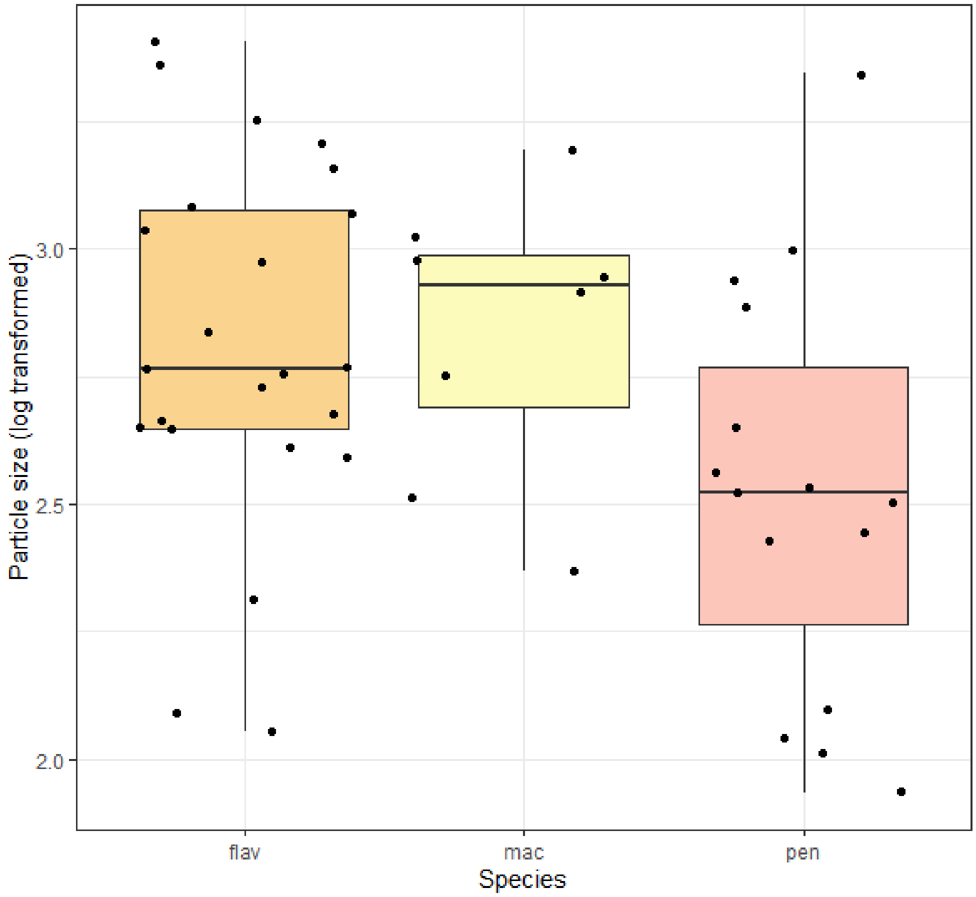

Figure 15: Species differences in optic lobe particle size (p = 0.031). 'flav' denotes V. flavopilosa, 'mac' V. maculifrons, and 'pen' V. pensylvanica.

The activity of serotonin in the optic lobe was also observed to have significant relationships to latency in terms of both average particles (p = 0.010) and percent area coverage of the ROI (p = 0.045). In both cases, the amount of serotonin increased with increasing latency, suggesting that less responsive colonies had lower serotonin in the optic lobe (Fig. 16). The relationship between latency and particle size also approached significance, but evidence of a negative relationship between particle size and latency is inconclusive (p = 0.069; Fig.17).

Full model results for latency and its relationships to the optic lobe can be found in Table 14.

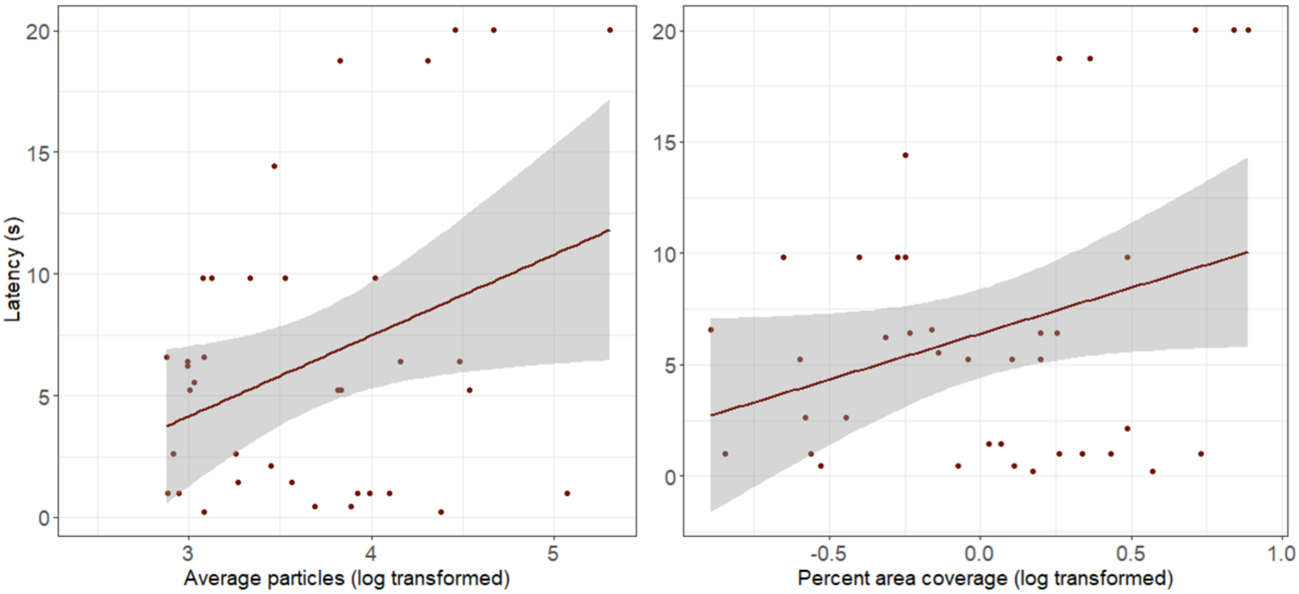

Figure 16: Relationships between latency and optic lobe serotonin. Left: latency plotted against average particles per z-step (p = 0.010). Right: latency against percent area coverage of selected ROI (p= 0.045). All anatomy variables log transformed.

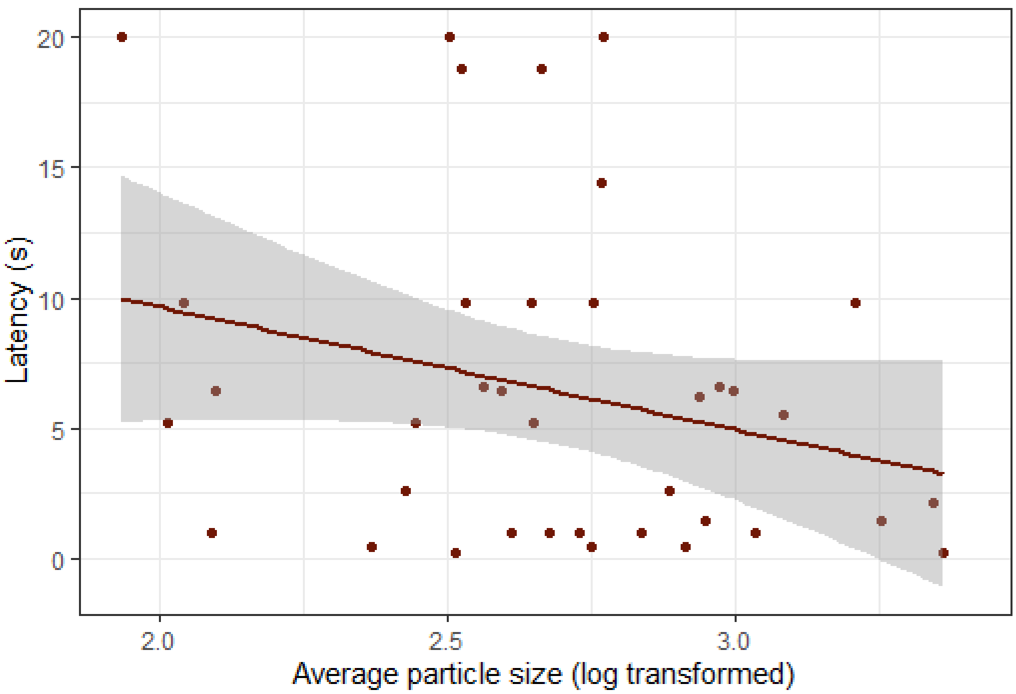

Figure 17: Relationship between latency and average particle size in the optic lobe (p = 0.069). Particle size has been log transformed.

Table 14: Model results for latency in the optic lobe. All anatomical variables log transformed. Model formula: Latency ~ log[anatomy variable] + (1|Nest)

Variable	**Cumulative R^2**	**Degrees of freedom**	**p-value**
Average particles	0.426	35.45	0.0104*
Particle size	0.296	36.32	0.0685$^{\cdot}$
Percent area coverage	0.391	34.33	0.0447*

Models comparing defense duration to serotonin in the optic lobe did not yield any significant results. However, the relationship between percent area coverage of optic lobe ROIs and defense duration approached significance (p = 0.0525), indicating possible but inconclusive evidence of a relationship. Full model results for duration in the optic lobe can be found in table 15.

.

Table 15: Model results comparing duration to serotonin in the optic lobe. All anatomical variables were log transformed. Model formula: Duration ~ log[anatomy variable] + (1|Nest)

Variable	**Cumulative R^2**	**Degrees of freedom**	**p-value**
Average particles	0.192	36.92	0.160
Particle size	0.142	36.93	0.604
Percent area coverage	0.200	36.38	0.0525.

Models comparing the total number of strikes to the target to serotonin in the optic lobe yielded significant results with respect to average particles and percent area coverage ($p = 0.021$; $p = 0.006$). Total strikes decreased with increasing particles and coverage in the optic lobe (Fig. 18). Full model results for total strikes and optic lobe serotonin can be found in Table 16. Two data points in the total strikes dataset were investigated as potential outliers, but sufficient evidence did not exist to warrant removal (Fig.18).

Table 16: Model results for total strikes with respect to serotonin in the optic lobe. All anatomical variables have been log transformed. Model formula: Total strikes ~ log[anatomy variable] + (1|Nest)

Variable	**Cumulative R^2**	**Degrees of freedom**	**p-value**
Average particles	0.207	36.86	0.021*
Particle size	$1.26e^{-6}$	37.00	0.99
Percent area coverage	0.245	36.86	0.006**

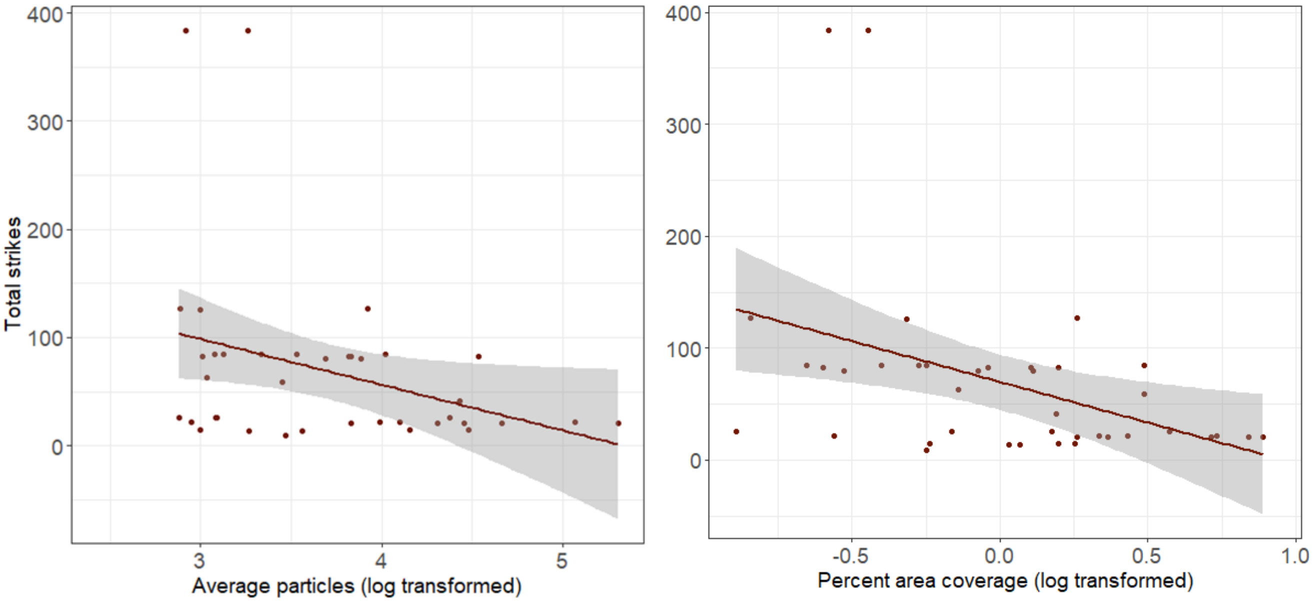

Figure 18: Left: relationship between total strikes and average serotonin particles in the optic lobe (p = 0.021). Right: relationship between total strikes and percent area coverage in the optic lobe (p = 0.006). All anatomical variables have been log transformed

Lateral protocerebrum

Models comparing serotonin in the lateral protocerebrum yielded no significant results for species or *V. flavopilosa* (parasite) proportion. Models for latency did not yield significant results. However, models constructed for the relationship between latency and particle size, and latency and percent area coverage both had p-values close to the threshold (p = 0.057 and p= 0.079, respectively). The relationship between latency and both variables appeared negative, with latency decreasing as particle size and percent area coverage increased (Fig. 19). Full model results for latency can be found in Table 17.

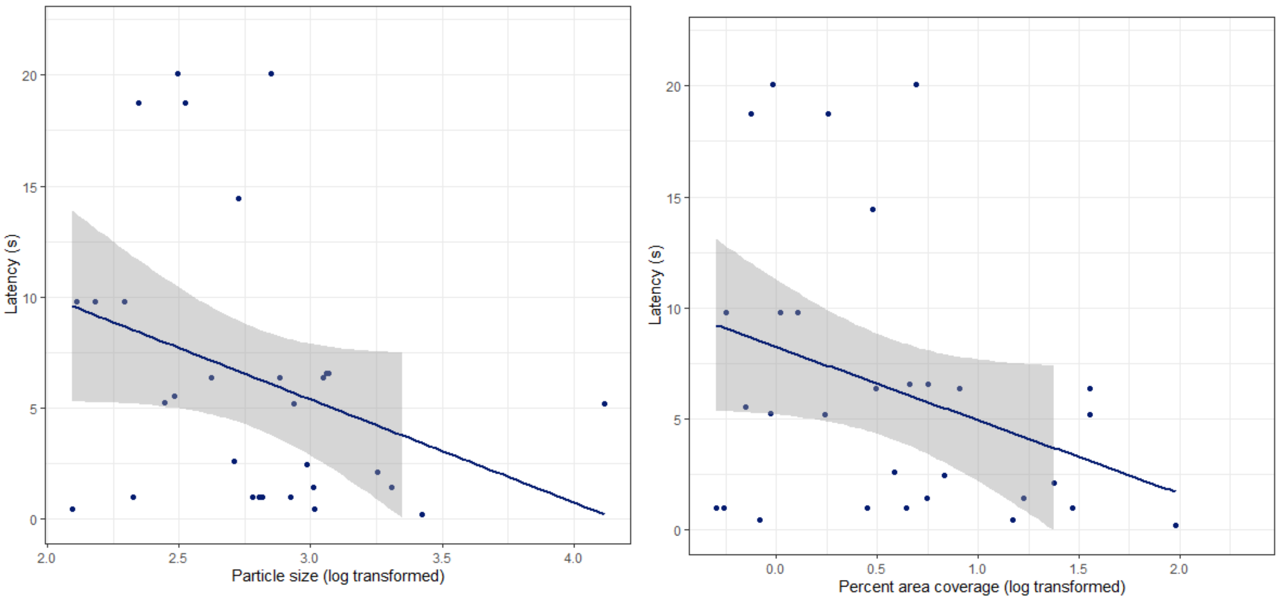

Figure 19: Serotonin in the lateral protocerebrum in relation to latency. Left: relationship between latency and particle size in the lateral protocerebrum (p = 0.057). Right: relationship between latency and percent area coverage (p = 0.079). All anatomical variables have been long transformed.

Table 17: Model results for latency and serotonin in the lateral protocerebrum. All anatomical variables have been log transformed. Model formula: Latency ~ log[anatomy variable] + (1|Nest)

Variable	**Cumulative R^2**	**Degrees of freedom**	**p-value**
Average particles	0.373	24.73	0.436
Particle size	0.450	24.41	0.057·
Percent area coverage	0.0421	29.62	0.0788·

Models comparing defense duration and serotonin in the lateral protocerebrum yielded significant results with percent area coverage ($p = 0.025$). The relationship between duration and area coverage was negative, with defense duration decreasing and percent area coverage increased (Fig. 20). Full model results for duration can be found in Table 18. No significant relationships were observed in the models comparing total strikes and serotonin in the optic lobe.

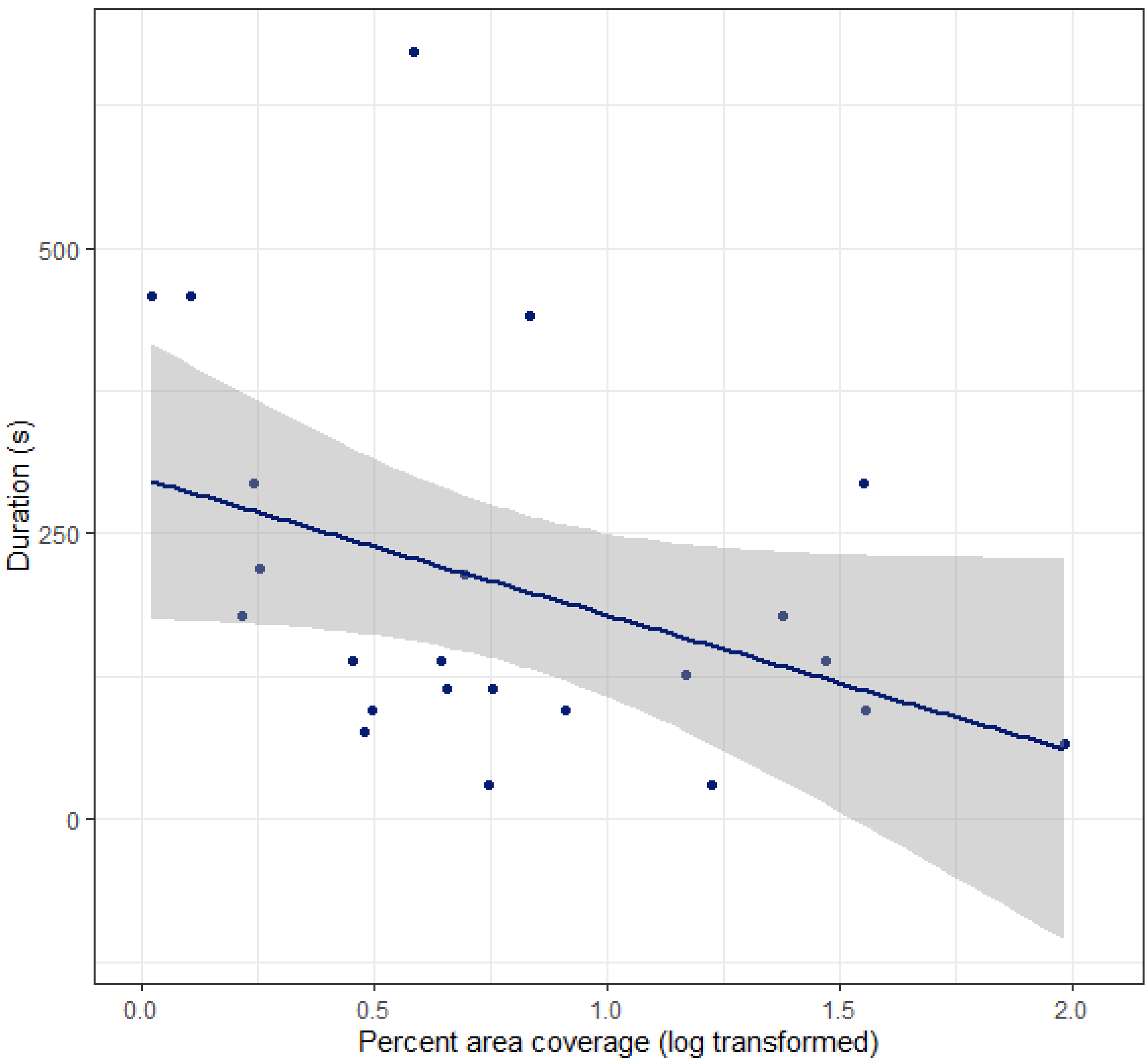

Figure 20: Relationship between serotonin percent area coverage and defense duration in the lateral protocerebrum (p = 0.025). Percent area coverage has been log transformed.

Table 18: Model results for duration and serotonin in the lateral protocerebrum. All anatomy variables have been log transformed. Model formula: Duration ~ log[anatomy variable] + (1|Nest)

Variable	**Cumulative R^2**	**Degrees of freedom**	**p-value**
Average particles	0.0749	29	0.13
Particle size	0.0775	29	0.123
Percent area coverage	0.156	29	0.0254*

Mushroom body calyces

No significance was observed for species differences with respect to serotonin in the mushroom body calyces. Models comparing *V. flavopilosa* (parasite) proportion and serotonin in the MB calyces yielded significant results with percent area coverage (p = 0.029), with serotonin area coverage increasing as parasite proportion increased (Fig. 21). Full model results for parasite proportion can be found in Table 19.

Table 19: Model results for social parasite intensity and serotonin in the mushroom body calyces. All anatomy variables have been log transformed. Model formula: Log[anatomy variable] ~ V. flavopilosa proportion + (1|Nest)

Variable	**Cumulative R^2**	**Degrees of freedom**	**p-value**
Average particles	0.486	42	0.146
Particle size	0.00148	42	0.802
Percent area coverage	0.106	42	0.029*

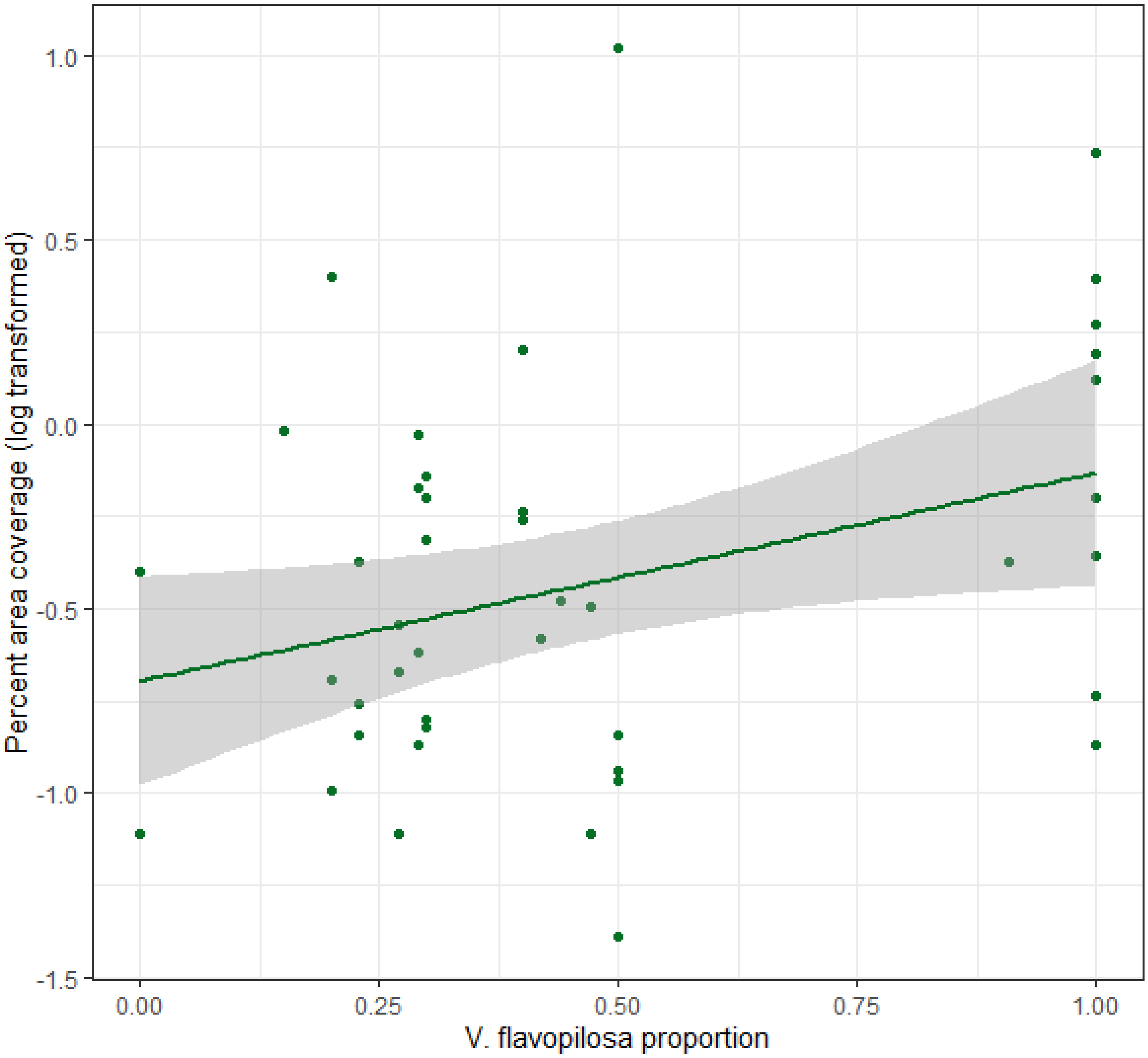

Figure 21:Relationship between percent area coverage and V. flavopilosa proportion in the mushroom body calyces ($p = 0.029$). All values for proportion taken from the cumulative parasite measurement on the day the specimen was collected. All variables have been log transformed.

Models comparing latency with serotonin in the MB calyces yielded positive results with particle size ($p = 0.045$) and approached significance with average particles ($p = 0.052$). The relationship between latency and particle size was negative, with response time decreasing as particle size increased (Fig. 22). The relationship between average particles and latency, however, appeared to be positive, with response time increasing as particles increased (Fig. 23). Full model results for latency and serotonin in the mushroom body calyces can be found in Table 20. No significant results were observed in the models comparing duration or total strikes to serotonin in the MB calyces.

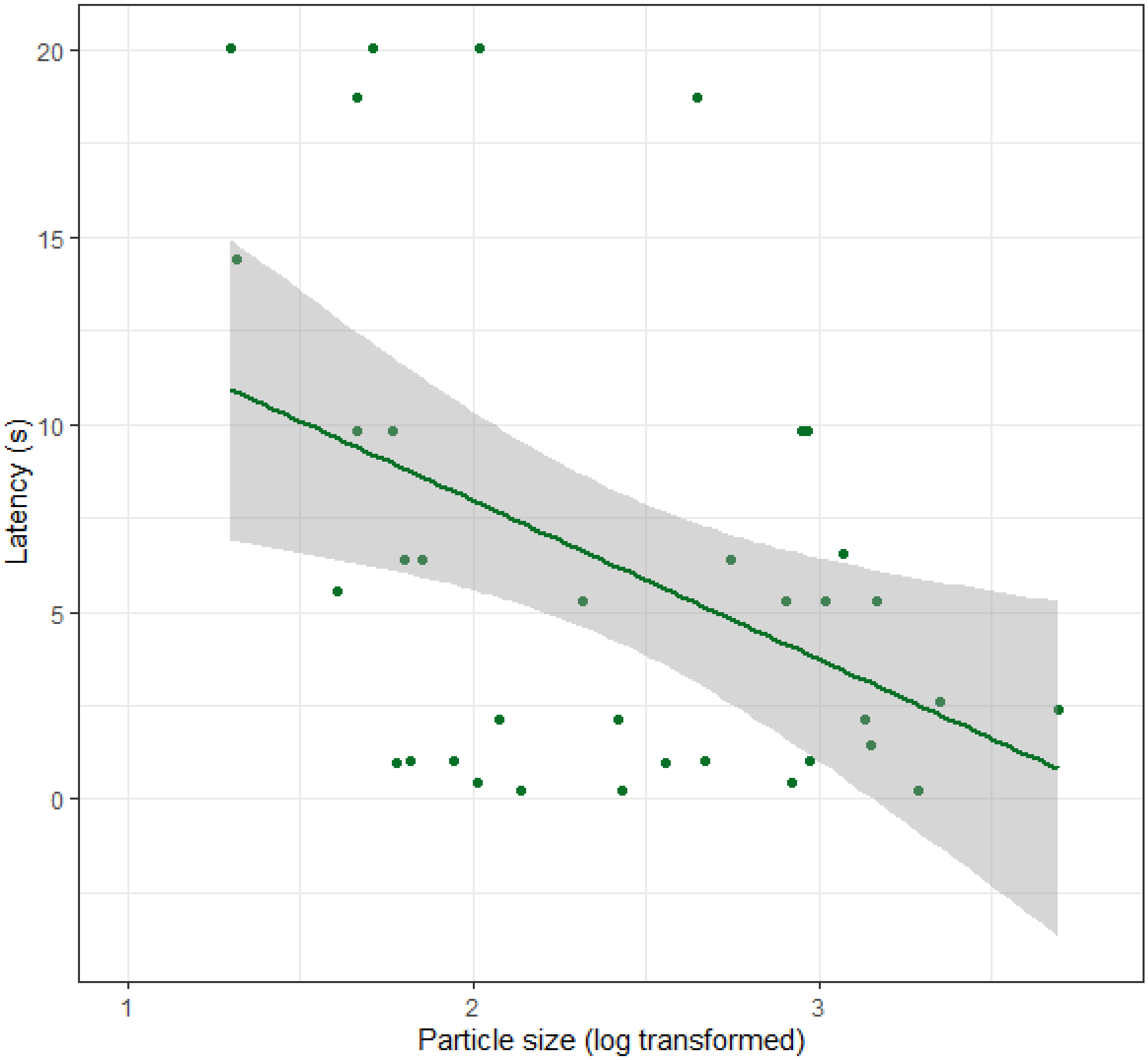

Figure 22: Relationship between latency and particle size in the mushroom body calyces. Higher latency indicates an increasingly delayed response to disturbance.

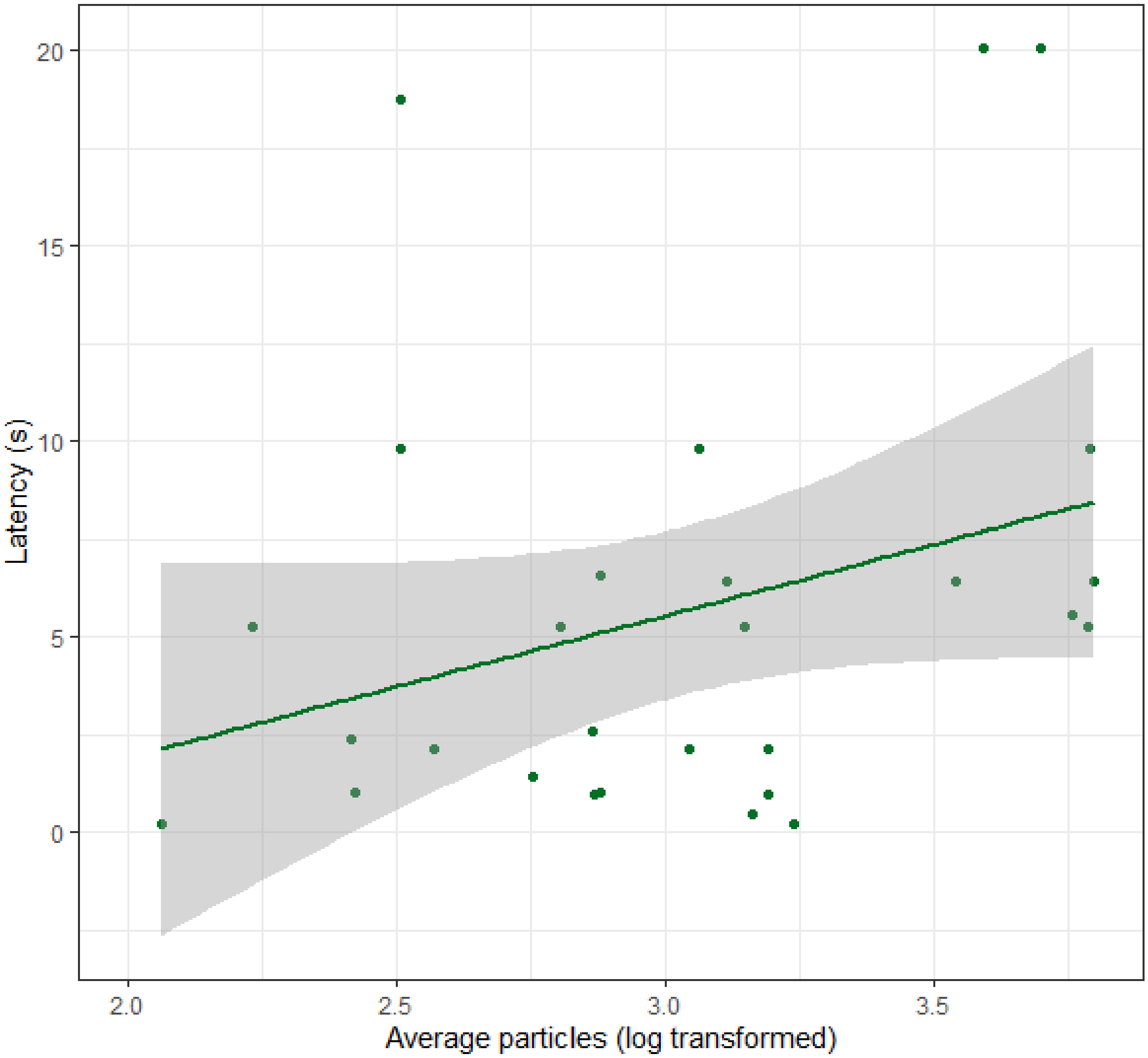

Figure 23: Relationship between latency and average particles in the mushroom body calyces. Increased latency indicates a delay in the response to disturbance.

Table 20: Model results for latency compared to serotonin in the mushroom body calyces. All anatomy variables have been log transformed. Model formula: Latency ~ log[anatomy variable] + (1|Nest)

Variable	**Cumulative R^2**	**Degrees of freedom**	**p-value**
Average particles	0.481	29.74	0.052·
Particle size	0.525	29.79	0.00448**
Percent area coverage	0.405	29.62	0.636

Discussion

The anatomical studies presented here represent, to our knowledge, the first confocal microscopy images of the brain of members of the genus *Vespula.* While a high degree of spread exists in the data for all three brain regions, the presence of significant relationships provides evidence of possible linkage between behaviour and serotonin content.

The characteristic band-like structures seen in the optic lobe align with imaging of this brain region in hymenopterans (Fig. 12; Schürmann and Klemm 1984). The presence of discrete particles of serotonin fluorescence in the lip of the MB calyces but not the collar observed in imaging of these specimens also indicates the expected presence of microglomeruli in these regions, and is similar to images done in other hymenopteran species (Fig. 14; Giraldo et al. 2013). The MB calyces are involved in processing both visual and auditory cues, and the decreased latency with increasing microglomerular size may indicate a more rapid response to alarm pheromone (Groh and Rössler 2019).

The lateral protocerebrum had a high degree of serotonergic immune activity, revealing large particle size and density (Fig.13). This region has been imaged via staining with fluorescent dye in other insect species, such as the silk moth *Bombyx mori,* and shows evidence of dense serotonergic neuropils when stained with this method (Namiki and Kanzaki 2019). The larger particles seen in my images may represent a cluster of smaller serotonergic neurons which appear indistinct due to binding of large amounts of serotonin, and the relatively low magnification at which our microscopy was performed (Fig. 13).

The only notable species difference between the three tested was for particle size in the optic lobe, which was notably lower in *V. pensylvanica* than the other two species (Fig. 15). Though species differences in the optic lobe were expected, I hypothesized that *V. flavopilosa,* being a facultative social parasite, would be different from the two free-living species. According to phylogeny presented by Lopez-Osorio et al. (2015), *V. pensylvanica* is also the most ancestral of the three species and shares a closer common ancestor with *V. germanica,* an invasive species in North America. This phylogenetic difference may present in the anatomy of the optic lobe, though confocal imaging of *V. germanica* has not been done. It should be noted that *V. pensylvanica* and *V. germanica* both sustain invasive populations, *V. pensylvanica* in Australia, New Zealand, and Hawaii, and *V. germanica* in Australia, New Zealand, and North America (Wilson-Rankin 2014; Warren and Promowicz 2025). Invasive populations have not been reported in *V. maculifrons* and *V. flavopilosa.* Further investigation into neurological differences between invasive and non-invasive yellowjackets may aid in defining characteristics that make invasive species successful.

Several measurements of serotonin in the optic lobe were also correlated to higher latency, ie. slower response to disturbance (Fig. 16; Table 14), as well as a lower number of total strikes (Fig. 18; Table 16). While the optic lobe has well-studied roles in attention and visual processing, any putative roles in aggression have not been demonstrated (Paulk et al. 2014; Uy et al. 2021). However, a significant correlation with higher response time may support the hypothesis presented by Bubak et al. (2020) that serotonergic regulation may attenuate aggression in some areas of the brain rather than increase it.

These results contrast with those of Nouvian et al. (2018), which found higher overall serotonin in the optic lobes was associated with higher colony-level aggression in honey bees.

However, this study was done using different methods, looking at serotonin levels from homogenized tissue rather than patterns of serotonergic neurons seen in intact tissue (Nouvian et al. 2018). This study also looked only at total strikes as an indicator of aggression, for which we did not find a correlation between any of our anatomy variables in the optic lobe. It is also possible a species difference exists in aggression mechanisms between honey bees and yellowjackets in the optic lobe, or that the relevant behavioural variables have different neural bases.

In the lateral protocerebrum, both particle size and percent area coverage were linked with lower latency (ie. faster response time) (Fig. 19; Table 17). One possible explanation to this relationship is sensitivity to alarm pheromone due to the lateral protocerebrum's roles in processing and responses to olfactory cues (Namiki and Kanzaki 2019). However, in this case it would also be expected for a relationship to be present with total strikes, as alarm pheromone produced by yellowjackets tends to linger on surfaces where it was applied (Reed and Landolt 2000). In addition, it's likely that at the time of the first strike alarm pheromone would not yet have been applied to the target (Reed and Landolt 2000). The lateral protocerebrum has also been demonstrated to have roles in mechanosensation, another possible explanation for its relationship to latency; the colonies with shorter latency may be responding more quickly to the mechanical disturbance of the nest (Pfuhl et al. 2014). Therefore, the hypothesis, stated in the introduction of this chapter, that serotonin in the lateral protocerebrum would have a relationship to colony aggression is partially supported.

In the mushroom body calyces, the only significant correlation between behaviour and anatomy was a negative relationship between latency and particle size, with no significant species differences being present (Fig. 23; Table 20). Unlike other areas of the insect brain, the

MB calyces contain microglomeruli, which increase in size with age and experience while decreasing in density (Stieb et al. 2010; Groh and Rössler 2019). Because of this organizational difference, it is expected that a relationship would be seen with particle size, but not average particles (Stieb et al. 2010). Though no relationship with duration or total strikes was observed, the relationship between disturbance responsiveness and particle size supports my hypothesis that serotonergic neurons in the MB calyces are involved in aggression at the colony level.

The only correlation observed between serotonin and *V. flavopilosa* presence was in the MB calyces, with ROI area coverage increasing as parasite proportion increased (Fig. 21; Table 19). In free-living social insects, MB calycal morphology has been shown to change with dominance interactions between individuals in other hymenopteran species (Pahlke et al. 2019). Social parasitism in paper wasps (*Polistes spp.*) has been demonstrated to cause an increase in dominance interactions between parasitized workers, as well as neurogenomic changes (Cini et al. 2020). Taken in conjunction with these results, the increase in serotonin observed in my study may indicate a change worthy of further investigation.

The effects of any anatomical variable on defense duration were largely inconclusive, with only the lateral protocerebrum being observed to have a significant effect (Table 18; Fig. 20). A negative relationship was present between duration and serotonergic activity, suggesting similarly to my optic lobe results that this region's serotonergic neurons may have an inhibitory effect (Bubak et al. 2020). However, the sample size for behavioural experiments presented here was small due to challenges faced during the field season (see chapter 2), which may account for the ambiguous results. Further investigation is needed.

References

Alekseyenko, O.V., Lee, C., Kravitz, E.A. 2010. Targeted Manipulation of Serotonergic Neurotransmission Affects the Escalation of Aggression in Adult Male *Drosophila melanogaster*. *PLoS One,* **5**(5): e10806. DOI: 10.1371/journal.pone.0010806.

Blenau, W., Thamm, M. 2011. Distribution of serotonin (5-HT) and its receptors in the insect brain with focus on the mushroom bodies. Lessons from *Drosophila melanogaster* and *Apis mellifera. Arthropod Struct. Dev.* **40:** 381-394. DOI: 10.1016/j.asd.2011.01.004.

Bubak, A.N., Watt, M.J., Yeager, J.D.W., Renner, K.J., Swallow, J.G. 2020. The stalk-eyed fly as a model for aggression – is there a conserved role for 5-HT between vertebrates and invertebrates? *J. Exp. Biol.* **223**(1): jeb132159. DOI: 10.1242/jeb.132159.

Cini, A., Branconi, R., Patalano, S., Cervo, R., Sumner, S. 2020. Behavioural and neurogenomic responses of host workers to social parasite invasion in a social insect. *Insectes soc*. **67:** 295-308. DOI: 10.1007/s00040-020-00765-6.

Dierick, H.A., Greenspan, R.J. 2007. Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. *Nat.Genet.* **39**(5): 679-682.

Giraldo, I.M., Patel, E., Gronenberg, W., Trainello, J.F.A. 2013. Division of labor and structural plasticity in an extrinsic serotonergic mushroom body neuron in the ant *Pheidole dentata. Neurosci. Lett.* **534:** 107-111. DOI: 10.1016/j.neulet.2012.11.057.

Groh, C., Rössler, W. 2019. Analysis of Synaptic Microcircuits in the Mushroom Bodies of the Honeybee. *Insects,* **11**(1): 43. DOI: 10.3390/insects11010043.

Jirakitpuwapat, W., Kumam, P., Deesuwan, T., Sanguansuttigul, P. 2020. Quantum Renyi entropy with applications in image processing. *Bangmod J. Math & Comp. Sci.* **1-2**(6): 25-37.

Landolt, P.J., Heath, R.R., Reed, H.C., Manning, K. 1995. Pheromonal Mediation of Alarm in the Eastern Yellowjacket *(Hymenoptera: Vespidae). Fla. Entomol.* **78**(1): 101. 10.2307/3495673.

Lopez-Osorio, F., Perrard, A., Pickett, K., Carpenter, J.M., Agnarsson, I. 2015. Phylogenetic tests reject Emery's rule in the evolution of social parasitism in yellowjackets and hornets *(Hymenoptera: Vespidae, Vespinae). R. Soc. Open Sci*. **2**(9): 150159. DOI: 10.1098/rsos.150159.

Molina, Y., O’Donnell, S. 2008. Age, sex, and dominance-related plasticity in the paperwasp *Mischocyttarus mastigophorous.* Dev. Neurobiol. **68(7):** 950-959. DOI: 10.1002/dneu.20633

Namiki, S., Iwabuchi, S., Pansopha Kono, P., Kanzaki, R. 2014. Information flow through neural circuits for pheromone orientation. *Nat. Commun.* **5**(1): 5919. DOI: 10.1038/ncomms6919

Namiki, S., Kanzaki, R. 2019. Morphology and physiology of olfactory neurons in the lateral protocerebrum of the silkmoth *Bombyx mori. Sci. Rep.* **9**(1): 16604. DOI: 10.1038/s41598-019-53318-8.

Pahlke, S., Jaumann, S., Seid, M.A., Smith, A.R. 2019. Brain differences between social castes precede group formation in a primitively eusocial bee. *Sci. Nat.* **106**(9-10): 49. DOI: 10.1007/s00114-019-1644-7.

Pfuhl, G., Zhao, X., Ian, E., Surlykke, A., Berg, B.G. 2014. Sound-sensitive neurons innervate the ventro-lateral protocerebrum of the heliothine moth brain. *Cell Tissue Res.* **355**(2): 289-302. DOI: 10.1007/s00441-013-1749-9.

Reed, H.C., Landolt, P.J. 2000. Application of Alarm Pheromone to Targets by Southern Yellowjackets *(Hymenoptera: Vespidae). Fla. Entomol.* **83**(2): 193. DOI: 10.2307/3496156.

Rogers, S.M., Matheson, T., Sasaki, K., Kendrick, K., Simpson, S.J., Burrows, M. 2004. Substantial changes in central nervous system neurotransmitters and neuromodulators accompany phase change in the locust. *J. Exp. Biol.* **207**(20): 3603-3617. DOI: 10.1242/jeb.01183.

Rozanski, A.N., Cini, A., Lopreto, T.E., Gandia, K.M., Hauber, M.E., Cervo, R., Uy, F.M.K. 2022. Differential investment in visual and olfactory brain regions is linked to the sensory needs of a wasp social parasite and its host. *J. Comp. Neurol.* **530**(4): 756-767. DOI: 10.1002/cne.25242

Sage, D., Donati, L., Soulez, F., Fortun, D., Schmit, G., Seitz, A., Guiet, R., Vonesch, C., Unser, M. 2017. DeconvolutionLab2: An open-source software for deconvolution microscopy. *Methods,* **115:** 28-41. DOI: 10.1016/j.ymeth.2016.12.015.

Sahoo, P., Wilkins, C., Yeager, J. 1997. Threshold selection using Renyi's entropy. *Pattern Recognition* **30**(1): 71-84. DOI: 10.1016/S0031-3203(96)00065-9.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kayning, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A. 2012. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**(7): 676-682. DOI: 10.1038/nmeth.2019.

Sullivan, J.M., Beltz, B.S. 2001. Neural pathways connecting the deutocerebrum and lateral protocerebrum in the brains of decapod crustaceans. *J. Comp. Neurol.* **441**(1): 9-22. DOI: 10.1002/cne.1394.

Uy, F.M.K., Jernigan, C.M., Zaba, N.C., Mehrotra, E., Miller, S.E., Sheehan, M.J., Stubbs, L. 2021. Dynamic neurogenomic responses to social interactions and dominance outcomes in female paper wasps. *PLoS Genet.* **17**(9): e1009474. DOI: 10.1371/journal.pgen.1009474.

Van der Woude, E., Smid, H. M. 2017. Maximized complexity in miniaturized brains: morphology and distribution of octopaminergic, dopaminergic and serotonergic neurons in the parasitic wasp, *Trichogramma evanescens*. *Cell Tissue Res.* **369**(3): 477-496. DOI: 10.1007/s00441-017-2642-8.

Van Nest, B.N., Wagner, A.E., Marrs, G.S., Fahrbach, S. E. 2017. Volume and density of microglomeruli in the honey bee mushroom bodies do not predict performance on a foraging task. *Develop. Neurobiol.* **77**(9): 1057-1071. DOI: 10.1002/dneu.22492.

Wilson-Rankin, E.E. 2014. Social context influences cue-mediated recruitment in an invasive social wasp. *Behav. Ecol. Sociobiol.* **68**(7): 1151-1161. DOI: 10.1007/s00265-014-1726-7.

Chapter 4: Conclusions and future directions

Conclusions

While the findings presented here represent a relatively small sample size, significant results were observed in both behavioural and anatomical experiments, as well as some observations made on yellowjackets and their behaviour in their native range. These results also represent the first confocal images of *Vespula* brains, and the first recorded instance of parasitism of *V. pensylvanica* by *V. flavopilosa.*

The results of colony searches over the 2023 and 2024 field seasons indicate that yellowjackets in central Canada prefer urban habitats—rural sites were also surveyed for this project, but were dropped due to lack of yellowjacket activity (see Appendix 1). The lack of wasp activity due to high precipitation observed in the 2024 field season (see chapter 2) also supports findings in existing literature regarding yellowjacket population cycles and conditions in which populations thrive or fail (Akre and Reed 1981). *V. maculifrons* was considerably less common in highly populated environments during the field seasons of this study, and may be less adapted to an urban lifestyle or more sensitive to environmental factors that impact yellowjacket life history due to their lower rates of foraging and slower rate of colony growth (Ross et al. 1981).

The results of our behavioural studies support the hypothesis that the two host species examined in this study (*V. pensylvanica* and *V. maculifrons*) respond differently to the invasion of the social parasite *V. flavopilosa,* with short-term fluctuations in *V. flavopilosa* presence increasing aggression in *V. maculifrons* and decreasing it in *V. pensylvanica* (Fig. 8). However, these short-term fluctuations may also indicate that *V. flavopilosa* is a species with colony-level aggression different from *V. pensylvanica* and *V. maculifrons.* There was a significant species difference between the two free-living species, with *V. pensylvanica* having higher defense duration, but being slower to respond to disturbances (Table 5; Fig. 5; Table 7; Table 8; Fig. 7). The only significant species difference found anatomically was in the optic lobe, with *V. pensylvanica* having lower particle density and smaller particle size (Fig. 15; Table 13). Interestingly, serotonin particle density and area coverage were positively correlated with higher latency (ie. more delayed response), despite being lower in *V. pensylvanica*. The link between species, behaviour, and serotonergic innervation of the optic lobe bears further investigation.

The differences in results for latency and duration presented in chapter 2 suggest that the neural mechanisms controlling these two behavioural variables are different, a hypothesis supported by the results of chapter 3. Latency was linked to particle size in the mushroom body calyces, with decreased latency (ie. faster response time) linked to particle size in a way consistent with the organization of microglomeruli present in the calyces of the mushroom bodies, and their reorganization with experience (Fig. 22; Stieb et al. 2010). Serotonergic neurons in the mushroom bodies have demonstrated roles in individual aggression, and these results support their role in aggression at the colony level as well (Blenau and Thamm 2011).

While the colony cycle played a strong role in the behaviour of colonies included in our study, we were unable to assess the role of age or colony senescence in our anatomical measurements. Due to specimen loss, the anatomical data presented here was largely from sampling late in the season, and not representative of the entire colony cycle. The role of colony senescence in serotonin level and distribution in the yellowjacket brain remains one of many directions for further study.

Future directions

In general, the genus *Vespula* represents an understudied system for social insect behaviour, urban ecology, and brood parasitism. Yellowjackets, as seen in our study, thrive in urban environments, displaying minimal population reduction and biodiversity loss compared to other genera, making them a good potential candidate for the study of insect wildlife in urban environments (Christine and Hochuli 2006; Komonen et al. 2020). In addition, yellowjackets' population cycling with weather patterns, namely precipitation observed in our study and supported by older literature, provides another area of interest for studying this genus (Akre and Reed 1981). Annual rainfall in the Canadian prairies has increased, and is projected to continue increasing due to climate change (Jeong et al. 2020). Due to their high trophic level among insects and their being relatively easy and cheap to sample, wasps have already been noted as having valuable potential as bioindicators (Brock et al. 2021). Yellowjackets present a potential use in monitoring the effects of climate change on insect populations in the Canadian prairies in the coming years.

Though my study measured colony behaviour in relation to social parasite proportion, potential confounding factors such as colony size, brood investment, and number of queens could not be taken into account. Though many studies measure colony size by excising the colony and examining the amount of mature comb, non-invasive measurements such as video measurements of forager traffic are more conducive to long-term colony studies (Jeanne and Bouwma 2002).

In order to control the introduction of a social parasite, or measure single vs multiple queen colonies, seeding a particular nest area with foundresses and observing colony growth has been done in paper wasps (*Polistes spp.*), and similar methods could be used to observe the colony cycle in other species of social wasp (Gamboa 1978). Laboratory observation of collected colonies has also been done in *V. maculifrons* and *V. flavopilosa* (MacDonald et al. 1980). Dominance interactions between individuals have been observed to change with workers of *Polistes sulcifer* with invasion of the social parasite *Polistes dominula* (Cini et al. 2020). Determining whether these changes are conserved across genera may provide some insight into the strategies host workers use to counteract social parasites.

The neuroanatomical results presented in this study are far from conclusive. The marginal relationships observed in the lateral protocerebrum between serotonin particle size and percent area coverage, and decreased latency, indicate a possible role of this region in the colony defense response, though a larger sample size would be required to confirm or deny the relationship. Another candidate for further anatomical study is dopamine, as a molecule that also has demonstrated roles in aggression, particularly around individual dominance hierarchies (Aonuma and Benelli 2023). Measuring dopamine levels and scaling in socially parasitized yellowjackets may also yield valuable insights into the biology of social parasitism.

References

Akre, R.D., Reed, H.C. 1981. Population cycles of yellowjackets in the Pacific Northwest. *Env. Entomol.* **10(3):** 267-274.

Brock, R.E., Cini, A., Sumner, S. 2021. Ecosystem services provided by aculeate wasps. *Biol. Rev.* **96**(4): 1645-1675. DOI: 10.1111/brv.12719

Christine, F.J., Hochuli, D.F. 2006. Responses of wasp communities to urbanization: effects on community resilience and species diversity. *J. Insect Conserv.* **13:** 213-221. DOI: 10.1007/s10841-008-9146-5

Gamboa, G.J. 1978. Intraspecific defense: advantage of social cooperation among paper wasp foundresses. *Science,* **199**(4336): 1463-1465.

Jeanne, R.L., Bouwma, A.M. 2002. Scaling in Nests of a Social Wasp: A Property of the Social Group. *Biol. Bull.* **202**(3): 289-295. DOI: 10.2307/1543481.

Jeong, D.I., Cannon, A.J., Morris, R.J. 2020. Projected changes to wind loads coinciding with rainfall for building design in Canada based on an ensemble of Canadian regional climate model simulations. *Clim. Change* **162**(2): 821-835. DOI: 10.1007/s10584-020-02745-y.

Komonen, A., Nirhamo, A., Tornainen, J. 2020. Social wasps (Vespinae) in urban gardens and woods. *Ann. Zool. Fennici.* **57:** 41-46.

Appendix: Pilot studies and testing of field methods

Testing of search and assay methods began in August of 2023. A sugar-based bait (grenadine) and a protein-based bait (sliced bologna sausage) were left out and observed for wasp activity. The protein-based bait was visited more often by yellowjackets and was chosen as the bait for search use in the future. Wasp activity checks were performed at a variety of candidate sites both within the city of Winnipeg and in the surrounding rural areas via observation of bait in 1-hour increments. Rural sites displayed very low levels of yellowjacket activity, and yellowjacket activity was consistently highest in central areas of the city with the exception of a point near the center of the Assiniboine Forest (Fig.1; Fig.2). Specimens were sampled and identified during activity checks, with *Vespula pennsylvanica* and *Vespula maculifrons* both being observed during the 2023 field season.

Over the 2023 field season, two nests were successfully located, with specimens from both identified as *V. pennsylvanica.* Assay methods were tested on these two nests, with a small amount of pilot data collected. A percussive stimulus facilitated by dropping a 1kg brick onto the nest from a height of 1m was unsuccessful in eliciting an aggressive response from either nest. The scratch stimulus used in 2024 behavioural testing successfully elicited an aggressive response from both nests.