DNA metabarcoding of prey reveals spatial, temporal, and diet partitioning of an island ecosystem by four invasive wasps

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Abstract

1. Invasive alien species can cause detrimental changes in native ecosystems, but our understanding of the interactions between multiple exotic species is limited. To evaluate the joint effect of multiple sympatric invaders on an ecosystem, we must first understand how they interact with each other.

2. Here, we quantified the spatial distribution, dietary composition and overlap of four invasive generalist vespid species (two *Vespula* and two *Polistes*) that co-occur on Ahuahu

off the north-east coast of New Zealand. We used DNA metabarcoding of larval faecal material to identify prey species, and mapped the locations of nests.

3. We observed clear spatial partitioning, with different wasp species aggregating in different habitats. Diet differed both among wasp species (diet partitioning) and among seasons (temporal partitioning). There was stronger competition (highest diet overlap) between *V. germanica* and *V. vulgaris* than between other wasp species, but partitioning resources minimised spatial and diet overlap between the four species.

4. Additionally, we obtained high-resolution diet inventories that help assess the combined ecological impact of invasion by multiple wasps. These species consumed mostly native and endemic invertebrates from multiple trophic levels, but particularly herbivores (Lepidoptera). We provide direct evidence that in combination they exploited a wide range of endemic invertebrates.

5. *Synthesis and applications.* Our study demonstrates resource partitioning among four invasive wasps, facilitating coexistence of these generalist invaders. We conclude that coexistence probably amplifies their ecological impact. Quantifying how multiple invaders exploit and partition resources provides insights into how communities assemble and helps to assess the cumulative effects of multiple invaders on the recipient community.

Keywords

Vespula, *Polistes*, diet overlap, invasive species, environmental DNA, Lepidoptera, resource competition, niche partitioning

Introduction

A fundamental question in invasion ecology is how multiple invaders interact in native ecosystems. When an invader colonises a novel habitat, one of the key factors for its success is access to resources (Tilman, 2004; Stachowicz & Byrnes, 2006). Accordingly, the presence of multiple invaders can result in competition between them. An invader may avoid this competition by shifting its ecological niche (e.g. foraging behaviour, habitat use, or activity patterns), thereby partitioning resources to minimise overlap with its sympatric counterpart (Schoener, 1974; Shea & Chesson, 2002). In the absence of niche shifting, either or both species may suffer deleterious effects (e.g. competitive exclusion, Gause, 1934; Griffen & Delaney, 2007). Understanding the degree of interspecific competition and the mechanisms that partition resources will provide insights into how communities assemble. It may also help invasion ecologists to predict which invaders are likely to co-exist, and to assess the cumulative effects of multiple invaders on the receiving community.

Invasive species can minimise competition with each other across many dimensions. Competing species can coexist in one region by utilizing different habitats (*spatial partitioning*, Chesson, 2000; Raby, Johnson, Kessel, Stewart, & Fisk, 2019). Species may also avoid competition by exploiting different food resources (*diet partitioning*, Schoener, 1974; Takahashi et al., 2020). Finally, should species rely on the same resource – be it space, food, or both – coexistence can be facilitated by exploiting this resource at different times (*temporal partitioning*, Chesson, 2000; Valeix, Chamaillé-Jammes, & Fritz, 2007; Mori, Ferretti, & Fattorini, 2019). Many studies have investigated resource partitioning between invaders and their native counterparts to estimate ecological impacts of invasion (e.g. Junker, Bleil, Daehler, & Blüthgen, 2010; Coccia et al., 2016; Culbertson & Herrmann, 2019). However, comparatively few have asked how multiple invaders partition resources among

themselves (e.g. Boyer & Rivault, 2006; Freed & Leisnham, 2014), despite the potentially important role of resource competition in determining coexistence.

Darwin (1859) proposed that close relatives should compete more intensely with one another than with distant relatives (*competition-relatedness hypothesis, CRH*), as they are more ecologically similar regarding habitat use, food resources and predators. Darwin's hypothesis could be applied to biological invasions in two ways. Firstly, phylogenetic relatedness between invasive species and native species may predict the strength of competitive interactions and subsequently the ecological impact of invasion. However, recent studies are mixed in their support for this (Venail et al., 2014; Alexandrou et al., 2015; Tan, Pu, Ryberg, & Jiang, 2015). Secondly, relatedness among multiple invaders should predict the extent to which they compete with each other and thereby the probability of coexistence. According to the CRH, competition should be strongest between closely related invaders, resulting in either the displacement of the inferior competitor or niche partitioning. The present study will help inform the debate about Darwin's hypothesis in regards to invader-invader relatedness.

Four invasive vespid species, *Vespula vulgaris*, *V. germanica*, *Polistes chinensis antennalis*, and *P. humilis*, have established on New Zealand's 'mainland' and many of the surrounding offshore islands (Beggs et al., 2011; Schmack et al., 2019; Schmack, Schleuning, Ward, & Beggs, 2020). The specific invasion history of vespid wasps on New Zealand's offshore islands is unknown, but all four species are found on many islands (Schmack et al., 2020) and they have been widespread throughout New Zealand for many decades (Clapperton, Moller, & Sandlant, 1989). They outcompete the native community for resources and prey heavily on native taxa (Beggs & Wardle, 2006; Beggs et al., 2011; Ward & Ramón-Laca, 2013). *Vespula* and *Polistes* exploit carbohydrate resources such as honeydew produced by scale insects (Beggs, 2001) and prey on invertebrates, with

Lepidoptera constituting one of their main protein resources (Beggs et al., 2011). Vespids invaders are likely to compete strongly with each other because they have similar resource requirements; demonstrated by competition between *Vespula vulgaris* and *V. germanica* for honeydew resources in southern beech (*Fuscospora* spp.) forests (Harris, 1991; Harris, Moller, & Winterbourn, 1994). However, little is known of the interactions in other habitats or between other invasive vespids species introduced to New Zealand.

The co-occurrence of four invasive wasp species on a small offshore island provides a unique opportunity to investigate resource partitioning among multiple closely related invaders. Because small islands are typically species depauperate and host less complex communities than mainland habitats of equivalent size (MacArthur & Wilson, 1963) they offer model ecosystems for studying invasion ecology against a less complicated ecological background (Wardle, 2002). By assessing the distribution of nests and diet of wasp species, we examined whether these four generalist invaders partition resources.

We evaluated the interactions of four sympatric wasp species on an offshore island to address the following research questions: (a) do the four species spatially partition the island? (b) do they partition food resources? To do this, we systematically mapped wasp nests in seven different habitats on the island, and we collected larval faecal samples from the nests, utilising next generation sequencing (NGS) of DNA to identify diet composition. Given New Zealand's Lepidopteran fauna is characterised by an exceptionally high rate of endemism (Hoare, 2010) and predation by invasive wasps represents a threat to indigenous biodiversity, we additionally asked which invasive wasp species consumed the most endemic Lepidoptera. Overall, this approach produces results at a high taxonomic resolution that can be utilised by conservation practitioners to assess the ecological impacts of invasive species.

Materials and methods

Study site and habitat types

We collected *Polistes* and *Vespula* nests on Ahuahu (Great Mercury Island), an island off north-eastern New Zealand (Fig. 1). We classified seven habitat types on Ahuahu based on vegetation cover using the Land Cover Data Base v. 4.1 (Land Resource Information Systems) coastal grassland; *Kanuka/Manuka* (*Kunzea ericoides*; *Leptospermum scoparium*) forest; exotic pine *Pinus radiata* plantation with native undergrowth; native restorations; residential gardens; clear-cutting; and mature native forest (dominated by Pohutukawa *Metrosideros excelsa*, Kohekohe *Dysoxylum spectabile*, Puriri *Vitex lucens*) (Wright, 1976). We used ArcGIS 10.5.1 (Environmental Systems Research Institute (ESRI) 2011) to calculate the cumulative area covered by these habitat types (1830 ha) which comprised 98% of the total island area (1860 ha). The remaining areas were sand and gravel.

Nest sampling

Two researchers searched 10 m x 500 m strip plots (0.5 ha) for nests during the peak of the wasp season (March 2019). We covered approximately 70% of the island, allocating our search effort proportional to the size of each habitat (Table 1) and avoiding inaccessible or unsafe areas. Our search effort totalled 52 hours over nine days between 9 am and 7 pm when foraging wasps were most active. We visually located nests by following the flight paths of foraging wasps. GPS coordinates and habitat of each nest site were recorded. We collected 64 active nests (Table 1). *Polistes* nests were cut from branches or scraped off rocks and placed in sealable plastic bags. *Vespula* nests were poisoned using a permethrin-based product 6 - 24 hours before being dug out of the ground and placed in sealable plastic boxes. All nests were transported in chilly bins and stored at -20°C within 5 hours.

DNA extractions

Of the nests collected for the first part of this study, 51 were used to identify prey species that

adult wasps have fed their young over an entire wasp season. We collected three faecal samples deposited at the base of a cell from each nest using tweezers, which were flame sterilized before and after the collection of each faecal pellet to avoid cross-contamination. Samples were collected from three nest layers containing different batches of brood (Spradbery, 1973) to explore seasonal (early, mid, late) differences in prey composition (see Supplementary Text S1, Fig. S1). One small nest of *P. chinensis* contained faecal matter sufficient for one sample only. Samples were placed directly in microcentrifuge tubes and disrupted for DNA extraction using a DNeasy Power Soil DNA extraction kit (Qiagen, Germany) following manufacturers instructions, except for the bead-beating step where we used a TissueLyser (Qiagen, Germany) at 30,000 rpm for 1 min. The DNA was extracted following manufacturers procedures. DNA concentrations in the resulting eluates were measured by fluorometry using a Qubit® with the dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA).

DNA amplification and sequencing

For DNA amplification, primers targeting a short (313 bp) gene region of the mitochondrial gene COI were used (namely m1COIintF: 5'-TCGTCGGCAGCGTCAGATGTGTAT
AAGAGACAGGGWACWGGWTGAACWGTWTAYCCYCC-3'; Leray et al., 2013) and HCO2198: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAAACTTCAGG
GTGACCAAAAAATCA-3'; Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 2013). This primer pair performs well across a range of metazoan samples (Leray et al., 2013), and has successfully been used for faecal samples of *Polistes* (Lefort et al., 2020). Primers include the Illumina adapter sequences (underlined) required for downstream sequencing. The following PCR conditions were applied to our DNA templates: 12.5 µL KAPA HiFi Hotstart ReadyMix (Kapa Biosystems, catalog number KK2602), 0.75 µL of each primer at 10 µM (m1COIintF

and HCO2198), 3.5 µL fecal DNA extraction and 7.5 µL PCR water to fill the reaction up to 25 µL. Negative controls containing DNA-free water instead of the DNA template were included in all PCR runs. PCR cycling conditions were 95 °C for 3 min followed by 14 cycles of 95°C for 10 s, 62°C (- 1°C per cycle) for 30 s and 72 °C for 60 s. We then conducted 25 cycles of 95°C for 10 s, 48°C for 30 s and 72°C for 60 s and a final elongation of 7 minutes at 72 °C. PCR products were electrophoresed in 1 % agarose gel stained with SYBR Safe (Thermo Fisher, US) and visualized by UV light. According to standard protocol (Support Illumina 2016), PCR products were purified using ZR-96 DNA Cleanup kits (Zymo Research). DNA concentrations in the resulting eluates were measured by fluorometry using a Qubit® with the dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA) and standardised to 2 µM before being submitted to New Zealand Genomics Ltd. (Auckland, New Zealand) for sequencing on an Illumina MiSeq instrument using 2-by-300-bp chemistry. Prior to sequencing, a unique combination of Nextera XT dual indices (Illumina Inc., USA) was attached to the DNA to allow multiplex sequencing; two sequencing runs were performed with 151 samples multiplexed per sequencing run.

Bioinformatics

We used USEARCH v 10 (Edgar, 2010) for quality filtering of DNA sequences. Primer sequences were removed by trimming 24 bp of each read and paired end reads were merged. Merged reads were filtered using an expected error threshold of one and replicates removed to identify unique sequences. Quality filtered reads were clustered at 100% identity to generate zero-radius operational taxonomic units (zOTUs) using the UNOISE algorithm in USEARCH. zOTUs were then compared to the Barcode of Life Database (BOLD). Only zOTUs for which read relative abundance within a sample was higher than 0.5% of the total reads per sample were retained to limit false positives. We used a conservative approach and

kept only zOTUs identified at species level above the percentage identity of 98 %. We checked suggested matches of Lepidoptera with a taxonomic index (Hoare, 2010). zOTUs identified as terrestrial and aquatic invertebrates or vertebrates were used for further analysis, while reads corresponding to fungi and plant DNA as well as *Vespula* and *Polistes* DNA were removed to avoid contamination. We assigned prey as native, endemic, or exotic using the New Zealand Organisms Register (www.nzor.org.nz).

Statistical analysis

All analyses were performed in RStudio; the main packages used were *vegan* (Oksanen et al., 2019), *mvabund* (Wang, Naumann, Wright, & Warton, 2012), *EcoSimR* (Gotelli & Ellison, 2013) and *spaa* (Zhang & Zhang, 2013). To answer whether wasp species partition spatially, we quantified whether the nests of a given species were more aggregated than expected by chance alone using Stone and Roberts' (1990) c-score (see Text S2).

The model-based approach *mvabund* uses simultaneous generalized linear models (ManyGLM; Wang et al., 2012), which specify the mean-variance relationship instead of relying on the assumptions of a distance-based approach. We used multivariate generalised linear models with binomial distribution to investigate differences in prey composition among wasp species, seasonal samples and seasonal samples of a given wasp species. Likelihood ratio tests (LRT) and resampled p-values were used to test effects of wasp species and seasonality on diet composition. In two different input data: a zOTU table containing presence/absence of all prey species and a zOTU table containing presence/absence of Lepidopteran prey species.

We quantified dietary niche width of wasps using Levin's index (Levin, 1968) and calculated trophic niche overlap between all pairs of wasp species (0 = no overlap, 1 = complete overlap) using the Pianka index (Pianka, 1973). We visualized diet overlap with

network diagrams generated using the *bipartite* package (v.2.15, Dormann, Gruber, & Fründ, 2008). We calculated mean prey richness (number of species per sample) for each wasp species and compared it among wasp species and nest layers, using a non-parametric Kruskal-Wallis test and the non-parametric Dunn's test (p -values adjusted with the Holm method) to determine significant differences between means.

When quantifying the ecological impact of wasps on the endemic community, we intentionally did not account for differences in sample size. Instead, we counted all endemic taxa identified as prey of the four wasp species.

Results

Spatial partitioning

We observed clear spatial partitioning among all four species of invasive wasps (Fig. 1, Table 1). Nests of all four species were significantly more aggregated than if they were randomly distributed across habitats as expected under null simulation (*P. chinensis*, $c_{observed} = 0.476$, $c_{simulated} = [0.824, 0.877]$, $p = 0.001$; *P. humilis*, $c_{observed} = 0.429$, $c_{simulated} = [0.713, 0.964]$, $p = 0.001$; *V. germanica*, $c_{observed} = 0$, $c_{simulated} = [0.667, 1]$, $p = 0.02$; *V. vulgaris*, $c_{observed} = 0.533$, $c_{simulated} = [0.783, 0.9]$, $p = 0.001$). For *V. vulgaris* nests ($n=16$), 50% of nests occurred in *Kanuka/Manuka* forest and 50% occurred in pine plantation. In contrast, *V. germanica* nests ($n=3$) were only found in coastal grassland. Both *P. humilis* and *P. chinensis* nests were exclusively found in open habitats. However, while *P. chinensis* occurred in four types of open habitat ($n=25$ in coastal grassland, $n=1$ in residential gardens, $n=9$ in native restorations, $n=3$ in clear cutting), *P. humilis* inhabited solely grassy habitats ($n=5$ in coastal grassland, $n=2$ in residential gardens). No nests of any species were found in mature native forest.

DNA sequencing and identified prey

DNA was successfully amplified and sequenced from 148 (n=67 for *P. chinensis*, n=24 for *P. humilis*, n=9 for *V. germanica*, n=48 for *V. vulgaris*) of the 151 faecal samples (three samples per nest, except for one small nest where faecal matter was sufficient for one sample only). We obtained an average of 4703 DNA reads ($\pm$ 497 SE) per sample. Out of 1,729 zOTUs, 262 (15%) could be assigned to species level using the Barcode of Life Database (BOLD); 85% yielded no match and were discarded before analysis. Seven zOTUs were assigned to the four predators (n = 2 *P. chinensis*, n = 2 *P. humilis*, n = 2 *V. vulgaris*, n = 1 *V. germanica*), and were discarded prior to analysis to account for potential cross-contamination. We identified 105 species belonging to 16 orders of animals (Table 2). Individual faecal samples contained between one and six prey orders and between one and 17 prey species (Table S1, for a list of prey species per wasp species). Out of the 105 identified prey species, 59 (i.e. 56%) occurred exclusively in the diet of different wasp species and only 10 were shared among all four wasp species. Lepidoptera, Diptera and Hymenoptera were important prey orders consumed by all four wasp species (Fig. 3). Of the 105 prey species identified, 44 (42%) were endemic, 18 (17%) were native and 41 (39%) were exotic. For two prey species, their status in New Zealand could not be identified (*Amblyseius* sp. JCS08, *Spilomelinae* gen. *spiloBioLep01*). Lepidoptera comprised the majority of identifications (46 taxa) and occurred in 95% of all samples (Table 2). Of the 45 Lepidopteran species identified, 18 were endemic species, 12 were native, 14 exotic and one was of unknown status (*Spilomelinae* gen. *spiloBioLep01*).

Rarefaction curves (Fig. 2) with the samples collected for this study did not reach an asymptote, suggesting that more prey species could have been identified with more samples. *V. germanica* had particularly few samples (n=3 nests equivalent to 9 samples). Nevertheless, we are confident that although diets may be incomplete, the main prey taxa

were detected. Furthermore, indices used to compare diets among wasps (i.e. species richness, niche width) are calculated taking sample size into account.

Diet and temporal partitioning

Diet composition (at the zOTU level) differed significantly between wasp species and seasonal samples (Table 3, Fig. 3). The diet of each wasp species varied across seasonal samples, indicating a change in prey species detected as the season progressed. Lepidoptera diet composition (at the zOTU level) differed significantly between wasp species and seasonal samples (Table 3, Fig. 3). The Lepidopteran diet of each wasp species varied across seasonal samples, indicating a change in Lepidopteran prey species detected as the season progressed.

Comparison of diet composition

Average prey richness per sample varied significantly among wasp species ($\chi^2 = 39$, $p = 0.001$) (Table 4, Fig. 4). *P. chinensis* fed on fewer species than *V. germanica* ($z = -3.09$, $p = 0.005$) and *V. vulgaris* ($z = -5.96$, $p < 0.001$). *P. humilis* fed on fewer species than *V. vulgaris* ($z = -2.80$, $p = 0.01$). Niche width (Table 4) and overlap varied among wasp species (Fig. 5). *V. germanica* had the widest and *V. vulgaris* the narrowest trophic niche. Pianka index for trophic niche overlap was highest between *V. germanica* and *V. vulgaris* (0.68). It was lowest between *P. chinensis* and *V. vulgaris* (0.27) as well as between *P. humilis* and *V. vulgaris* (0.27). Trophic niche overlap was 0.33 between *P. chinensis* and *P. humilis*, 0.45 between *P. chinensis* and *V. germanica*, and 0.3 between *P. humilis* and *V. germanica*.

Lepidoptera were an important part of the diet of all wasp species, although they consumed different families and species (Fig. 3, Fig. 5). While *P. humilis* and *P. chinensis* preyed predominantly on Geometridae (80% and 46%, respectively), *V. germanica* and *V.*

vulgaris consumed mainly Tortricidae (37% and 57%, respectively) (Fig. S2). Lepidopteran prey richness per sample differed significantly among wasp species ($Chi = 18$, $p = 0.001$), as did niche width (Table 4) and overlap of Lepidopteran diet. *P. chinensis* fed on fewer Lepidopteran species than *V. vulgaris* ($z = -4$, $p < 0.001$) and *P. humilis* ($z = -3$, $p < 0.05$). This trend was not significant in *V. germanica* ($z = -1$, $p > 0.05$). *P. humilis* had the widest and *V. vulgaris* the narrowest Lepidopteran trophic niche.

V. vulgaris consumed the most endemic species overall ($n=36$) and the most endemic Lepidoptera species ($n=16$). In contrast, *V. germanica* fed on 12 endemic species, of which five were endemic Lepidoptera. *P. humilis* and *P. chinensis* consumed 10 and 15 endemic species respectively, of which eight and seven were endemic Lepidoptera (Fig. 6).

Discussion

We provide evidence of spatial, trophic and temporal partitioning among four invasive social wasps co-occurring on Ahuahu, New Zealand. Using DNA metabarcoding, we identified 105 prey species in the seasonal diet of the four wasp species. This highlights the large number of species, particularly Lepidoptera, that are potentially impacted when generalist introduced predators co-occur. Although we could not assign the majority of zOTUs to species level, we did reveal trophic interactions with soft-bodied species that would have been impossible to detect using morphological techniques. For example, we recorded the basket-cocoon parasitoid *Meteorus pulchricornis* and their Lepidopteran hosts (Berry & Walker, 2004) in the same samples for all four wasp species. It is likely that wasps preyed on Lepidoptera larvae parasitised by *M. pulchricornis* rather than directly preying on the parasitoid. Our interpretation of wasp diet is a conservative description because of the limited availability of reference sequences for New Zealand, particularly for endemic species. This improves the robustness of our ecological conclusions (Calderón-Sanou et al., 2019)

although we note that differences in barcoding efforts for taxonomic groups may have led to over- or underrepresentation of some groups.

Spatial partitioning

Wasps partitioned Ahuahu spatially as different wasp species aggregated in different habitats. Only two out of seven habitat types were occupied by more than one wasp species. Maximum overlap occurred in grassland habitat, where *P. chinensis*, *P. humilis* and *V. germanica* were present. Mature native forest was not utilised by any species, but we did find *V. vulgaris* nests in both the more open exotic pine plantation and in native *Kanuka/Manuka* forest that had an abundant honeydew resource produced by the endemic scale insect *Coelostomidia wairoensis* (Gardner-Gee & Beggs, 2012). This is consistent with prior research suggesting that dense canopy cover without honeydew resources inhibits wasp invasion on islands (Schmack et al., 2020). *P. chinensis* was the only species found in native restorations and clear cutting, which are human-modified habitats characterized by young native vegetation and recent succession of exotic weeds, respectively. *P. humilis* nests were less abundant than *P. chinensis* nests and restricted to grassy habitat.

Spatial partitioning may result from intense competition between closely related species. *V. germanica* was only found in coastal grassland. It is probably displaced from *Kanuka/Manuka* forest by *V. vulgaris* competing for honeydew. Competitive exclusion in this habitat is in line with earlier studies from New Zealand's honeydew-producing southern beech (*Fuscospora* spp.) forests, which demonstrated that *V. vulgaris* outcompetes *V. germanica* for honeydew (Harris, 1991; Harris et al., 1994). The co-existence of both *Vespula* species at broader spatial scales suggests alterations to other aspects of their niche, preventing total displacement (Crowder & Snyder, 2010).

Diet and temporal partitioning

There was also evidence of both diet and temporal partitioning amongst the four wasp species. Seasonal changes in diet composition are likely due to seasonal differences in prey availability. For example, the second most consumed species in our study was Plantain moth *Scopula rubraria*, which are most abundant from summer to mid-autumn (Gerard, Philip, Ferguson, & Eden, 2017). We recorded twice as many occurrences of *S. rubraria* in late season samples compared to earlier in the season. We have interpreted a difference between nest layers/circles as a seasonal difference in diet. However, an alternative explanation is that differences between layer/circle may instead reflect differences in DNA degradation over time. Further research is needed to resolve this, but we have no reason to expect that DNA degradation would vary within taxa (such as Lepidoptera). Trophic partitioning has been demonstrated in a range of closely related sympatric species. For example, partitioning of food resources allowed invasive round gobies *Apollonia melanostoma* and bighead gobies *Neogobius kessleri* to coexist in the Danube river (Borza, Erős, & Oertel, 2009). Similarly, diet partitioning occurred in four sympatric Gomphocerinae grasshopper species in subalpine grassland in the French Alps (Ibanez et al., 2013). Future assessments on whether an introduced species will establish should consider the potential for resource partitioning with other species.

Polistes are known to feed predominantly on Lepidoptera (Kasper et al., 2004; Todd et al., 2015; Ward & Ramon-Laca, 2013), while *Vespula* also consume other invertebrates such as Diptera and Araneae (Harris & Oliver, 1993; Kasper et al., 2004). Our research confirms this, but we also show that the four wasp species partition within Lepidoptera; while both *Polistes* mainly fed on Geometridae, *V. germanica* and *V. vulgaris* mostly consumed Tortricidae. This contrasts with *Polistes* in New Zealand kiwifruit orchards, which collected more Noctuidae rather than Geometridae (Todd et al., 2015). We suggest that the diet of

generalist Vespids reflects differences in prey availability in different habitats and seasons, although foraging preferences likely play a role as well.

Prey richness and niche width differed considerably among wasp species. *V. germanica* had the most species rich diet and the widest trophic niche, while both *Polistes* species had a relatively narrow niche. Likewise, *V. germanica* in Australia had a wide niche that entirely included the comparatively narrow niche of *P. humilis* (Kasper et al., 2004). Theoretically, the species with an included niche must be a superior competitor for the shared resource on a per capita basis to coexist with its more generalist counterpart (Chase, 1996; Guevara & Avile's, 2011). Our findings are consistent with theoretical predictions about included niche and may at least partly explain why *V. germanica*, *P. humilis* and *P. chinensis* can coexist in the same habitat. Alternatively, our results could demonstrate that instead of being the stronger competitor, *P. humilis* avoids competition with *V. germanica* by separating spatially.

We observed the highest diet overlap between *V. vulgaris* and *V. germanica*, indicating stronger competition between these closely related species than between *Vespula* and *Polistes*. This supports Darwin's (1859) *competition-relatedness hypothesis*. In contrast, we found *P. chinensis* shared more prey species with *V. vulgaris* than with its congeneric *P. humilis*. In fact, other studies also show that phylogenetic relatedness does not always predict the nature and strength of competition among species (Venail et al., 2014; Alexandrou et al., 2015; Lyu, Liu, Venail, & Zhou, 2017), but rather that species interactions and resource overlap shape the assembly and evolution of communities (Wilcox, Schwartz, & Lowe, 2018).

Ecological impact

Coexistence of multiple generalist invaders is likely to intensify their ecological effect (Crowder & Snyder, 2010). The four wasp species in our study fed on a broad range of both herbivores (e.g. Lepidoptera) and predators (e.g. Araneae), potentially affecting the native community on multiple trophic levels. Furthermore, the majority of prey species were endemic or native to New Zealand. By partitioning resources, the four species in combination would impact a wide range of endemic species in the ecosystem. *Vespula vulgaris* are known to cause ecological damage to New Zealand's native ecosystems (Beggs et al., 2011), particularly to the Lepidopteran fauna (Beggs & Rees, 1999). We conclude that coexistence of *V. vulgaris* and three other vespid wasps probably amplifies their ecological impact because they partition resources spatially and trophically.

Conclusion

DNA metabarcoding of faecal material revealed novel insights into the nature and strength of trophic interactions among four sympatric social wasp species. We conclude that these invaders were avoiding competition with each other by shifting their ecological niche, partitioning resources to minimise overlap with their counterparts. There was stronger competition (highest diet overlap) between *V. germanica* and *V. vulgaris* than between other wasp species. Indeed, in one habitat we found evidence of competitive exclusion between the two *Vespula* species, probably because of competition for honeydew resources. In contrast, *P. chinensis* shared more species with *V. vulgaris* than with its congener *P. humilis*. We therefore provide further evidence that competitive interactions and resource overlap rather than taxonomic relatedness shape the structuring of ecological communities. We suggest coexistence of these generalist invaders on Ahuahu intensified their ecological effect, particularly on endemic Lepidoptera that comprised a large part of the diet of all wasp species. By providing a better understanding of resource partitioning by these four generalist

421 species, we have gained an improved assessment of the total ecological impact when there
422 are multiple invaders.

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Authors' Contributions

JMS and JRB designed the study. JMS collected wasp nests, performed laboratory analysis, analysed the data, prepared the figures and led the writing. SB provided the method. GL and CAG supported laboratory analysis. All authors contributed critically to the drafts and gave final approval for publication.

Data Availability Statement

Data available via the Dryad Digital Repository <https://doi:10.5061/dryad.r4xgxd2bq> (Schmack et al., 2021). .

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633 Table 1. Number of wasp nests of *P. chinensis*, *P. humilis*, *V. germanica*, and *V. vulgaris* collected from seven habitat types on Ahuahu off the
634 east coast of New Zealand. For each habitat type, we give its size, the proportion of the total island area it covers, the search effort spent finding
635 nests and its proportion of the total search effort for the whole island.

Habitat type	Size		Search effort		Number of nests per species			
	[ha]	[%]	[h]	[%]	<i>P. chinensis</i>	<i>P. humilis</i>	<i>V. germanica</i>	<i>V. vulgaris</i>
Coastal grassland	683	37.3	15	28.9	26	6	3	0
Residential garden	2	0.1	2	3.9	1	2	0	0
Clear cutting	5	0.3	2	3.9	3	0	0	0
Native restorations	38	2.1	4	7.7	7	0	0	0
<i>Kanuka/Manuka</i> forest	297	16.2	9	17.3	0	0	0	8
Pine plantation	772	42.2	18	34.6	0	0	0	8
Mature native forest	29	1.6	2	3.9	0	0	0	0

636 Table 2. Numbers and percentages of prey identified from 148 wasp faecal samples of four invasive vespid wasps co-occurring on Ahuahu off
637 the east coast of New Zealand: *Polistes chinensis* (Pc), *P. humilis* (Ph), *Vespula germanica* (Vg) and *V. vulgaris* (Vv). The most common taxa
638 for each order and the number of samples it was identified from is given, along with its status in New Zealand.

Order (# species)	Percentage of total diet				Number of prey taxa				Most common species (# samples it was recorded in)	NZ Status
	Pc	Ph	Vg	Vv	Pc	Ph	Vg	Vv		
Number of nests	24	8	3	16	24	8	3	16		
Number of samples	70	24	9	48	70	24	9	48		
Insecta										
Lepidoptera (45)	77	87.5	46.9	62	27	19	13	31	<i>Ctenopseustis obliquana</i> (72)	endemic
Hymenoptera (6)	2.8	7.2	3.1	5.2	5	3	2	6	<i>Meteorus pulchricornis</i> (22)	exotic
Diptera (13)	3.2	2	22.4	5.2	3	2	8	10	<i>Oxysarcodexia varia</i> (21)	exotic
Hemiptera (8)	7.3	0	9.2	11.5	6	0	2	4	<i>Kikihia ochrina</i> (43)	endemic
Orthoptera (3)	0	0	8.2	0.2	0	0	2	1	<i>Hemiandrus pallitarsis</i> (6)	endemic
Coleoptera (2)	0.6	0	0	0.2	1	0	0	1	<i>Eucolaspis sp. PRCD2015c</i> (2)	endemic
Neuroptera (2)	0	0	0	2.5	0	0	0	2	<i>Wesmaelius subnebulosus</i> (6)	exotic
Psocodea (2)	1.3	0	1	0	2	0	1	0	<i>Liposcelis paeta</i> (4)	exotic
Annelida										
Haplotaxida (1)	0.3	0	0	0.2	1	0	0	1	<i>Enchytraeus buchholzi</i> (2)	exotic
Mollusc										
Stylommatophora (1)	0	0	0	0.2	0	0	0	1	<i>Athoracophorus bitentaculatus</i> (1)	endemic
Octopoda (1)	0	0	1	0	0	0	1	0	<i>Argonauta nodosa</i> (1)	native

Arachnida

Araneae (12)	1.6	0	5.1	12.6	2	0	3	12	<i>Eriophora pustulosa</i> (28)	native
Mesostigmata (2)	0	2	0	0	0	2	0	0	<i>Amblyseius sp. JCS08</i> (2)	exotic
Opliliones (1)	0.3	0	0	0	1	0	0	0	<i>Phalangium opilio</i> (1)	exotic

Vertebrata

Rodentia (1)	5.7	1.3	0	0.2	1	1	0	1	<i>Mus musculus</i> (21)	exotic
Charadriiformes (1)	0	0	3.1	0	0	0	1	0	<i>Chroicocephalus novaehollandiae</i> (3)	native

639 Table 3. Analysis of deviance tables for differences in diet among four invasive wasp species
640 (*P. chinensis*, *P. humilis*, *V. germanica* and *V. vulgaris*); seasonal samples across all wasp
641 species and seasonal samples of each wasp species are given. Differences were calculated
642 among all prey and within the main prey order Lepidoptera.

	Prey composition		Lepidopteran prey composition	
	LRT	p	LRT	p
Wasp species	1508.5	0.001***	1084.9	0.001***
<i>P. humilis</i>	125.41	0.001***	89.88	0.001***
<i>V. germanica</i>	189.03	0.001***	75.96	0.001***
<i>V. vulgaris</i>	306.43	0.001***	219.79	0.001***
Time of season	424.0	0.002**	224.6	0.039*
<i>Mid-season</i>	75.71	0.006**	43.81	0.002**
<i>Late season</i>	97.29	0.002**	69.81	0.001***
Wasp species x Time of season	224.1	0.003**	164.9	0.001***
<i>P. humilis x mid season</i>	41.93	0.007**	32.97	0.003**
<i>V. germanica x mid season</i>	28.90	0.006**	13.45	0.032*
<i>V. vulgaris x mid season</i>	31.82	0.008**	22.07	0.005**
<i>P. humilis x late season</i>	26.17	0.004**	21.97	0.003**
<i>V. germanica x late season</i>	26.47	0.003**	9.00	0.127
<i>V. vulgaris x late season</i>	29.91	0.008**	19.21	0.003**

643
644

645 Table 4. Diet indices of *P. chinensis*, *P. humilis*, *V. germanica* and *V. vulgaris* based on DNA
646 metabarcoding of faecal samples collected from nests on Ahuahu, New Zealand. Given are
647 the mean prey species richness and Levin's index of trophic niche width. This was calculated
648 for all prey taxa and for only Lepidopteran prey taxa.

Species	Prey richness ($\pm$ SE)	Niche width	Lepidopteran prey richness ($\pm$ SE)	Lepidopteran niche width
<i>P. chinensis</i>	4.73 $\pm$ 0.05	9.54	3.64 $\pm$ 0.04	6.02
<i>P. humilis</i>	6.33 $\pm$ 0.16	11.13	5.54 $\pm$ 0.15	8.48
<i>V. germanica</i>	10.89 $\pm$ 0.76	16.56	5.11 $\pm$ 0.38	5.94
<i>V. vulgaris</i>	9.27 $\pm$ 0.08	9.32	5.75 $\pm$ 0.06	3.53

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Figure 1. Location of nests of four invasive wasp species (*Polistes chinensis*, *P. humilis*, *Vespula vulgaris* and *V. germanica*) systematically searched for on Ahuahu, located 9 km off the north-east coast of New Zealand. The pale cream colour denotes sand/gravel, a habitat that was not included in this study.

Figure 2. Rarefaction curves of prey taxa identified in faecal samples collected from vespid nests on Ahuahu, New Zealand.

Figure 3. Diet composition (% prey taxa detected) of four invasive vespid species inhabiting Ahuahu, New Zealand. The Lepidopteran families detected are also shown. Pie charts show the percentages of endemic, native and exotic Lepidoptera taxa.

Figure 4. Boxplot of mean prey richness per sample identified in faecal samples from *P. chinensis* (n=70), *P. humilis* (n=24), *V. germanica* (n=9) and *V. vulgaris* (n=48). Presented are 25th and 75th percentiles (lower and upper box boundaries), median (line inside box) and 10th and 90th percentiles (lower and upper error lines).

668 Figure 5. Network diagram showing the diet overlap between four invasive wasp species
 669 coexisting on Ahuahu. Presented are the nature (denoted by grey bars) and strength (denoted
 670 by thickness) of predation on 105 prey species identified from faecal samples. Note that
 671 strength of predation is proportional to sample size. Photos of wasps courtesy of Bryce
 672 McQuillan.

Figure 6. Network diagram showing the predation of endemic Lepidopteran species by four invasive wasp species coexisting on Ahuahu. Presented are the nature (denoted by grey bars) and strength (denoted by bar thickness) of predation on 18 endemic Lepidoptera identified from faecal samples. Note that strength of predation is proportional to sample size. Photos of wasps and Lepidoptera courtesy of Bryce McQuillan and Olivier Ball, respectively.