STABLE ISOTOPE INSIGHTS INTO THE TROPHIC INTERACTIONS OF INVASIVE ANTS: ARE SUGAR AND SHARING PROMOTING ABUNDANCE?

A Dissertation

by

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ABSTRACT

The complex interplay between trophic interactions, species abundance, and competition helps explain the distribution of a species and can have important evolutionary consequences. Stable isotope analysis is one of the leading tools that ecologists use to quantify trophic interactions. Although authors commonly estimate trophic position from stable isotopes, there is incredible variation in the baselines and methodologies used in the literature. I conducted a literature review to determine the causes and consequences of this variation. Baseline and methodology significantly impacted the trophic position estimates of individual species, as well as conclusions about food web structure. Increased sample size may mitigate some of the variation caused by different baselines and methodologies, but an alarmingly large proportion of studies collected only one sample or did not report how many they collected at all, highlighting a critical need to increase stable isotope sample size in trophic ecology research. Next, I used stable isotope analysis and direct observation to determine the relationship between trophic position and abundance of the invasive tawny crazy ant (*Nylanderia fulva*). Classical food web theory predicts that individuals at the base of the food web will be more abundant than those at the top, but I found that tawny crazy ant densities were highest when ants were more predaceous. Rather than feeding as herbivores, tawny crazy ants were actually highly omnivorous. Another factor that may promote the spread and establishment of many invasive ant species is their proclivity towards intraspecific cooperation. To quantify cooperation between colonies in the field,

I developed methods to label food using stable isotope tracers. I then applied these methods to examine inter- and intracolonial cooperation using the red imported fire ant (*Solenopsis invicta*; hereafter fire ant). My results suggest that multiple-queen fire ant colonies are not cooperating and may engage in high levels of intraspecific competition with neighboring colonies and may exhibit low levels of nepotism within the colony. My dissertation helps develop best practices for stable isotope analysis, which is a critical tool in ecological research. It also tests fundamental questions about food web structure and kin selection theory.

DEDICATION

To the ants and other insects that drove me to start this crazy journey in the first place.

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NOMENCLATURE

TDF	The trophic discrimination factor, or the shift in the proportion of the heavier to the lighter isotope from one trophic level to the next
DX90	The number of days in the month with a maximum temperature exceeding or equal to 32.2˚C
DP10	The number of days in the month with greater than or equal to 25.4mm of precipitation

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1. INTRODUCTION

1.1. Trophic interactions, competition, and species abundance

Trophic interactions describe who eats whom in an ecological community, and they are a major force determining community structure and influencing ecosystem processes (Winemiller and Polis 1996, Holt and Loreau 2002). For example, selective grazing by large ungulate herbivores can result in a greater abundance of less palatable, chemically-defended plant species (Augustine and McNaughton 1998), which can in turn impact soil animal diversity and belowground processes such as decomposition (Bardgett et al. 1998, Stark et al. 2000). One way that trophic interactions shape community structure is by influencing the abundance of a species through space and time. Elton (1927) was one of the first ecologists to formally link trophic interactions and species abundance with his 'pyramid of numbers' concept, by which the number of individuals representing each trophic level tends to decrease moving up the food chain (i.e., autotrophs are more abundant than herbivores which are more abundant than predators, etc.). Lindeman (1942) explained this decrease in abundance by estimating that only ~10% of energy can be transferred from one trophic level to the next, so the biomass of higher order consumers is unavoidably constrained compared with organisms in lower trophic levels. The 'pyramid of numbers' holds true for many empirical food webs, but others, especially aquatic food webs, show a more 'inverted pyramid' structure, in which there are higher numbers of heterotrophs than autotrophs at the base (Del Giorgio et al. 1999, Snyder and Evans 2006, Hall 2011, McCauley et al. 2018,

Woodson et al. 2018). The reasons for this inversion are context-dependent, but include higher turnover rates in short-lived autotrophs in aquatic systems (Cebrian 1999, Del Giorgio et al. 1999) and moderate levels of omnivory (McCauley et al. 2018). Understanding the relationship between trophic interactions and abundance can provide important insights into community structure and function.

Another factor that impacts trophic interactions and constrains species abundance is competition (Connell 1961, Jeffries and Lawton 1984, Denno et al. 1995, Davis et al. 1998, Cunningham et al. 2009). For example, the combined effects of inter- and intraspecific competition on a shared host plant reduced the survivorship of two planthopper species by nearly 66% (Denno et al. 2000). Competition is typically density-dependent, which means that competition strengthens as population size increases and shared resources become limiting (McClure 1980, Denno et al. 1995, Hunter et al. 1997, Denno et al. 2000, Ohgushi 2005). Organisms employ a variety of strategies to avoid the negative effects of competition, including dispersal to an environment with fewer competitors (Branch 1975, Vasconcellos-Neto and Monteiro 1993, Denno et al. 2000), territoriality to prevent potentially deadly encounters (Branch 1975, Cunningham et al. 2009), and resource partitioning (MacArthur and MacArthur 1961, Schoener 1974).

The complex interplay between trophic interactions, species abundance, and competition helps explain the distribution of a species (Davis et al. 1998, Cunningham et al. 2009), and it can also have important evolutionary consequences. Strong competition between two species in overlapping ranges can cause character displacement, in which traits differ between the species where they co-occur (sympatric range) but are similar

where they occur separately (allopatric range) (Brown and Wilson 1956). For example, the beak size of two Darwin's finch species diverged dramatically over just 22 years due to intense competition over a shared resource (Grant and Grant 2006). This divergence due to competition can increase speciation (Pritchard and Schluter 2001) and underlies many ecological predictions about biotic diversity (Hutchinson 1959). Determining the interactions between diet, competition, and abundance of a species can, therefore, provide important insights into community structure and the evolution of a species.

1.2. Invasive ants

Because of the success and prevalence of invasive ants, studying them can yield important insights into the relationships between abundance, trophic interactions, and competition. Invasive ants can have drastic effects in their introduced range, often displacing native ants and lowering overall biodiversity (Porter and Savignano 1990, Holway 1998, McGlynn 1999, Holway et al. 2002, Berman et al. 2013, LeBrun et al. 2013). Argentine ants (*Linepithema humile*), for example, reduced native ant diversity from 23 species to only two in just eight years in parts of California (Holway 1999). Initial invasion of the red imported fire ant (*Solenopsis invicta*; hereafter fire ant) in Texas reduced biodiversity by approximately 70% (Porter and Savignano 1990). Their negative impacts may still persist in some habitats after more than 20 years (LeBrun et al. 2012). The successful establishment of an invasive ant species is largely impacted by their trophic interactions (Holway et al. 2002). Many invasive ants are omnivorous, feeding on a broad variety of arthropods and often supplementing their diet with

carbohydrates such as nectar or honeydew (Holway et al. 2002, Tillberg et al. 2007, Wilder et al. 2011b, Abbott et al. 2014). Moreover, competition is an important factor influencing the abundance and distribution of ant species throughout a habitat (Cerda et al. 2013).

Ants commonly form mutualisms with honeydew-producing insects in nature (Way 1963, Helms 2013). In exchange for protection from natural enemies and/or competitors, honeydew-producing insects provide ants with honeydew: a carbohydrate-rich excretion. Honeydew contains high concentrations of sucrose, glucose, and fructose, with low levels of amino acids. Access to additional carbohydrates in the form of honeydew or extrafloral plant nectar leads to an increase in colony growth, worker survival, and foraging activity in many ant species (Davidson 1997, Abbott 2005, Wetterer et al. 2006, Wilder et al. 2011a, Helms 2013). For example, *S. invicta* colonies with access to aphid honeydew produced 50% more workers than colonies without access to aphid honeydew (Wilder et al. 2011a). Ant colonies that feed on carbohydrate-rich resources are also more active foragers and often find food items more quickly than ant species that do not feed on carbohydrate-rich resources (Oster and Wilson 1978, Davidson 1997). Due to the benefits of honeydew consumption, ant mutualisms with honeydew-producing insects may aid in invasive ant spread (Holway et al. 2002, Tillberg et al. 2007, Savage et al. 2011, Wilder et al. 2011b, Helms 2013). In fact, many invasive ant species tend honeydew-producing insects in their invaded range (Benois et al. 1973, Aldana et al. 1995, Davidson 1997, Holway et al. 2002, Menke et al. 2010), and they often more aggressively dominate these resources than colonies in their native

range (Tillberg et al. 2007, Wilder et al. 2011b). For example, colonies of *S. invicta* in the United States dominated over 75% of observed hemipteran aggregations compared with only 2% in their native Argentina (Wilder et al. 2011b). Ant colonies that tend honeydew-producing insects often defend these insects from natural enemies and/or potential competitors, resulting in top-down effects that can influence community composition (Styrsky and Eubanks 2007, 2010). For example, cotton plants with fire ant-tended aphids produced 16% more bolls and 25% more seeds than cotton plants without aphids due to fire ant removal of caterpillars on cotton plants with aphids (Styrsky and Eubanks 2010). Because of their broad diet breadth, invasive ants can have an even larger influence on their invaded community.

Another factor that may promote the spread and establishment of many invasive ant species is their proclivity towards intraspecific cooperation (Holway et al. 2002, Krushelnycky et al. 2010). Many invasive ant species are unicolonial, meaning that colonies show reduced intraspecific aggression and an absence of boundaries between nests (Helanterä et al. 2009). By cooperating instead of competing with conspecifics, unicolonial ants avoid the costs of intraspecific competition, which allows them to reach higher densities (Porter et al. 1992, Giraud et al. 2002) and achieve greater ecological dominance by more effectively outcompeting other species (Holway et al. 2002, Holway and Suarez 2004, LeBrun et al. 2013). For example, when experimental colonies of the unicolonial Argentine ant cooperated instead of competed, they reduced the fecundity and survival in native ant colonies of *Forelius mccooki* by over 80% and 87% respectively (Holway and Suarez 2004). Additionally, the lack of boundaries within a

unicolonial population allows nests to easily relocate throughout the habitat depending on their needs and availability of resources (Holway and Case 2000, Elias et al. 2005, Heller and Gordon 2006, Krushelnycky et al. 2010). Supercolonies of the Argentine ant, for example, often move nests to locations near newly available food resources (Holway and Case 2000), causing approximately 96% of all nests to be abandoned after one to four months (Heller and Gordon 2006). By contrast, multicolonial species that compete with conspecifics likely need to usurp another colony in order to move locations within an environment (Krushelnycky et al. 2010). The benefits of unicoloniality may explain why many (but not all i.e., Eyer et al. 2020) invasive ant species are unicolonial (Passera 1994, Holway et al. 2002).

1.3. Quantifying trophic interactions using stable isotope analysis

Despite the importance of trophic interactions, they can be difficult to assess, particularly in difficult-to-observe organisms such as ants. Stable isotope analysis is one of the leading tools that ecologists use to quantify trophic interactions (Fry 2006). According to a *Web of Science* search in March 2020, stable isotope analysis has been used in over 5,486 published studies of trophic position between 1988-2019. Stable isotope analysis employs naturally occurring, stable (non-radioactive) forms of biologically relevant elements, such as nitrogen and carbon. The heavier isotopes of nitrogen and carbon (^{15}N and ^{13}C) occur rarely in nature and accumulate in consumer tissues based on the resources that they consume. Due to a stepwise increase in the ratio of heavy to light nitrogen isotopes (^{15}N:^{14}N, expressed as $\delta^{15}N$) from one trophic level to

the next, $\delta^{15}N$ values provide an estimate of a consumer's trophic position, or the position that an organism occupies in the food chain (DeNiro and Epstein 1981, Minagawa and Wada 1984). This method has been used extensively to estimate ant trophic position (Fiedler et al. 2007, Feldhaar et al. 2010, Gibb and Cunningham 2011, Resasco et al. 2012, Zhang et al. 2015, Hanna et al. 2017, Iakovlev et al. 2017) and compare invasion-associated changes in ant diet between native and introduced ranges (Tillberg et al. 2007, Wilder et al. 2011b). In general, consumers are enriched in the heavier isotope (have higher $\delta^{15}N$) relative to their food resource, and lower $\delta^{15}N$ values typically correspond to a more herbivorous diet (i.e., increased consumption of carbohydrate resources).

Although stable isotope analysis is commonly employed by ecologists to estimate trophic position, there is remarkable variation in the models, techniques, and baselines used to estimate trophic position from stable isotope data. All estimates of trophic position from stable isotope data require some kind of reference point to be meaningful. One reason is because the inherent levels of isotopes vary strongly over space and time, and these variations are naturally reflected in the isotope values of the animals that live in these environments (Rounick and Winterbourn 1986, Zohary et al. 1994, Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999, Post 2002). This makes it difficult to directly compare consumer isotope values across locations, as differences in values could be due to diet or inherent variation in isotopes in the environment. By estimating trophic position relative to material collected from the same environment at approximately the same time (an isotope "baseline"), stable isotope

values become biologically meaningful (Cabana and Rasmussen 1994, Vander Zanden and Rasmussen 1999, Ponsard and Arditi 2000, Post 2002). Trophic position can be estimated relative to this baseline material using a specific formula (Vander Zanden et al. 1999, Post 2002, Hussey et al. 2014) or by statistical comparison (Croteau et al. 2005, Da Silva et al. 2005, Hebert et al. 2009, Kohzu et al. 2009, Reum and Marshall 2013, Starrfelt et al. 2013, Hette-Tronquart et al. 2018, Quezada-Romegialli et al. 2018). Despite the importance of an appropriate baseline material and formula/ statistical analysis to accurately estimate trophic position from stable isotope data, there is widespread variation in what is used in the literature. The consequences of this variation have not been explored, but could dramatically alter conclusions about a species' trophic interactions. In this study, I evaluate the causes and consequences of this variation for stable isotope research as a first step in developing best-practices for this important ecological tool.

It is also possible to use stable isotopes as labels to directly measure nutrient fluxes between organisms. By artificially 'spiking' a food with an appropriate concentration of a heavy isotope, ecologists can trace the movement of this isotope through consumers and identify the flow of nutrients through an ecosystem (Fry 2006).Grabmaier et al. (2014), for example, used isotopically spiked earthworms to quantify the flow of nitrogen between earthworms, plants, and aphids on the plants. When an isotope label is detected in an organism that was not fed the labeled resource, this serves as direct evidence of nutrient exchange (Feldhaar et al. 2010). After feeding a nitrogen tracer to ant colonies of *Pheidole bicornis*, Fischer et al. (2003) found highly

enriched values in the myrmecophytic *Piper* plants that housed the colonies, indicating nutrient exchange between ant colonies and their host plant mutualists. The use of isotope tracers could be used to quantify resources sharing between ant nests, particularly in the case of unicolonial ant species, which are thought to share indiscriminately with each other. Although the methods for isotope tracers are relatively well described in other taxa (Mulholland et al. 2000, Van den Meersche et al. 2004, Dyckmans et al. 2005, Fry 2006), this technique has rarely been applied to ants (Fischer et al. 2003, Defossez et al. 2010, Wagner and Fleur Nicklen 2010), and never to large colonies of ground-nesting species. Myrmecologists have used stable isotope analysis of natural abundance values of ^{15}N and ^{13}C to characterize colony trophic position and describe food webs (Blüthgen et al. 2003, Tillberg et al. 2007, Feldhaar et al. 2010, Wilder et al. 2011b), but isotope tracers allow for a more direct measurement of nutrient fluxes between organisms. In this study, I develop methods that can be applied to examine resource sharing between large, ground-nesting ant species in the field.

1.4. Study organisms

Two of the most invasive ants in Texas are the tawny crazy ant (*Nylanderia fulva*) and the red imported fire ant (*S. invicta*). The tawny crazy ant is an invasive species native to Argentina and Brazil that has caused considerable damage to native fauna in its introduced range (Zenner-Polania 1994, Aldana et al. 1995, LeBrun et al. 2013, Wetterer et al. 2014, Lester and Gruber 2016). For example, initial invasion of the tawny crazy ant reduced arthropod species richness by approximately 74% at a nature

reserve in Colombia (Aldana et al. 1995). Tawny crazy ants form a single supercolony throughout their invaded range in North America, in which workers regularly share collected resources with each other and occupy transitory nests (Wang et al. 2016, Eyer et al. 2018). The tawny crazy ant has become a major pest in most of the Gulf Coast states, with ant worker numbers that often exceed the combined biomass of all other ants by nearly two-fold (LeBrun et al. 2013). At high abundance, tawny crazy ants displace the previously dominant red imported fire ant and reduce arthropod diversity in grassland ecosystems (LeBrun et al. 2013). Tawny crazy ants regularly undergo local population explosions and crashes (Wetterer et al. 2014), which may be due to changes in the availability of plant- and insect-produced honeydew. Tawny crazy ants have been seen tending honeydew-producing insects (Zenner-Polania 1990, Aldana et al. 1995, Sharma et al. 2013), and worker activity is reduced on plants where honeydew-producing hemipterans have been removed (Sharma et al. 2019). However, the relationship between tawny crazy ant diet and abundance in the field has not been examined, and may have important implications for tawny crazy ant control. I examine the relationship between tawny crazy ant trophic position, densities, and worker foraging activity. Based on past research, I expect that tawny crazy ant densities will be highest and foraging will be most active when colonies have a more herbivorous diet (i.e., a low trophic position).

Fire ants are another invasive pest in many parts of the world (CABI 2019), with negative economic and ecological impacts (Porter and Savignano 1990, Tschinkel 2006, LeBrun et al. 2012). Fire ants occur as two social forms: the polygyne form (i.e.,

colonies with multiple egg-laying queens) and the monogyne form (i.e., colonies with only a single egg-laying queen; Ross 1993, Ross et al. 1996, Tschinkel 2006, Gotzek et al. 2007). The monogyne form is highly aggressive towards conspecifics (Vander Meer et al. 1990) and defends distinct colony boundaries (Tschinkel et al. 1995). In contrast, introduced polygyne colonies show reduced intraspecific aggression (Morel et al. 1990, Vander Meer et al. 1990) and are thought to inhabit large areas of interconnected nests (Bhatkar and Vinson 1987). For these reasons, introduced polygyne colonies of this species are often referred to as unicolonial (e.g., (Morel et al. 1990, Vander Meer et al. 1990, Greenberg et al. 1992, Porter et al. 1992, Holway et al. 2002, Plowes et al. 2007). There is evidence, however, that boundaries exist at least on some level between neighboring nests of introduced polygyne fire ants (Weeks et al. 2004, Goodisman et al. 2007, Krushelnycky et al. 2010), suggesting that polygyne fire ants are not unicolonial in their invaded range. Determining the extent of boundaries between nests of polygyne fire ants tests fundamental questions in kin selection theory and has important implications for their management and overall impacts on invaded communities. Unicolonial species often have a much greater ecological impact than their multicolonial counterparts (Helanterä et al. 2009, Krushelnycky et al. 2010), and it is thought that polygyne fire ants may have stronger impacts on invaded communities than monogyne fire ants (Porter and Savignano 1990). If polygyne colonies are competing with each other, however, their effects on invaded communities may be weaker than expected. I use a stable isotope tracer to label a carbohydrate resource and quantify resource sharing between fire ant colonies of both social forms in the field. I also examine within-colony sharing

between workers and nestmate or non-nestmate brood in polygyne colonies in the laboratory. If polygyne fire ants are truly unicolonial, they should freely exchange the labeled resource between colonies in the field. Moreover, workers should share indiscriminately with brood, regardless of kinship (i.e., nestmate or non-nestmate).

1.5. Objectives

In this study, I apply stable isotope analysis to examine the relationship between trophic interactions, abundance, and competition using invasive ants with four primary objectives:

1. Determine the causes and consequences of the variation in baselines and methodologies used by ecologists to estimate trophic position from stable isotope data.
2. Examine the relationship between tawny crazy ant trophic position, density, and foraging activity.
3. Develop methods for use of a stable isotope tracer in large, ground-nesting ant colonies.
4. Correlate kinship with between- and within-colony sharing in the red imported fire ant.

1.6. References

Abbott, K. L. 2005. Supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on an oceanic island: forager activity patterns, density and biomass. Insectes Sociaux **52**:266-273.

Abbott, K. L., P. T. Green, and D. J. O'Dowd. 2014. Seasonal shifts in macronutrient preferences in supercolonies of the invasive Yellow Crazy Ant *Anoplolepis gracilipes* (Smith, 1857) (Hymenoptera: Formicidae) on Christmas Island, Indian Ocean. Austral Entomology **53**:337-346.

Aldana, R. C., M. L. Baena, and P. Chacón de Ulloa. 1995. Introducción de la hormiga loca (*Paratrechina fulva*) a la Reserva Natural Laguna de Sonso (Valle del Cauca, Colombia). Boletin del Museo de Entomología de la Universidad del Valle **3**:15-28.

Augustine, D. J., and S. J. McNaughton. 1998. Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. The Journal of Wildlife Management **62**:1165-1183.

Bardgett, R., D. Wardle, and G. Yeates. 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biology & Biochemistry **30**:1867-1878.

Benois, A., J. Lafont, and J. Marro. 1973. Incidence des facteurs écologiques sur le cycle annuel et l'activité saisonnière de la fourmi d'Argentine, *Iridomyrmex humilis* Mayr (Hymenoptera, Formicidae), dans la région d'Antibes. Insectes Sociaux **20**:267-295.

Berman, M., A. Andersen, C. Hély, and C. Gaucherel. 2013. Overview of the distribution, habitat association and impact of exotic ants on native ant communities in New Caledonia. PLoS One **8**:e67245.

Bhatkar, A., and S. Vinson. 1987. Colony limits in *Solenopsis invicta* Buren. Pages 599-600 *in* J. Eder and H. Rembold, editors. Chemistry and biology of social insects. Munich: Verlag J. Paperny.

Blüthgen, N., G. Gebauer, and K. Fiedler. 2003. Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. Oecologia **137**:426-435.

Branch, G. 1975. Mechanisms reducing intraspecific competition in *Patella spp*.: migration, differentiation and territorial behaviour. The Journal of Animal Ecology **44**:575-600.

Brown, W. L., and E. O. Wilson. 1956. Character displacement. Systematic zoology **5**:49-64.

Cabana, G., and J. B. Rasmussen. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature **372**:255.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences **93**:10844-10847.

CABI. 2019. *Solenopsis invicta* (red imported fire ant). In: Invasive Species Compendium. Wallingford, UK: CAB Inernational.

Cebrian, J. 1999. Patterns in the fate of production in plant communities. The American Naturalist **154**:449-468.

Cerda, X., X. Arnan, and J. Retana. 2013. Is competition a significant hallmark of ant (Hymenoptera: Formicidae) ecology. Myrmecological News **18**:131-147.

Connell, J. H. 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology **42**:710-723.

Croteau, M. N., S. N. Luoma, and A. R. Stewart. 2005. Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnology and Oceanography **50**:1511-1519.

Cunningham, H. R., L. J. Rissler, and J. J. Apodaca. 2009. Competition at the range boundary in the slimy salamander: using reciprocal transplants for studies on the role of biotic interactions in spatial distributions. Journal of Animal Ecology **78**:52-62.

Da Silva, D. S., M. Lucotte, M. Roulet, H. Poirier, D. Mergler, E. O. Santos, and M. Crossa. 2005. Trophic structure and bioaccumulation of mercury in fish of three natural lakes of the Brazilian Amazon. Water, Air, and Soil Pollution **165**:77-94.

Davidson, D. W. 1997. The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. Biological Journal of the Linnean Society **61**:153-181.

Davis, A. J., L. S. Jenkinson, J. H. Lawton, B. Shorrocks, and S. Wood. 1998. Making mistakes when predicting shifts in species range in response to global warming. Nature **391**:783-786.

Defossez, E., C. Djiéto-Lordon, D. McKey, M.-A. Selosse, and R. Blatrix. 2010. Plant-ants feed their host plant, but above all a fungal symbiont to recycle nitrogen. Proceedings of the Royal Society B: Biological Sciences **278**:1419-1426.

Del Giorgio, P. A., J. J. Cole, N. F. Caraco, and R. H. Peters. 1999. Linking planktonic biomass and metabolism to net gas fluxes in northern temperate lakes. Ecology **80**:1422-1431.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta **45**:341-351.

Denno, R. F., M. S. McClure, and J. R. Ott. 1995. Interspecific interactions in phytophagous insects: competition reexamined and resurrected. Annual Review of Entomology **40**:297-331.

Denno, R. F., M. A. Peterson, C. Gratton, J. Cheng, G. A. Langellotto, A. F. Huberty, and D. L. Finke. 2000. Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. Ecology **81**:1814-1827.

Dyckmans, J., C. M. Scrimgeour, and O. Schmidt. 2005. A simple and rapid method for labelling earthworms with ^{15}N and ^{13}C. Soil Biology and Biochemistry **37**:989-993.

Elias, M., R. Rosengren, and L. Sundström. 2005. Seasonal polydomy and unicoloniality in a polygynous population of the red wood ant *Formica truncorum*. Behavioral Ecology and Sociobiology **57**:339-349.

Elton, C. 1927. Animal ecology. The Macmillan Company, New York.

Eyer, P.-A., B. McDowell, L. N. Johnson, L. A. Calcaterra, M. B. Fernandez, D. Shoemaker, R. T. Puckett, and E. L. Vargo. 2018. Supercolonial structure of invasive populations of the tawny crazy ant *Nylanderia fulva* in the US. BMC Evolutionary Biology **18**:209.

Eyer, P. A., E. M. Espinoza, A. J. Blumenfeld, and E. L. Vargo. 2020. The underdog invader: Breeding system and colony genetic structure of the dark rover ant (*Brachymyrmex patagonicus* Mayr). Ecology and Evolution **10**:493-505.

Feldhaar, H., G. Gebauer, and N. Blüthgen. 2010. Stable isotopes: past and future in exposing secrets of ant nutrition (Hymenoptera: Formicidae). Myrmecological News **13**:3-13.

Fiedler, K., F. Kuhlmann, B. Schlick-Steiner, F. Steiner, and G. Gebauer. 2007. Stable N-isotope signatures of central European ants–assessing positions in a trophic gradient. Insectes Sociaux **54**:393-402.

Fischer, R. C., W. Wanek, A. Richter, and V. Mayer. 2003. Do ants feed plants? A ^{15}N labelling study of nitrogen fluxes from ants to plants in the mutualism of *Pheidole* and *Piper*. Journal of Ecology **91**:126-134.

Fry, B. 2006. Stable isotope ecology. Springer.

Gibb, H., and S. A. Cunningham. 2011. Habitat contrasts reveal a shift in the trophic position of ant assemblages. Journal of Animal Ecology **80**:119-127.

Giraud, T., J. S. Pedersen, and L. Keller. 2002. Evolution of supercolonies: the Argentine ants of southern Europe. Proceedings of the National Academy of Sciences **99**:6075-6079.

Goodisman, M. A., K. A. Sankovich, and J. L. Kovacs. 2007. Genetic and morphological variation over space and time in the invasive fire ant *Solenopsis invicta*. Biological Invasions **9**:571-584.

Gotzek, D., D. D. Shoemaker, and K. G. Ross. 2007. Molecular variation at a candidate gene implicated in the regulation of fire ant social behavior. PLoS One **2**:e1088.

Grabmaier, A., F. Heigl, N. Eisenhauer, M. G. van der Heijden, and J. G. Zaller. 2014. Stable isotope labelling of earthworms can help deciphering belowground–aboveground interactions involving earthworms, mycorrhizal fungi, plants and aphids. Pedobiologia **57**:197-203.

Grant, P. R., and R. B. Grant. 2006. Evolution of character displacement in Darwin's finches. Science **313**:224-226.

Greenberg, L., S. Vinson, and S. Ellison. 1992. Nine-year study of a field containing both monogyne and polygyne red imported fire ants (Hymenoptera: Formicidae). Annals of the Entomological Society of America **85**:686-695.

Hall, R. J. 2011. Eating the competition speeds up invasions. Biology Letters:rsbl20100766.

Hanna, C., I. Naughton, C. Boser, and D. A. Holway. 2017. Aphid-tending ants on introduced fennel: can resources derived from non-native plants alter the trophic position of higher-order consumers? Ecological Entomology **42**:61-66.

Hebert, C. E., D. C. Weseloh, L. T. Gauthier, M. T. Arts, and R. J. Letcher. 2009. Biochemical tracers reveal intra-specific differences in the food webs utilized by individual seabirds. Oecologia **160**:15-23.

Helanterä, H., J. E. Strassmann, J. Carrillo, and D. C. Queller. 2009. Unicolonial ants: where do they come from, what are they and where are they going? Trends in Ecology & Evolution **24**:341-349.

Heller, N. E., and D. M. Gordon. 2006. Seasonal spatial dynamics and causes of nest movement in colonies of the invasive Argentine ant (*Linepithema humile*). Ecological Entomology **31**:499-510.

Helms, K. R. 2013. Mutualisms between ants (Hymenoptera: Formicidae) and honeydew-producing insects: Are they important in ant invasions. Myrmecological News **18**:61-71.

Hette-Tronquart, N., T. Oberdorff, E. Tales, A. Zahm, and J. Belliard. 2018. Biological impacts of local vs. regional land use on a small tributary of the Seine River (France): insights from a food web approach based on stable isotopes. Environmental Science and Pollution Research **25**:23583-23594.

Holt, R. D., and M. Loreau. 2002. Biodiversity and ecosystem functioning: the role of trophic interactions and the importance of system openness. Pages 246-262 *in* A. Kinzig, S. Pacala, and D. Tilman, editors. The functional consequences of biodiversity: empirical progress and theoretical extensions. Princeton University Press.

Holway, D. A. 1998. Effect of Argentine ant invasions on ground-dwelling arthropods in northern California riparian woodlands. Oecologia **116**:252-258.

Holway, D. A. 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. Ecology **80**:238-251.

Holway, D. A., and T. J. Case. 2000. Mechanisms of dispersed central-place foraging in polydomous colonies of the Argentine ant. Animal Behaviour **59**:433-441.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, and T. J. Case. 2002. The causes and consequences of ant invasions. Annual Review of Ecology and Systematics **33**:181-233.

Holway, D. A., and A. V. Suarez. 2004. Colony-structure variation and interspecific competitive ability in the invasive Argentine ant. Oecologia **138**:216-222.

Hunter, M. D., G. C. Varley, and G. R. Gradwell. 1997. Estimating the relative roles of top-down and bottom-up forces on insect herbivore populations: a classic study revisited. Proceedings of the National Academy of Sciences **94**:9176-9181.

Hussey, N. E., M. A. MacNeil, B. C. McMeans, J. A. Olin, S. F. Dudley, G. Cliff, S. P. Wintner, S. T. Fennessy, and A. T. Fisk. 2014. Rescaling the trophic structure of marine food webs. Ecology Letters **17**:239-250.

Hutchinson, G. E. 1959. Homage to Santa Rosalia or why are there so many kinds of animals? The American Naturalist **93**:145-159.

Iakovlev, I., T. Novgorodova, A. Tiunov, and Z. Reznikova. 2017. Trophic position and seasonal changes in the diet of the red wood ant *Formica aquiloniaas* indicated by stable isotope analysis. Ecological Entomology **42**:263-272.

Jeffries, M., and J. Lawton. 1984. Enemy free space and the structure of ecological communities. Biological Journal of the Linnean Society **23**:269-286.

Kohzu, A., T. Iwata, M. Kato, J. Nishikawa, E. Wada, N. Amartuvshin, B. Namkhaidorj, and N. Fujita. 2009. Food webs in Mongolian grasslands: the analysis of ^{13}C and

^{15}N natural abundances. Isotopes in Environmental and Health Studies **45**:208-219.

Krushelnycky, P. D., D. A. Holway, and E. G. LeBrun. 2010. Invasion processes and causes of success. Pages 245-260 *in* L. Lach, C. L. Parr, and K. L. Abbott, editors. Ant ecology. Oxford University Press.

LeBrun, E., R. Plowes, and L. Gilbert. 2012. Imported fire ants near the edge of their range: disturbance and moisture determine prevalence and impact of an invasive social insect. Journal of Animal Ecology **81**:884-895.

LeBrun, E. G., J. Abbott, and L. E. Gilbert. 2013. Imported crazy ant displaces imported fire ant, reduces and homogenizes grassland ant and arthropod assemblages. Biological Invasions **15**:2429-2442.

Lester, P. J., and M. A. Gruber. 2016. Booms, busts and population collapses in invasive ants. Biological Invasions **18**:1-11.

Lindeman, R. 1942. The Trophic-Dynamic Aspect of Ecology. Ecology **23**:399-417.

MacArthur, R. H., and J. W. MacArthur. 1961. On bird species diversity. Ecology **42**:594-598.

McCauley, D. J., G. Gellner, N. D. Martinez, R. J. Williams, S. A. Sandin, F. Micheli, P. J. Mumby, and K. S. McCann. 2018. On the prevalence and dynamics of inverted trophic pyramids and otherwise top-heavy communities. Ecology Letters **21**:439-454.

McClure, M. S. 1980. Competition between exotic species: scale insects on hemlock. Ecology **61**:1391-1401.

McGlynn, T. P. 1999. The worldwide transfer of ants: geographical distribution and ecological invasions. Journal of Biogeography **26**:535-548.

Menke, S. B., A. V. Suarez, C. V. Tillberg, C. T. Chou, and D. A. Holway. 2010. Trophic ecology of the invasive argentine ant: spatio-temporal variation in resource assimilation and isotopic enrichment. Oecologia **164**:763-771.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta **48**:1135-1140.

Morel, L., R. K. Vander Meer, and C. S. Lofgren. 1990. Comparison of nestmate recognition between monogyne and polygyne populations of *Solenopsis invicta* (Hymenoptera: Formicidae). Annals of the Entomological Society of America **83**:642-647.

Mulholland, P. J., J. L. Tank, D. M. Sanzone, W. M. Wollheim, B. J. Peterson, J. R. Webster, and J. L. Meyer. 2000. Food resources of stream macroinvertebrates determined by natural-abundance stable C and N isotopes and a ^{15}N tracer addition. Journal of the North American Benthological Society **19**:145-157.

Ohgushi, T. 2005. Indirect interaction webs: herbivore-induced effects through trait change in plants. Annual Review of Ecology, Evolution, and Systematics **36**:81-105.

Oster, G. F., and E. O. Wilson. 1978. Caste and ecology in the social insects. Princeton University Press.

Passera, L. 1994. Characteristics of tramp species. Pages 23-43 *in* D. F. Williams, editor. Exotic ants. Biology, impact, and control of introduced species. Westview Press.

Plowes, R. M., J. G. Dunn, and L. E. Gilbert. 2007. The urban fire ant paradox: native fire ants persist in an urban refuge while invasive fire ants dominate natural habitats. Biological Invasions **9**:825-836.

Ponsard, S., and R. Arditi. 2000. What can stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) tell about the food web of soil micro-invertebrates? Ecology **81**:852-864.

Porter, S., and D. Savignano. 1990. Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. Ecology **71**:2095-2106.

Porter, S. D., H. G. Fowler, and W. P. Mackay. 1992. Fire ant mound densities in the United States and Brazil (Hymenoptera: Formicidae). Journal of Economic Entomology **85**:1154-1161.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**:703-718.

Pritchard, J. R., and D. Schluter. 2001. Declining interspecific competition during character displacement: summoning the ghost of competition past. Evolutionary Ecology Research **3**:209-220.

Quezada-Romegialli, C., A. L. Jackson, B. Hayden, K. K. Kahilainen, C. Lopes, and C. Harrod. 2018. tRophicPosition, an R package for the Bayesian estimation of trophic position from consumer stable isotope ratios. Methods in Ecology and Evolution **9**:1592-1599.

Resasco, J., D. J. Levey, and E. I. Damschen. 2012. Habitat corridors alter relative trophic position of fire ants. Ecosphere **3**:art100.

Reum, J. C., and K. N. Marshall. 2013. Evaluating $\delta^{15}N$–body size relationships across taxonomic levels using hierarchical models. Oecologia **173**:1159-1168.

Ross, K. G. 1993. The breeding system of the fire ant *Solenopsis invicta*: effects on colony genetic structure. The American Naturalist **141**:554-576.

Ross, K. G., E. L. Vargo, and L. Keller. 1996. Social evolution in a new environment: the case of introduced fire ants. Proceedings of the National Academy of Sciences **93**:3021-3025.

Rounick, J., and M. Winterbourn. 1986. Stable carbon isotopes and carbon flow in ecosystems. Bioscience **36**:171-177.

Savage, A. M., S. D. Johnson, K. D. Whitney, and J. A. Rudgers. 2011. Do invasive ants respond more strongly to carbohydrate availability than co-occurring non-invasive ants? A test along an active *Anoplolepis gracilipes* invasion front. Austral Ecology **36**:310-319.

Schoener, T. W. 1974. Resource partitioning in ecological communities. Science **185**:27-39.

Sharma, S., E. A. Buss, G. S. Hodges, and D. H. Oi. 2019. Effect of soil treatments for cottony cushion scale (Hemiptera: Monophlebidae) control on *Nylanderia fulva* (Hymenoptera: Formidicae) survival and trailing activity. Florida Entomologist **102**:202-206.

Sharma, S., D. Oi, and E. Buss. 2013. Honeydew-producing hemipterans in Florida associated with *Nylanderia fulva* (Hymenoptera: Formicidae), an invasive crazy ant. The Florida Entomologist **96**:538-547.

Snyder, W., and E. Evans. 2006. Ecological effects of invasive arthropod generalist predators. Annual Review of Ecology, Evolution, and Systematics **37**:95-122.

Stark, S., D. A. Wardle, R. Ohtonen, T. Helle, and G. W. Yeates. 2000. The effect of reindeer grazing on decomposition, mineralization and soil biota in a dry oligotrophic Scots pine forest. Oikos **90**:301-310.

Starrfelt, J., K. Borgå, A. Ruus, and E. Fjeld. 2013. Estimating trophic levels and trophic magnification factors using Bayesian inference. Environmental Science & Technology **47**:11599-11606.

Styrsky, J. D., and M. D. Eubanks. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. Proceedings of the Royal Society of London B: Biological Sciences **274**:151-164.

Styrsky, J. D., and M. D. Eubanks. 2010. A facultative mutualism between aphids and an invasive ant increases plant reproduction. Ecological Entomology **35**:190-199.

Tillberg, C., D. Holway, E. LeBrun, and A. Suarez. 2007. Trophic ecology of invasive Argentine ants in their native and introduced ranges. Proceedings of the National Academy of Sciences of the United States of America **104**:20856-20861.

Tschinkel, W. R. 2006. The fire ants. Harvard University Press.

Tschinkel, W. R., E. S. Adams, and T. Macom. 1995. Territory area and colony size in the fire ant *Solenopsis invicta*. Journal of Animal Ecology **64**:473-480.

Van den Meersche, K., J. J. Middelburg, K. Soetaert, P. Van Rijswijk, H. T. Boschker, and C. H. Heip. 2004. Carbon-nitrogen coupling and algal-bacterial interactions during an experimental bloom: Modeling a ^{13}C tracer experiment. Limnology and Oceanography **49**:862-878.

Vander Meer, R., M. Obin, and L. Morel. 1990. Nestmate recognition in fire ants: monogyne and polygyne populations. Pages 322-328. Applied myrmecology: a world perspective. Westview Press.

Vander Zanden, J. M., and J. B. Rasmussen. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology **80**:1395-1404.

Vander Zanden, M. J., J. M. Casselman, and J. B. Rasmussen. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature **401**:464.

Vasconcellos-Neto, J., and R. F. Monteiro. 1993. Inspection and evaluation of host plant by the butterfly *Mechanitis lysimnia* (Nymph., Ithomiinae) before laying eggs: a mechanism to reduce intraspecific competition. Oecologia **95**:431-438.

Wagner, D., and E. Fleur Nicklen. 2010. Ant nest location, soil nutrients and nutrient uptake by ant-associated plants: does extrafloral nectar attract ant nests and thereby enhance plant nutrition? Journal of Ecology **98**:614-624.

Wang, Z., L. Moshman, E. Kraus, B. Wilson, N. Acharya, and R. Diaz. 2016. A review of the tawny crazy ant, *Nylanderia fulva*, an emergent ant invader in the southern United States: is biological control a feasible management option? Insects **7**:77.

Way, M. J. 1963. Mutualism between ants and honeydew-producing homoptera. Annual Review of Entomology **8**:307-344.

Weeks, R. D., L. Wilson, and S. Vinson. 2004. Resource partitioning among colonies of polygyne red imported fire ants (Hymenoptera: Formicidae). Environmental Entomology **33**:1602-1608.

Wetterer, J., O. Davis, and J. Williamson. 2014. Boom and bust of the tawny crazy ant, *Nylanderia fulva* (Hymenoptera: Formicidae), on St. Croix, US Virgin Islands. The Florida Entomologist **97**:1099-1103.

Wetterer, J. K., X. Espadaler, A. L. Wetterer, D. Aguin-Pombo, and A. M. Franquinho-Aguiar. 2006. Long-term impact of exotic ants on the native ants of Madeira. Ecological Entomology **31**:358-368.

Wilder, S., D. Holway, A. Suarez, and M. Eubanks. 2011a. Macronutrient content of plant-based food affects growth of a carnivorous arthropod. Ecology **92**:325-332.

Wilder, S., D. Holway, A. Suarez, E. LeBrun, and M. Eubanks. 2011b. Intercontinental differences in resource use reveal the importance of mutualisms in fire ant invasions. Proceedings of the National Academy of Sciences of the United States of America **108**:20639-20644.

Winemiller, K. O., and G. A. Polis. 1996. Food webs: what can they tell us about the world? Pages 1-22 Food Webs. Springer.

Woodson, C. B., J. R. Schramski, and S. B. Joye. 2018. A unifying theory for top-heavy ecosystem structure in the ocean. Nature Communications **9**:23.

Zenner-Polania. 1990. Biological aspects of the hormiga-loca, *Paratrechina-(Nylanderia)-fulva* (Mayr), in Colombia. Pages 290-297 *in* R. K. Vander Meer, K. Jaffe, and A. Cedeno, editors. Applied myrmecology: a world perspective. Westview Press.

Zenner-Polania, I. 1994. Impact of *Paratrechina fulva* on other ant species. Pages 121-132 *in* D. F. Williams, editor. Exotic ants. Biology, impact, and control of introduced species. Westview Press.

Zhang, S., Y. Zhang, and K. Ma. 2015. Mutualism with aphids affects the trophic position, abundance of ants and herbivory along an elevational gradient. Ecosphere **6**:1-11.

Zohary, T., J. Erez, M. Gophen, I. Berman-Frank, and M. Stiller. 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. Limnology and oceanography **39**:1030-1043.

2. WIDESPREAD VARIATION IN STABLE ISOTOPE TROPHIC POSITION ESTIMATES: PATTERNS, CAUSES, AND POTENTIAL CONSEQUENCES

2.1. Overview

Stable isotope analysis has emerged as one of the leading techniques to estimate the trophic position of organisms. According to a *Web of Science* search in March 2020, stable isotope analysis has been used in over 5,486 studies of trophic position published between 1988-2019. There is, however, remarkable variation in the models, techniques, and baselines used to estimate trophic position from stable isotope data. The consequences of this lack of standardization are unknown but could result in biased or erroneous conclusions. We conducted a literature review to quantify the variation in baselines and models/ methodologies used to estimate trophic position from stable isotope data. Next, we assessed the potential consequences of this variation on individual species estimates and overall food web structure by extracting hundreds of published trophic positions and applying various baselines and methodologies to existing stable isotope datasets. Out of the 10 baselines and eight models/ methodologies we identified in the literature, authors that focused on aquatic ecosystems tended to use herbivores as a baseline and a single source food web formula (55% and 58%, respectively, of all aquatic studies). By contrast, authors that focused on terrestrial ecosystems tended to use either no baseline (i.e., unadjusted $\delta^{15}N$ as a proxy for trophic position) or primary producers (34% and 23% of all terrestrial studies) with statistical analyses (41%) and baseline subtraction (24%) as methodologies. Baseline and methodology significantly

affected trophic position estimates of individual species, as well as conclusions about food web structure. Published trophic positions and trophic positions estimated from the same dataset often varied by over two trophic levels depending on the baseline or methodology used, but differences due to baseline and/or methodology were not consistent across all species. For example, compound-specific isotope analysis produced significantly lower estimates than all other methodologies in some species, but significantly higher estimates in other species. Statistical conclusions about overall food web structure were the same when analyzing isotope datasets with more than three samples per trophic group, suggesting that increased sample size may mitigate some of the variation caused by different baselines and methodologies. A power analysis revealed that studies needed to collect at least five to thirty-six samples on average per trophic group if using bulk isotope analysis and at least two samples if using compound-specific isotope analysis. Based on our review of the literature, however, an alarmingly large proportion of studies collected only one sample in at least one trophic group (41% of all studies) or did not report how many they collected at all (26%), highlighting a critical need to increase stable isotope sample size and publication transparency in trophic ecology research. We recommend moving away from biological interpretations of absolute trophic positions (as these are prone to a number of biases), and instead focus on comparisons of these values relative to other organisms collected from the same habitat. Authors should collect a minimum of five samples per trophic group (but 10 for best-practices) from as many trophic groups as possible to increase statistical power and redundancy in comparisons.

2.2. Introduction

A fundamental question in ecology is "Who eats whom?" Trophic relationships are often complex and highly variable over space and time, with many species feeding opportunistically on temporarily available resources, shifting their diet ontogenetically, and consuming prey from multiple trophic levels (Darnell 1961, Paine 1980, Polis and Strong 1996, Pfennig and Murphy 2000). One way to capture these complex relationships is by quantifying an organism's trophic position on a continuous scale. Continuous measures of trophic position are typically interpreted relative to traditional food web categories, with 1 corresponding to primary producers such as plants, 2 to primary consumers such as herbivores, 3 to primary predators, and >4 to higher order predators that often feed on other predators (i.e., intraguild predation). Assigning trophic positions along a continuous "trophic spectrum" allows ecologists to quantitatively measure and compare the relative strength and direction of feeding interactions in the real world (Darnell 1961, Paine 1980, Polis and Strong 1996). For example, Jardine (2016) found that the Australian longfinned eel, an opportunistic top predator in rivers and streams, occupied a higher trophic position in temperate systems than in tropical systems (trophic levels of 4.7 vs. 3.8 respectively) due to a greater diversity of herbivorous prey in tropical systems. Information based on these trophic position estimates has been used to assess the sustainability of fishing practices (Roell and Orth 1998, Blaber et al. 2000, Caddy and Garibaldi 2000, Kitchell et al. 2000, Cooke and Cowx 2006), to determine the biomagnification of important contaminants such as mercury (Bowles et al. 2001, Croteau et al. 2005, Pethybridge et al. 2012, Coelho et al.

2013, Lavoie et al. 2013, Azevedo-Silva et al. 2016) and to demonstrate invasion-associated changes in food web structure (Vander Zanden et al. 1999a, Geiger et al. 2005, Gratton and Denno 2006, Tillberg et al. 2007, Britton et al. 2010).

Because of its flexibility and utility, stable isotope analysis has become one of the leading techniques for assessing trophic position. The ratio of stable isotopes of nitrogen ($\delta^{15}N$) is often used to estimate trophic position due to shifts in the proportion of heavy to light nitrogen isotopes from one trophic level to the next (DeNiro and Epstein 1981, Minagawa and Wada 1984, Fry 1988). In general, consumers are enriched in the heavier isotope (have higher $\delta^{15}N$) relative to their food resource. Because these isotopes are assimilated into an organism's body over time, they provide a more quantitative measure of resource use over time than other techniques such as gut content analysis or direct observation, which produce only a snapshot of what the organism most recently consumed (Fry 2006, Traugott et al. 2013). Sampling for stable isotope analysis is relatively easy and can also be non-destructive, so the diets of many species, including those that are critically endangered (Evans et al. 2012, Molina-Burgos et al. 2018, Cameron et al. 2019) can be reconstructed over a broad spatial and temporal scale (Tillberg et al. 2007, Caut et al. 2008, Wilder et al. 2011, McMeans et al. 2019). Stable isotope analysis can even be used to examine the trophic interactions of long-extinct organisms, including large, herbivorous mammals from the Pleistocene (Feranec and MacFadden 2000) and 5,500-year-old fish (Rowell et al. 2010), which would otherwise be impossible to study. According to a *Web of Science* search in March 2020, stable isotope analysis has been used in over 5,486 published studies of trophic position

between 1988-2019. There is, however, remarkable variation in the models, techniques, and baselines used to estimate trophic position from stable isotope data. The consequences of this lack of standardization are unknown but could be far-reaching. Trophic position estimates from stable isotope data are currently being used to substantiate restoration efforts (Senior et al. 2013, James et al. 2020), control important animal and human disease vectors (Rasgon 2008, Stapp and Salkeld 2009, Gilbreath et al. 2013, Heylen et al. 2019), and inform wildlife conservation (Darimont and Reimchen 2002, Alves-Stanley et al. 2010, Merkle et al. 2011, Evans et al. 2012). It is therefore critical to establish a more standardized method to generate accurate and reliable trophic positions and conclusions from stable isotope data. Our primary objectives for this paper, therefore, were 1) to conduct a literature review to quantify the variation in methods and techniques used to estimate trophic position from stable isotope data, and 2) to assess the potential consequences of this variation on absolute estimates for individual species and conclusions about food web structure.

2.2.1. Baselines and models: Important components to estimate trophic position from stable isotopes

All estimates of trophic position from stable isotope data require some kind of reference point to be meaningful. Isotopic concentrations and ratios in abiotic components of ecosystems (e.g., water, inorganic sediments, rocks) vary over space and time, and plants and animals will, to varying degrees, reflect the isotopic ratios of elements assimilated from their environments (Rounick and Winterbourn 1986, Zohary

et al. 1994, Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999, Post 2002). This environmental variation in isotopes makes it difficult to directly compare consumer isotope values across locations, as differences in values could be due to diet, differences in baseline levels of isotopes at each location, or some combination of the two. By estimating trophic position relative to material collected from the same environment at approximately the same time (an isotope "baseline"), stable isotope values become biologically meaningful (Cabana and Rasmussen 1994, Vander Zanden and Rasmussen 1999, Ponsard and Arditi 2000, Post 2002). Trophic position can be estimated relative to this baseline material using a specific formula (Vander Zanden et al. 1999b, Post 2002, Hussey et al. 2014) or by statistical comparison (Croteau et al. 2005, Da Silva et al. 2005, Hebert et al. 2009, Kohzu et al. 2009, Reum and Marshall 2013, Starrfelt et al. 2013, Hette-Tronquart et al. 2018, Quezada-Romegialli et al. 2018). Therefore, two of the most important decisions an ecologist faces when using stable isotopes are 1) what baseline material to use, and 2) how trophic position should be estimated relative to this baseline.

2.2.1.1. Decision 1: Choosing an appropriate baseline

The purpose of a baseline is to correct for inherent differences in isotope levels that characterize a given location, so the isotope values of an ideal baseline should be relatively constant over time (Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999, Post 2002). When the isotope values of a baseline show high temporal variation (typically in smaller organisms with high tissue-turnover rates), the trophic

level estimate of a higher order consumer may depend more on when the baseline was sampled rather than the actual diet of the consumer. For example, Cabana and Rasmussen (1996) showed that the temporal variance of $\delta^{15}N$ in phytoplankton and particular organic matter (POM) in lakes was ten times greater than that of a large vertebrate or fish. As a consequence, estimates of food chain length differed by as much as eight trophic levels depending on whether phytoplankton (high tissue turnover) or large primary consumers (low tissue turnover) were used as a baseline (Couey 1935, Cabana and Rasmussen 1996).

Despite the recommendation to use longer-lived primary consumers (Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999, Post 2002), there is still widespread variation in baselines used by aquatic ecosystem studies. Many aquatic ecosystem studies refer to POM or phytoplankton as baselines (Renaud et al. 2011, Vinagre et al. 2012, Quiroga et al. 2014, Lorrain et al. 2015, Pethybridge et al. 2018), which is sometimes due to a lack of longer-lived primary consumers in some habitat types (Jardine et al. 2014). There appears to be even more variation in how ecologists select baselines for terrestrial ecosystem studies, perhaps due to fewer comparative analyses (Sabat et al. 2013). Many terrestrial studies use soil as a baseline (Sampedro and Domínguez 2008, Gibb and Cunningham 2011, Crotty et al. 2014, Ingimarsdóttir et al. 2014, Gibb et al. 2015, Haynert et al. 2017), but others use leaf litter because soil isotope values can vary by depth (Ponsard and Arditi 2000, Schneider et al. 2004, Potapov et al. 2019b). Still others use vascular plants as a baseline (Ladygina et al. 2008, König et al. 2011, Woodcock et al. 2012, Fetcher et al. 2015, Hamer et al. 2015, Des

Roches et al. 2016, Kim et al. 2019), but there is disagreement over what spatial scale best reflects environmental isotope levels (Woodcock et al. 2012). Compound-specific isotope analysis (hereafter CSIA), which uses the isotope values of specific amino acids within a consumer, bypasses some of these difficulties because a baseline is no longer necessary (Chikaraishi et al. 2007, Chikaraishi et al. 2009, Steffan et al. 2013). However, CSIA requires extraction of specific amino acids from consumer tissues, a process that is much more intensive compared with bulk analysis in which samples are often simply dried, ground, and run through a mass spectrometer. Because of the increased processing and difficulty of CSIA, this technique is available in far fewer isotope laboratories and, therefore, less widely used. Baselines can have a significant effect on trophic level estimates (Iken et al. 2010, Guzzo et al. 2011, Frisch et al. 2016, Potapov et al. 2019a), so it is important to understand the potential causes and consequences for why different baselines are used to create more standardized recommendations for each study system.

2.2.1.2. Decision 2: Models to estimate trophic position relative to the baseline

After an appropriate baseline is chosen, trophic position can be estimated relative to the baseline using a specific formula (Vander Zanden et al. 1999b, Post 2002, Hussey et al. 2014), or it can be inferred using a variety of frequentist or Bayesian statistical approaches (Croteau et al. 2005, Da Silva et al. 2005, Hebert et al. 2009, Kohzu et al. 2009, Reum and Marshall 2013, Starrfelt et al. 2013, Hette-Tronquart et al. 2018, Quezada-Romegialli et al. 2018). Kohzu et al. (2009), for example, used ANOVA and Tukey's HSD to show that Mongolian people and dogs had $\delta^{15}N$ values that were not

significantly different from each other, but that both were significantly higher than herbivorous mammals in Mongolian grasslands, concluding that people and dogs are apex predators in this system.

As with baselines, there are many different formulas and statistical approaches used to estimate trophic position in the literature (see Table 2.1 for a list and description of major categories). Because each methodology often requires different inputs (i.e., single source vs. multiple sources), and sometimes completely different measurements (i.e., bulk isotope analysis vs. CSIA), trophic position estimates of the same material can dramatically differ depending on the methodology used. Post (2002), for example, showed that estimates using two different models varied by over two trophic levels. Even small deviations, however, could affect biological interpretations of the same data. For example, Molina-Burgos et al. (2018) used Bayesian statistics to estimate that the endangered Darwin's frog (*Rhinoderma darwinii*) occupied a trophic position of 2.9 (secondary consumer). If this estimate differed by just a few tenths of a trophic level and the trophic position was 2.4 instead of 2.9, it would imply that Darwin's frogs relied on more plant material, which might influence conservation strategies for this species. Ideally, a specific methodology should be chosen based on the biology of the organism; a single source formula can be used if the organism feeds primarily on one resource, whereas a two source formula would be more appropriate if the organism feeds on two different resources. It can be difficult, however, to select the best methodology without prior knowledge of an organism's diet, but prior knowledge of diet is not always possible. Authors may also choose one methodology over another because the estimates

lie outside of the traditional trophic spectrum (i.e., between 1 to 5). Letvin et al. (2017), for example, switched formulas because the first produced, in their opinion, biologically unrealistic trophic position estimates (i.e., trophic positions > 6). Although such decisions may be common, the consequences of various methodologies on trophic positions and overall conclusions reported in the literature are unknown.

Table 2.1 Most common methodologies used to estimate trophic position from stable isotope data.

Methodology	Formula	General description
Single source	$TP_{animal} = \lambda + \frac{\delta^{15}N_{animal} - \delta^{15}N_{base}}{\Delta n}$	Trophic position is calculated relative to a single baseline using a single isotope (usually $\delta^{15}N$). λ = trophic level of the baseline (TL = 1 for primary producers, TL = 2 for herbivores, etc.).
Two source	1) $\alpha = \frac{\delta^{13}C_{animal} - \delta^{13}C_{base1}}{\delta^{13}C_{base1} - \delta^{13}C_{base2}}$ 2) $TP_{animal} = \lambda + \frac{\delta^{15}N_{animal} - \delta^{15}N_{base1}\times\alpha + \delta^{15}N_{base2}\times(\alpha - 1)}{\Delta n}$	Trophic position is calculated relative to two possible baselines using a two isotopes ($\delta^{15}N$, $\delta^{13}C$). λ = trophic level of the baseline (TL = 1 for primary producers, TL = 2 for herbivores, etc.).
Baseline subtraction	**Variation 1**: $\delta X_{corrected} = \delta X_{animal} - \delta X_{base}$ **Variation 2**: $\delta X_{corrected} = \delta X_{animal\ SiteB} - (\delta X_{base\ SiteB} - \delta X_{base\ SiteA})$	Consumer isotope values (either $\delta^{15}N$ or $\delta^{13}C$) are normalized across sites by subtracting the isotope values of the baseline (i.e., soil, leaf litter, or primary producers).
Statistics	NA	Consumer isotope values (either $\delta^{15}N$, $\delta^{13}C$ or both) are statistically compared relative to other trophic groups collected from the same habitat. Includes both frequentist and Bayesian approaches. See Appendix S1 for complete list of approaches used in the literature.
Compound-specific isotope analysis (CSIA)	$TP_{animal} = 1 + \frac{\delta^{15}N_{animal\ Glu} - \delta^{15}N_{animal\ Phe} + \beta}{\Delta n}$	Trophic position is calculated using the isotope values of specific amino acids. Typically glutamic acid (Glu) and phenylalanine (Phe) are used. β is the difference between $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ of a primary producer. β = -3.4‰ for aquatic primary producers β = +8.4 for terrestrial primary producers
None	NA	Consumer isotope values ($\delta^{15}N$) are directly compared relative to other trophic groups collected from the same habitat.

2.2.1.3. Application to individual species and overall food web

Estimates of trophic position can be used to determine the resource use of individual species (e.g., Ménard et al. 2007, Wilder et al. 2011, Kloskowski and Trembaczowski 2015, Jardine 2016, Evangelista et al. 2017) or to assess overall food web structure using functional groups instead of individual species (e.g., Fry 1988,

Kohzu et al. 2009, König et al. 2011, Di Beneditto et al. 2012, Coelho et al. 2013, Fetcher et al. 2015, Bowes et al. 2020). Both applications are extremely useful in trophic ecology research. For example, trophic position estimates of individual species have informed conservation of endangered species (Darimont and Reimchen 2002, Alves-Stanley et al. 2010, Molina-Burgos et al. 2018) and helped to identify the various hosts of mosquitoes (Rasgon 2008, Gilbreath et al. 2013), fleas (Stapp and Salkeld 2009), and ticks (Heylen et al. 2019). Food web structure based on trophic position estimates of functional groups has been used to examine biomagnification of important contaminants within a food web (Bowles et al. 2001, Di Beneditto et al. 2012, Coelho et al. 2013, Schneider et al. 2015, Azevedo-Silva et al. 2016), to determine the effects of humans on ecosystem health (Mavraki et al. 2019, Oczkowski et al. 2020, Pingram et al. 2020), and to quantify recovery after restoration (Senior et al. 2013, James et al. 2020). Variation in the baseline and methodology used to estimate trophic position across studies could, therefore, impact conclusions about individual species, as well as overall food web structure. Additionally, conclusions based on these estimates are often compiled and compared across studies (Pauly et al. 1998, Das et al. 2003, Pethybridge et al. 2018, Potapov et al. 2019a), but the variation due to different baselines and methodologies used to calculate these estimates is often not considered (but see Potapov et al. 2019a).

Our goals are to quantify the variation in baselines and methodologies used to estimate trophic position from stable isotope data and then assess the consequences of this variation on conclusions about individual species and overall food web structure. Previous reviews have addressed other issues pertaining to stable isotope analysis in

trophic ecology, including trophic fractionation values (Vander Zanden and Rasmussen 2001, Post 2002, Vanderklift and Ponsard 2003, Spence and Rosenheim 2005, Caut et al. 2009), bulk vs. compound-specific isotope analysis (Steffan et al. 2013, Chikaraishi et al. 2014, Bowes and Thorp 2015, Ishikawa 2018), environmental and biological factors influencing isotopic variation (Oelbermann and Scheu 2002, Amundson et al. 2003, McCutchan et al. 2003, Boecklen et al. 2011, Wilson et al. 2011), and lipid removal (Post et al. 2007, Arostegui et al. 2019), but no study that we know of has systematically addressed how a lack of standardization in the models and baselines used to estimate trophic position from stable isotope data may affect the results and interpretation of stable isotope analysis studies.

2.2.1.4. A note on trophic discrimination factors

The trophic discrimination factor (TDF), or the shift in the proportion of the heavier to the lighter isotope from one trophic level to the next, is also critically important to accurately estimate trophic position from stable isotope data. Early reviews of a variety of taxa suggested that $\delta^{15}N$ increases on average by approximately 3.4‰ from a food resource to a consumer (Minagawa and Wada 1984, Post 2002), but it has since been demonstrated that the TDF fluctuates largely depending on a number of different factors, including taxonomy and the process by which nitrogen is excreted from the body (Vanderklift and Ponsard 2003), species identity of the consumer and of the resource consumed (Bastos et al. 2017, Blanke et al. 2017), feeding mode and life stage (Spence and Rosenheim 2005), starvation time prior to feeding (Adams and Sterner

2000), and tissue analyzed (i.e., muscle or whole body) (McCutchan et al. 2003). Although we note its importance, the issue of appropriate TDFs has been reviewed extensively elsewhere (Adams and Sterner 2000, Post 2002, McCutchan et al. 2003, Vanderklift and Ponsard 2003, Martinez del Rio et al. 2009, Bastos et al. 2017, Blanke et al. 2017), so we focus on several other aspects of isotope ecology that have received less attention, particularly that of the model/ methodology and baseline used to estimate trophic position.

2.3. Methods

2.3.1. Quantifying variation in the literature

2.3.1.1. Literature search

We identified ecological studies estimating trophic position from stable isotope data with key word searches using the *Web of Science* using the terms *stable isotop*, trophic position* OR *trophic level* OR *trophic,* and *food web* OR *food web structure* OR *food chain*. The search was concluded in March 2020. We found 5,486 papers in our initial search published between 1988-2019. In order to compare the methodologies used in aquatic and terrestrial ecosystem studies, we conducted a second search to identify terrestrial ecosystem studies contained within these 5,486 papers using the original search terms plus the terms *terrestrial* NOT *aquatic* OR *marine* OR *freshwater*. This search generated 375 papers. We removed any entry that was repeated or without a combined title, abstract, and author information. After removing repeated or incomplete

entries, our finalized list consisted of 5,251 papers, of which 4,904 focused on aquatic ecosystems and 347 focused on terrestrial ecosystems.

2.3.1.2. Extracting methodologies and baselines

Because of the large number of studies included in our initial search, we classified common baselines and methodologies used by reviewing a subset of the 5,251 papers. In total, we reviewed 200 aquatic ecosystem studies and 100 terrestrial ecosystem studies from our original search. To be included in the review, a study must have estimated the trophic position of an animal or group of animals using stable isotope data. Out of the 300 studies reviewed, we extracted information from 200 publications, of which 121 focused on aquatic ecosystems and 79 focused on terrestrial ecosystems.

To understand potential causes for why a certain baseline or methodology were used, we also extracted several variables in each study for use in a categorical analysis. Variables included: (1) general category describing the approach used to estimate trophic position; (2) ecosystem studied (aquatic or terrestrial); (3) material used as the baseline (if applicable); and (4) smallest number of samples collected for any given trophic group as analyzed in the study.

Studies were published between 1988-2019. Of the terrestrial studies examined, most focused on forests, rainforests, and grasslands (~40%, 10%, and 10%, respectively, of all terrestrial studies), whereas aquatic ecosystem studies focused on lakes, rivers, and seas (~27%, 16%, and 13%, respectively, of all aquatic studies). Studies were conducted around the world (approx. 48 countries), with most aquatic studies conducted in the

United States or Canada (~14% and 15%, respectively, of all aquatic studies) and most terrestrial studies conducted in the United States (~19% of all terrestrial studies). Most aquatic studies focused on fish (~88% of all aquatic studies), whereas terrestrial studies focused on invertebrates (~83% of all terrestrial studies), with ants being the focus of 25% of all terrestrial studies.

2.3.1.3. Statistical analysis

We conducted all statistical analyses in R v 3.6.1 (R Core Team 2019). We used log-linear models to evaluate the relationship between the explanatory variables from the publications that we reviewed, and $P = 0.05$ was taken as level of significance. The null hypothesis was that categorical variables were independent of each other, and therefore, there was no significant relationship between the two. Log-linear models were constructed by generating frequency tables and forming generalized linear models with a Poisson distribution using the *glm* function in base R statistical software. Frequency was treated as the dependent variable. Independent variables included year published, ecosystem studied (aquatic or terrestrial), methodology used to estimate trophic position, material used as the baseline (if applicable), and pairwise interactions between these categories.

2.3.2. Evaluating the consequences on estimates of individual species

In order to determine if baseline and methodology influence absolute trophic position estimates of individual species, we used two approaches: (1) we extracted

trophic position estimates from publications of commonly studied species that were calculated using various methodologies and/or baselines, and (2) we applied several methodologies and baselines to existing isotope datasets and compared the results.

2.3.2.1. Published trophic position estimates of common taxa

First, we identified commonly studied taxa by mining study species from the abstracts in our initial literature search. We downloaded abstracts, DOIs, titles, publication year, and author information for all 5,251 studies from our initial literature search. Any studies missing a DOI were assigned the *Web of Science* unique ID code. We also split texts into aquatic (4,904 papers) and terrestrial (347 papers) files for separate analyses. To create aquatic and terrestrial corpora, abstract texts were tokenized using the R package *tidytext* (Silge and Robinson 2016) with a custom word-tokenizing function utilizing the scispaCy "en_core_sci_lg" model v0.2.4 (https://allenai.github.io/scispacy/). The spaCy model was accessed from within R using the *spacyr* package v1.2.1 (Benoit et al. 2018). We pulled species names from abstracts by referencing each corpus to a species database that we compiled from multiple sources. We downloaded taxonomic databases from the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov/; Wheeler et al. 2007), Catalogue of Life (CoL; www.catalogueoflife.org/col; Roskov et al. 2020), and Integrated Taxonomic Information System (ITIS; retrieved 04-10-2020 www.itis.gov). We also manually added any unmatched species names and included a list of species from a recent meta-analysis of stable isotope records by Potapov et al. (2019a). All

databases were combined to generate a single species database with 1,605,531 species records. We found species names referenced in the abstracts of approximately 50% of all studies (2,277 aquatic studies and 155 terrestrial studies). We then organized species according to taxonomic class.

Using our list of common species, we extracted trophic position estimates from published studies. Often, studies used only a single methodology and baseline to estimate the trophic position of a given species. However, there were several studies that compared estimates using different methodologies or baselines, so these studies included multiple different estimates for each species. We only included studies that reported absolute trophic position estimates in tables, plots, appendices, or supplemental information. Whenever estimates were reported only in plots, we used ImageJ software to extract data (Schneider et al. 2012). We classified several additional variables from each study, including: (1) methodology or methodologies used to estimate trophic position; (2) baseline used (when applicable); (3) location of sample collection; (4) habitat; and (5) year of sample collection. For all fish taxa, we also included trophic positions reported on FishBase based on stomach contents (Froese and Pauly 2000).

In total, we analyzed 834 trophic position estimates of 63 species taken from 101 publications. Species represented two kingdoms (Animalia and Plantae), four phyla, nine classes, 23 orders, and 39 families, but most species came from four classes that are well-represented in the literature: Actinopterygii (58% of all estimates), Mammalia (17%), Insecta (12%), and Aves (4%). Organisms were collected in 21 different habitats from 37 regions/ countries, with lakes, arctic marine, ocean, and agricultural fields

representing the largest proportion of estimates (41%, 20%, 10%, and 8%, respectively). We identified six methodologies for estimating trophic position (single source, two source, three source, Bayesian statistics, CSIA, and stomach content analysis) and four baseline categories used with bulk isotope analysis (basal resource, primary consumer, secondary consumer, and multiple trophic levels).

2.3.2.2. Calculating trophic position using stable isotope datasets

We also applied several of the most common methodologies (see Results: *Methodologies used by study system*) to stable isotope datasets to determine the effects of baseline and methodology on trophic position estimates when applied to the same data. The methodologies used included the following: (1) two variations of the single source food web formula (*variation 1*: autotroph at base; *variation 2*: herbivore at base); (2) two source food web formula (when both $\delta^{15}N$ and $\delta^{13}C$ values were available); (3) baseline subtraction; (4) CSIA (when available). Additional information about each methodology can be found in Table 2.1. These methodologies represent the most common approaches that ecologists use to estimate trophic position from stable isotope data.

Based on the results from our searches on *Web of Science*, we selected papers with associated stable isotope datasets that were available online. Due to only three available isotope datasets based on this search, we also searched the *Dryad* data repository (https://datadryad.org/stash/) using the search term *stable isotope analysis*, and we downloaded the *Bilagay* dataset associated with the *tRophicPosition* package in

R (Quezada-Romegialli et al. 2018), which was gathered and compiled by Chris Herrod and his research group (Docmac et al. 2017). We only used datasets that reported raw isotope values for each sample (rather than means for each taxa) so that appropriate statistical analyses could be conducted. When possible, we tried to find datasets that included compound-specific isotope analysis measurements to compare with bulk isotope analysis.

In total, we used nine studies, of which four focused on aquatic ecosystems (Ishikawa et al. 2014, Quezada-Romegialli et al. 2018, Fox et al. 2019, Price et al. 2019), four focused on terrestrial ecosystems (Sanders and Platner 2007, Pringle and Fox-Dobbs 2008, Steffan et al. 2013, Korobushkin et al. 2014), and one included data for an aquatic and a terrestrial ecosystem (Chikaraishi et al. 2014); thus, we analyzed five datasets for each ecosystem type (10 total datasets). To facilitate easier reading in figures and analyses, we assigned each dataset a shortened code name: *Ishi* (Ishikawa et al. 2014), *Bilagay* (Quezada-Romegialli et al. 2018), *Fox* (Fox et al. 2019), *Price* (Price et al. 2019), *Sanders* (Sanders and Platner 2007), *Pringle* (Pringle and Fox-Dobbs 2008), *Steffan* (Steffan et al. 2013), *ChikA* (aquatic dataset from Chikaraishi et al. 2014), and *ChikT* (terrestrial dataset from Chikaraishi et al. 2014). See Appendix A S5 for detailed explanation of each dataset. Datasets that did not include species identification (i.e., specimens were identified to taxonomic order only) were excluded for this portion of the analyses.

2.3.2.3. Statistical analysis

To analyze absolute trophic position estimates extracted from the literature and calculated using isotope datasets, we constructed linear mixed-effects models using the *lme4* package (Bates et al. 2015). The significance of each fixed factor and any pairwise interactions was tested using ANOVA with Satterthwaite's method in the *lmerTest* package (Kuznetsova et al. 2017). Trophic position was treated as the dependent variable in all analyses.

For trophic position estimates taken from the literature, methodology and taxonomic class of each species were treated as fixed effects, whereas species, year of sampling, and location were treated as random effects. Because baseline changed only within the bulk isotope methodologies (i.e., single source, two source, three source, and Bayesian statistics), we analyzed the effect of baseline in a separate analysis on bulk isotope estimates only. We also separately analyzed each of the most represented classes (Actinopterygii, Aves, Insecta, and Mammalia) to further examine any class-specific differences that were difficult to determine in the overall analysis. Within each class, we examined the effect of methodology and species (and any pairwise interaction whenever possible) on trophic position estimates. All Aves trophic positions were calculated with only one methodology (single source) but with varying baselines, so we analyzed the effect of baseline instead of methodology for this class.

For trophic position estimates calculated from isotope datasets, we used repeated-measures ANOVA with restricted maximum likelihood (REML). We treated methodology and species as fixed effects, and individual and dataset as random effects.

Additional information about specific models and results for random effects terms can be found in Appendix A S3.

2.3.3. Evaluating the consequences on food web structure

We also wanted to determine if conclusions about the overall food web change according to methodology or baseline. We used the same 10 stable isotope datasets as described above. We grouped species according to the trophic groups reported in the original publication (see Appendix A S5 for details).

2.3.3.1. Statistical analysis

For each dataset, we conducted separate ANOVAs within each methodology to test for differences between trophic groups. Whenever groups were collected from multiple sites, we also determined the effect of location on mean trophic position. If residuals were not normally distributed (via Shapiro-Wilk test, significance of $P = 0.05$), we used Kruskal-Wallis nonparametric tests instead of ANOVAs. To identify the number of unique trophic groups, we determined pairwise differences between each trophic group (significance of $P = 0.05$) using Tukey's HSD as a post-hoc procedure to ANOVA and Dunn's test as a post-hoc to Kruskal-Wallis using the package *FSA* v0.8.25 (Ogle et al. 2019).

2.3.4. Power and sample size analysis

Sample size affects statistical power, which determines the reliability of overall conclusions. To determine the fewest possible replicates required to detect differences between trophic levels for each methodology at the recommended 80% power, we also conducted a sample size analysis using the ANOVA analyses of trophic groups as described above. The pooled standard deviation from each ANOVA was used as the assumed standard deviation (all non-normally distributed data were not included in this analysis). An $\alpha = 0.05$ was used with a maximum difference between trophic positions of 1 and a power level of 0.80. All power and sample size analyses were conducted using the package *pwr* v1.2-2 (Champely et al. 2018).

We tested all data for heterogeneity of variances and normality. All plots were generated using *ggplot2* (Wickham 2016).

2.4. Results

2.4.1. Quantifying variation in the literature

2.4.1.1. Methodologies used by study system

Based on our literature search, the use of stable isotope analysis has nearly doubled in the past ten years (Figure 2.1), with a major focus on aquatic ecosystems compared with terrestrial ecosystems. In our review of a subset of these studies, we identified eight major methodologies used in the literature to estimate trophic position from stable isotope data (Figure 2.2). Categories included none (or direct comparison of raw isotope values), single source food web formula, two source food web formula,

baseline subtraction, statistical analyses, compound-specific isotope analysis (CSIA), a combination of methodologies, or not specified. There was no significant relationship between methodology used and year published ($\chi^2 = 179.609$, df = 168, $P = 0.256$). We found a significant relationship between methodology and study system ($\chi^2 = 76.079$, df = 7, $P < 0.001$; Figure 2.2). Authors that focused on aquatic ecosystems used a single source formula more often than any other method, whereas authors that focused on terrestrial ecosystems used statistical analyses or baseline subtraction significantly more often than any other method. Approximately 58% of all aquatic studies employed a single source formula compared with only 13% of all terrestrial studies. Meanwhile, approximately 41% of all terrestrial studies used statistical analyses (compared with 9% of aquatic studies), and 24% of terrestrial studies used baseline subtraction (compared with 3% of aquatic studies). See Appendix A S1 for a complete list of statistics used.

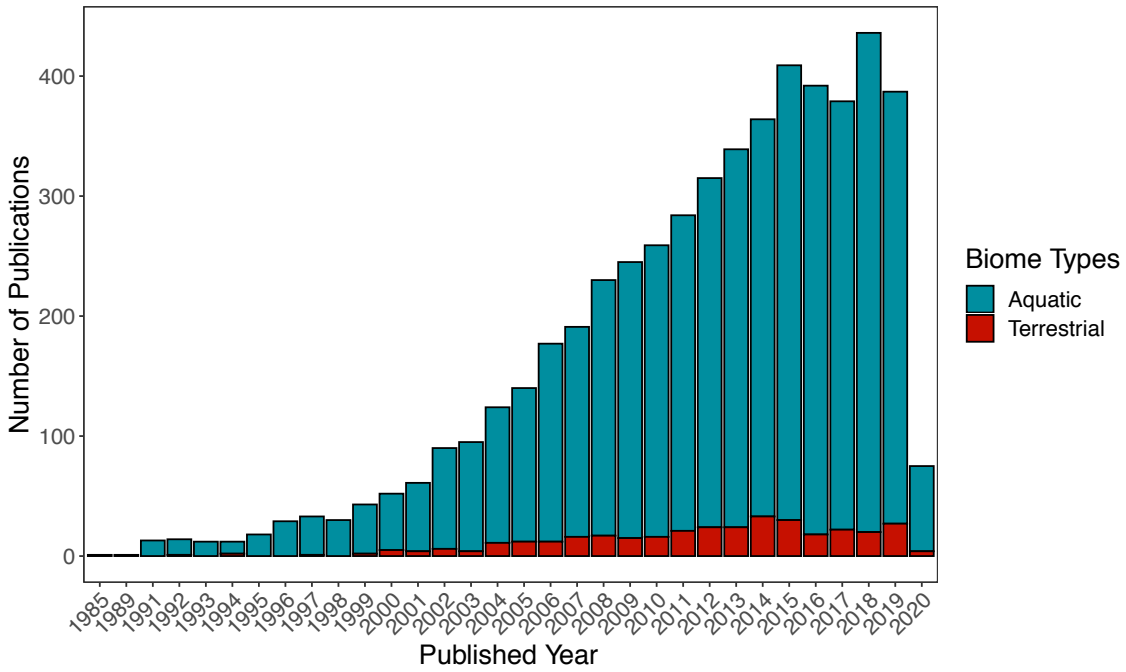


Figure 2.1 Number of publications by year and ecosystem type (Aquatic or Terrestrial) based on 5,251 stable isotope studies of trophic position from the *Web of Science*.

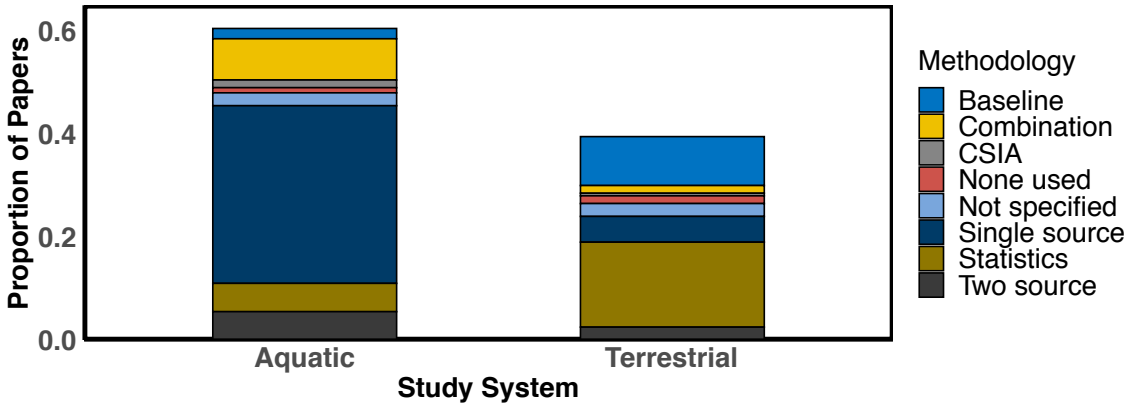


Figure 2.2 Proportion of papers (out of 200 total publications) and the methodology used by study system. Compound-specific isotope analysis abbreviated to "CSIA."

2.4.1.2. Baseline used by study system

Based on our review of a subset of the overall literature, we identified 10 major types of baselines used in the literature (Figure 2.3), including primary producers, leaf litter, particulate organic matter (POM), periphyton, detrivores, herbivores, predators, sediment/ soil, a combination of the above, none (i.e., no baseline was used), or not specified. There was no significant relationship between baseline used and year published ($\chi^2 = 154.633$, df = 216, $P = 0.999$). There was a significant interaction between ecosystem type and baseline ($\chi^2 = 94.113$, df = 9, $P < 0.001$). Authors that focused on aquatic ecosystems tended to use herbivores as a baseline (55% of all aquatic studies compared with 3% terrestrial studies), whereas authors that focused on terrestrial ecosystems tended to use either no baseline (34% of terrestrial compared with 12% aquatic) or primary producers as a baseline (23% of terrestrial compared with 13% aquatic).

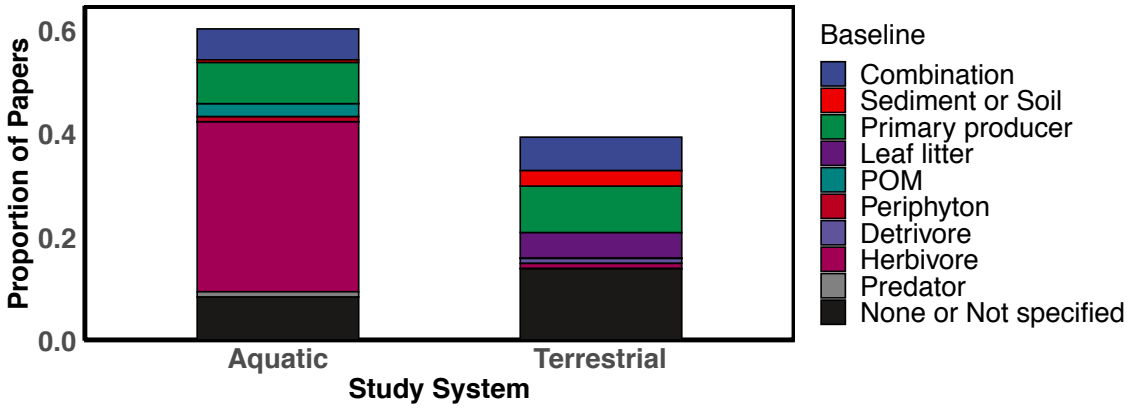


Figure 2.3 Proportion of papers (out of 200 total publications) and the baseline used by study system.

2.4.2. Evaluating the consequences on estimates of individual species

2.4.2.1. Published trophic position estimates of common taxa

Based on our analysis of 5,251 abstracts, fish species from the Actinopterygii (the ray-finned fishes) were the most common type of organism in aquatic studies (~1,750 abstracts; Figure 2.4), with a large number of studies focusing on lake trout (*Salvelinus namaycush*), arctic char (*Salvelinus alpinus*), and yellow perch (*Perca flavescens*; Table 2.2). Aves and Mammalia were identified in 342 and 318 abstracts, respectively, with many studies focusing on marine seabirds (including the black-legged kittiwake *Rissa tridactyla*) and marine mammals (including the ringed seal *Phoca hispida*). In contrast with aquatic studies, there were few species shared among terrestrial studies (Table 2.2). Studies focused more generally on the taxonomic classes Entognatha (primarily soil-dwelling springtail species) and Insecta, which appeared in 56 and 50 terrestrial abstracts, respectively (Figure 2.4). Of the species that appeared most often (i.e., European beech *Fagus sylvatica*, European ash *Fraxinus excelsior,* red fox *Vulpes vulpes*, and white-tailed deer *Odocoileus virginianus*), none of these studies included absolute trophic position estimates. For terrestrial organisms, therefore, we extracted estimates from comparative studies that included multiple trophic positions for each species (i.e., Chikaraishi et al. 2011).

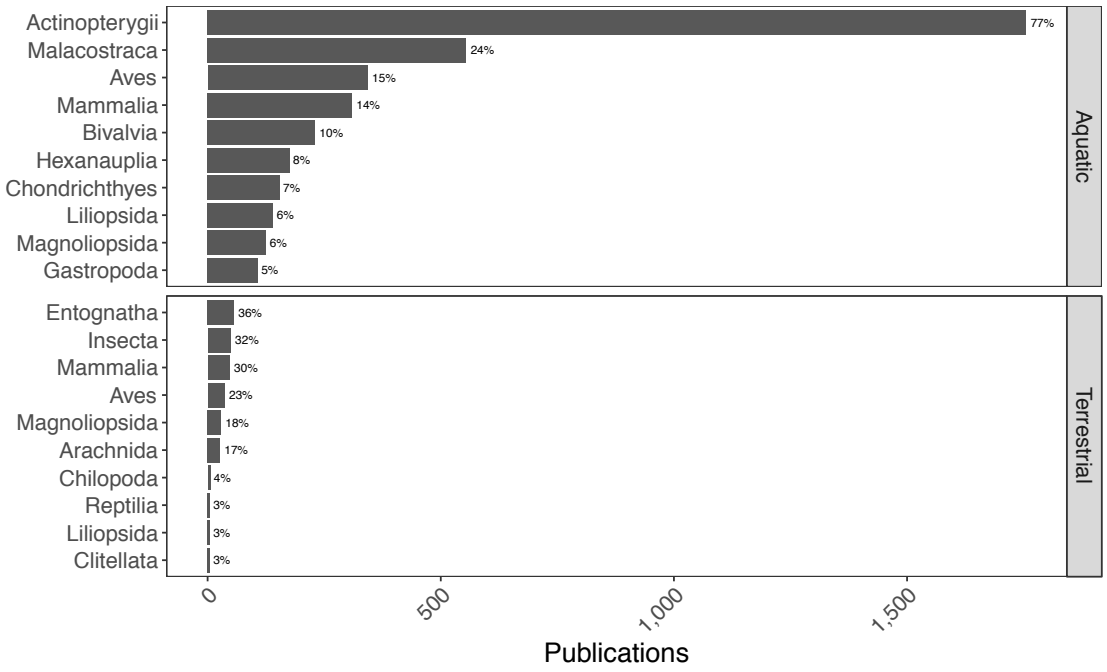


Figure 2.4 Taxonomic classes by ecosystem type (Aquatic or Terrestrial) identified in abstracts based on 5,251 stable isotope studies of trophic position from the *Web of Science*.

Table 2.2 Top 20 species by ecosystem type (Aquatic or Terrestrial) identified in abstracts based on 5,251 stable isotope studies of trophic position from the *Web of Science*.

Aquatic			Terrestrial		
Species	**Total papers**	**% of total:**	**Species**	**Total papers:**	**% of total:**
Salvelinus namaycush	41	1.8	*Fagus sylvatica*	5	3.23
Salvelinus alpinus	34	1.49	*Fraxinus excelsior*	4	2.58
Perca fluviatilis	30	1.32	*Vulpes vulpes*	3	1.94
Perca flavescens	29	1.27	*Alces alces*	3	1.94
Oncorhynchus mykiss	29	1.27	*Odocoileus virginianus*	3	1.94
Salmo trutta	28	1.23	*Chaetomium globosum*	3	1.94
Euphausia superba	26	1.14	*Folsomia fimetaria*	2	1.29
Boreogadus saida	23	1.01	*Heteromurus nitidus*	2	1.29
Cyprinus carpio	23	1.01	*Folsomia candida*	2	1.29
Phragmites australis	22	0.97	*Lepidocyrtus cyaneus*	2	1.29
Fulmarus glacialis	19	0.83	*Protaphorura fimata*	2	1.29
Zostera marina	19	0.83	*Drosophila melanogaster*	2	1.29
Posidonia oceanica	19	0.83	*Formica aquilonia*	2	1.29
Esox lucius	18	0.79	*Aphaenogaster araneoides*	2	1.29
Larus hyperboreus	18	0.79	*Linepithema humile*	2	1.29
Dreissena polymorpha	18	0.79	*Cervus elaphus*	2	1.29
Oncorhynchus tshawytscha	17	0.75	*Peromyscus leucopus*	2	1.29
Rissa tridactyla	17	0.75	*Alopex lagopus*	2	1.29
Uria lomvia	17	0.75	*Acinonyx jubatus*	2	1.29
Phoca hispida	17	0.75	*Canis lupus*	2	1.29

When all trophic positions extracted from the literature were analyzed together, trophic position estimates did not significantly differ depending on methodology ($F_{5, 113.544} = 2.008$, $P = 0.0827$), but there was a significant interaction between methodology and taxonomic class ($F_{11, 384.085} = 21.703$, $P < 0.001$; Figure 2.5). When we examined the most well represented classes separately, we found that CSIA produced significantly lower trophic position estimates than all other methodologies in Actinopterygii and Mammalia, but it produced significantly higher trophic position estimates in Insecta (Table 2.3, Figure 2.5). However, this effect was not consistent across all species, as there was also a significant interaction between methodology and species for Actinopterygii and Mammalia (Table 2.3).

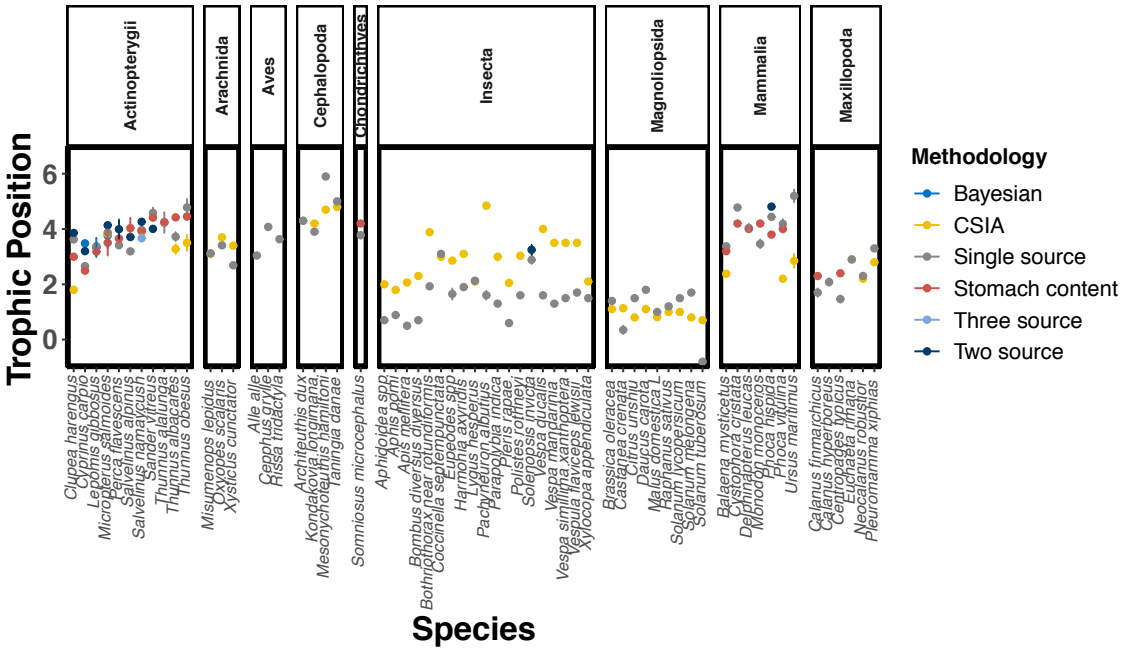


Figure 2.5 Trophic positions extracted from the literature by species, taxonomic class, and methodology. Each point represents a single trophic position measurement. Compound-specific isotope analysis abbreviated to "CSIA."

Table 2.3 Significant effects on trophic position estimates of species from the most well represented taxonomic classes: Actinopterygii, Insecta, Mammalia, and Aves. Methodologies include single source, two source, three source, Bayesian statistics, CSIA, and stomach content analysis. All trophic positions of species within the Aves class were estimated using a single source formula with varying baselines, so baseline was analyzed instead of methodology for Aves. Baselines within the Aves class include basal resource, primary consumer, and consumer. SS represents the total sum of squares, and MS represents mean square.

Actinopterygii

	SS	MS	df	F	*P*
Methodology	6.096	1.219	5, 62.616	6.052	**<0.001**
Species	15.887	1.589	10, 189.276	7.886	**<0.001**
Methodology x Species	9.816	0.446	22, 225.487	2.215	**0.002**

Insecta

	SS	MS	df	F	*P*
Methodology	43.198	21.599	2, 77.342	79.145	**<0.001**
Species	46.365	2.576	18, 6.134	9.439	**0.005**

Mammalia

	SS	MS	df	F	*P*
Methodology	9.987	3.329	3, 107.293	10.392	**<0.001**
Species	4.657	0.776	6, 113.187	2.423	**0.031**
Methodology x Species	2.215	0.316	7, 113.288	0.988	0.444

Aves

	SS	MS	df	F	*P*
Baseline	0.035	0.018	2, 21.867	0.913	0.416
Species	2.053	1.027	2, 21.239	53.391	**<0.001**
Baseline x Species	0.333	0.083	4, 21.216	4.327	**0.010**

Baseline had a significant effect on trophic position estimates from bulk isotope analysis, but there was no interaction between baseline and methodology (Table 2.4; Appendix A S4). When secondary consumers were used as a baseline, estimated trophic positions were significantly lower than when basal resources or primary consumers were used. Baseline also significantly affected trophic position estimates within Aves;

however, this effect varied by species (Table 2.3). For example, trophic position estimates were lowest when consumers were used as a baseline for little auk (*Alle alle*) and black-legged kittiwake (*Rissa tridactyla*) but highest for black guillemot (*Cepphus grylle*) compared with all other baselines.

Table 2.4 Influence of bulk isotope methodologies and baseline on trophic position estimates from ten taxonomic classes in the stable isotope literature. Methodologies include single source, two source, three source, and Bayesian statistics. Baselines include basal resource, primary consumer, secondary consumer, and multiple trophic levels. SS represents the total sum of squares, and MS represents mean square.

	SS	MS	df	F	*P*
Bulk Methdology	1.720	0.573	3, 79.762	2.433	0.071
Baseline	3.400	1.133	3, 371.792	4.811	**0.003**
Class	23.342	2.918	8, 63.655	12.384	**<0.001**
Bulk Methdology x Baseline	1.540	0.385	4, 265.099	1.634	0.166
Bulk Methodology x Class	0.000	0.000	1, 385.053	0.001	0.979

2.4.2.2. Calculating trophic position using stable isotope datasets

As expected, there were significant differences in trophic position estimates by species (e.g., lacewings vs. aphids) across all stable isotope datasets ($F_{131,\ 2.000}$ = 111.436, P = 0.009; Figure 2.6). Methodology also significantly impacted trophic position ($F_{4,\ 3370.100}$ = 144.922, P < 0.001). More importantly, however, we found a significant interaction between methodology and species ($F_{379,\ 3370.100}$ = 23.482, P < 0.001), indicating that differences due to methodology were not consistent across all species. Many authors interpret trophic position estimates relative to traditional food chain levels (i.e., 1 = autotroph, 2 = herbivore, 3 = primary predator, etc.), so this

variation in trophic position estimates could affect our biological interpretations of the data. For example, trophic position estimates for the Japanese spiny lobster (*Panulirus japonicus*) in the *ChikA* dataset (aquatic dataset from Chikaraishi et al. 2014) differed by nearly two trophic levels depending on what baseline and methodology were used: spiny lobster would be considered a tertiary predator if using CSIA (trophic position of 3.9), an omnivore if using a single source formula with an herbivore as a baseline (2.62), and a strict herbivore if using a single source formula with an autotroph as a baseline (1.96; Figure 2.6).

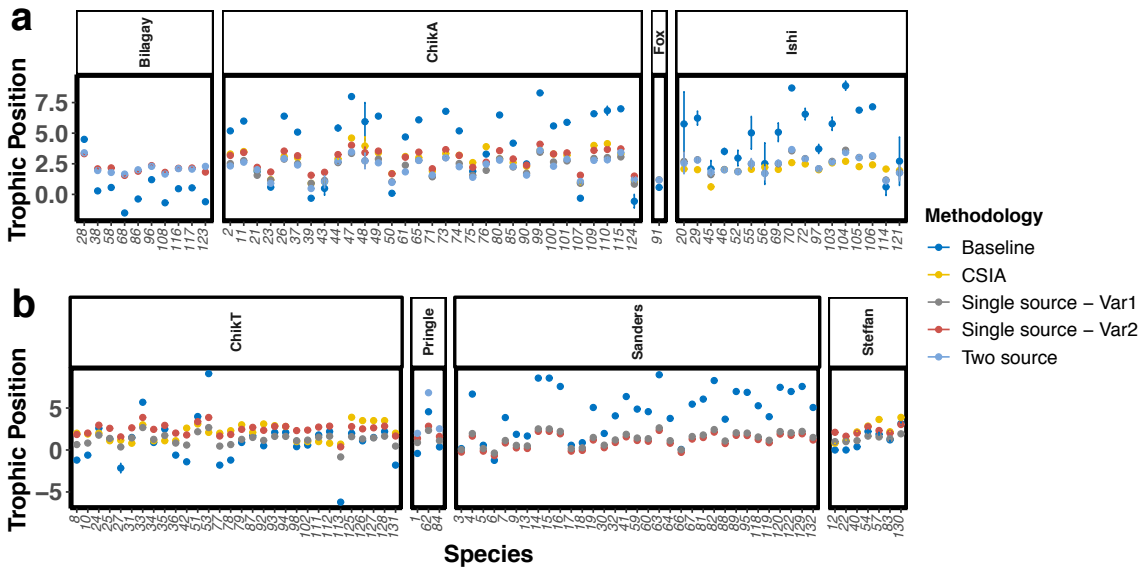


Figure 2.6 Trophic position of each species by methodology using eight stable isotope datasets. a) Aquatic datasets, and b) Terrestrial datasets. Points and error bars represent means ± SE. Species names that correspond to each number can be found in Appendix A S6. Although ten datasets were analyzed, two datasets (*Price* and *Koro*) are not presented here because samples were not identified to species. All trophic positions were calculated using the formulas included in Table 2.1. Single source – Var 1 was calculated using primary producers as the baseline, and Single source – Var 2 was calculated using herbivores as the baseline. Δn = 3.4‰ for all single source and two source calculations. For all compound-specific isotope analysis (CSIA) calculations Δn = 7.6‰, β = -3.4‰ for aquatic organisms, and β = +8.4‰ for terrestrial organisms. Additional information for each dataset can be found in Appendix A S5.

When datasets were analyzed separately, overall trophic position differed significantly by methodology in all five terrestrial datasets and in three out of five aquatic datasets (Table 2.5). However, differences in methodologies were not consistent across all datasets. For example, in the *Steffan* dataset, baseline subtraction produced trophic position estimates that were not significantly different from those produced with

the single source formula (autotroph as base), but these methodologies were significantly different from each other in the *Koro* dataset (Table 2.6). CSIA measurements were available in four datasets (two terrestrial and two aquatic). In both terrestrial datasets, CSIA produced estimates that were the same as the single source formula (herbivore as base). In both aquatic datasets, CSIA produced estimates that were not significantly different from any other methodology. We also examined the effects of different baselines (autotroph vs herbivore) on overall estimates using the single source formula in five datasets (four terrestrial and one aquatic; Table 2.6). We detected significant differences in estimates due to baseline variation in four out of five cases (three terrestrial and one aquatic).

Table 2.5 Effect of methodology on trophic position estimates in ten stable isotope datasets. ANOVA results reported for the *Steffan* dataset only because this was the only dataset with normally distributed residuals (via Shapiro-Wilk test, significance of $P = 0.05$). Results from Kruskal-Wallis nonparametric tests reported for all other datasets.

Effect of methodology (ANOVA)

Steffan

	df	SS	MS	F	*P*
Methodology	3	55.34	18.447	52.843	**<0.001**
Residuals	159	55.51	0.349		

Effect of methodology (Kruskal-Wallis)

	χ^2	df	*P*
Sanders	32.329	2	**<0.001**
Koro	113.83	1	**<0.001**
Pringle	280.34	3	**<0.001**
ChikT	31.566	3	**<0.001**
ChikA	25.332	4	**<0.001**
Ishi	3.1367	3	0.371
Bilagay	52.326	2	**<0.001**
Price	5.6323	2	0.05984
Fox	287	2	**<0.001**

Table 2.6 Trophic positions for each methodology based on ten stable isotope datasets. Different letters represent significant differences in trophic position estimates between methodologies within each dataset based on Tukey's HSD or Dunn's Test (significance of $P = 0.05$).

Ecosystem	Dataset	Methodology	TP	SE	Significant letters
Aquatic	Bilagay	Baseline subtraction	1.84	0.08	a
		Single source (herbivore base)	2.54	0.02	b
		Two source	2.57	0.03	c
	ChikA	Baseline subtraction	4.09	0.45	a
		CSIA	2.71	0.18	ab
		Single source (herbivore base)	2.86	0.13	a
		Single source (autotroph base)	2.2	0.13	b
		Two source	2.19	0.14	b
	Fox	Baseline subtraction	0.25	0.04	a
		Single source (autotroph base)	1.1	0.01	b
		Two source	1.08	0.01	b
	Ishi	Baseline subtraction	3.15	0.34	a
		CSIA	1.71	0.07	a
		Single source (autotroph base)	1.93	0.1	a
		Two source	1.91	0.1	a
	Price	Baseline subtraction	1.23	0.11	a
		Single source (autotroph base)	1.36	0.03	a
		Two source	1.28	0.09	a
	ChikT	Baseline subtraction	0.88	0.48	a
		CSIA	2.04	0.18	b
		Single source (herbivore base)	2.43	0.13	b
		Single source (autotroph base)	1.23	0.13	a
	Koro	Baseline subtraction	5.69	0.21	a
		Single source (autotroph base)	2.67	0.06	b
Terrestrial	Pringle	Baseline subtraction	3.06	0.15	a
		Single source (herbivore base)	2.4	0.05	c
		Single source (autotroph base)	1.9	0.05	d
		Two source	5.3	0.15	b
	Sanders	Baseline subtraction	4.37	0.46	a
		Single source (herbivore base)	0.98	0.14	b
		Single source (autotroph base)	1.29	0.14	b
	Steffan	Baseline subtraction	1.14	0.2	a
		CSIA	2.48	0.18	b
		Single source (herbivore base)	2.24	0.06	b
		Single source (autotroph base)	1.33	0.06	a

2.4.3. Evaluating the consequences on food web structure

When we examined the effect of methodology on the number of statistically different trophic groups, we found variation in five out of ten datasets (two terrestrial and three aquatic; Table 2.7 and Figure 2.7). For example, in the *ChikT* dataset, there were four statistically distinct trophic groups detected when using CSIA, but none of these trophic groups were significantly different from each other when using any of the other methodologies. In almost all cases, this variation was due to differences between CSIA and all other methodologies, suggesting improved resolution from reduced variance using CSIA compared with bulk analysis. The one exception was in the *Price* dataset, in which there were no statistically different trophic groups when using the two source formula, but there were three significantly different trophic groups detected when using all other methodologies. The number of trophic groups detected was the same regardless of which baseline was used (Table 2.7).

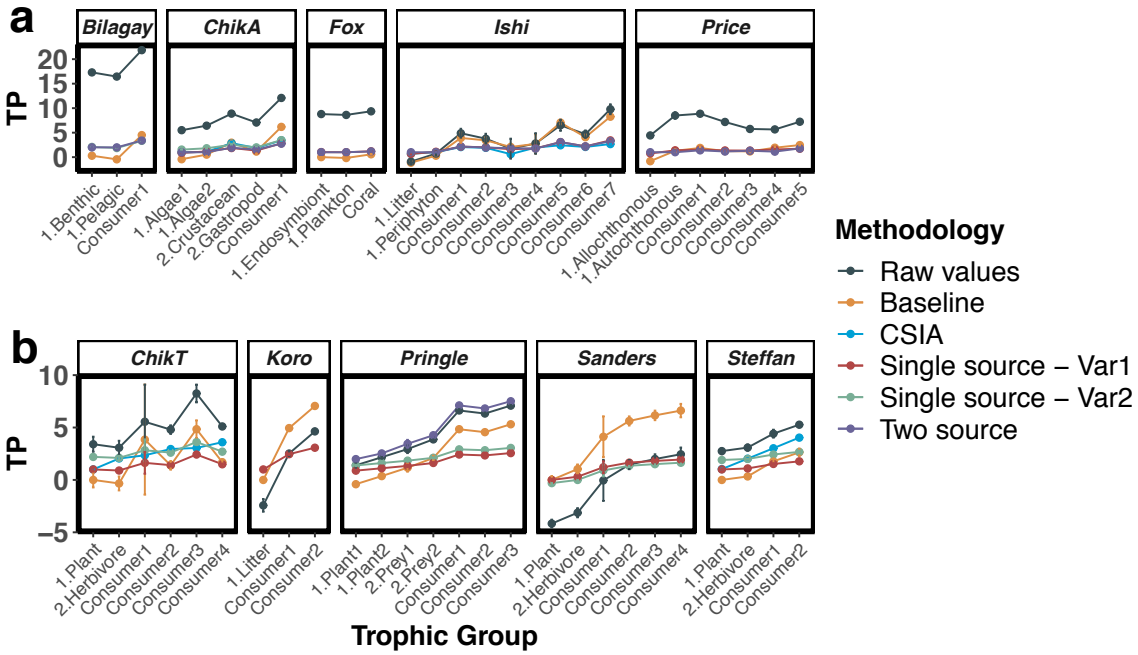


Figure 2.7 Trophic groups by methodology using ten stable isotope datasets. a) **Terrestrial datasets and** b) **Aquatic datasets, with estimated trophic position (TP) by trophic group and methodology. Points and error bars represent means ± SE. All trophic positions were calculated using the formulas included in Table 2.1. Single source – Var 1 was calculated using primary producers as the baseline, and Single source – Var 2 was calculated using herbivores as the baseline. Δn = 3.4‰ for all single source and two source calculations. For all compound-specific isotope analysis (CSIA) calculations Δn = 7.6‰, β = -3.4‰ for aquatic organisms, and β = +8.4‰ for terrestrial organisms. Additional information for each dataset can be found in Appendix A S5.**

Table 2.7 Summary of the effects of different baselines and methodologies on food web structure. Number of significantly different trophic groups and effects of sampling location (when applicable) are reported for each methodology and dataset, as well as the minimum sample size collected for at least one trophic group.

System	Data	Structural measurements	Raw	CSIA	Single source - Var1 (autotroph as base)	Single source - Var2 (herbivore as base)	Two source	Baseline subtraction	Differences in food web structure by methodology?	Min sample size
Terrestrial	ChikT	# trophic groups	1	4	1	1	-	1	Yes	2
	Koro	# trophic groups Diff. by location?	3 No	- -	3 Yes	- -	- -	3 Yes	Yes	2
	Pringle	# trophic groups	4	-	4	4	4	4	No	20
	Sanders	# trophic groups	2	-	2	2	-	2	No	3
	Steffan	# trophic groups Diff. by location?	2 Yes	4 Yes	2 No	2 Yes	- -	2 No	Yes	3
Aquatic	Bilagay	# trophic groups Diff. by location?	3 Yes	- -	- -	3 Yes	3 Yes	3 Yes	No	10
	ChikA	# trophic groups	3	2	3	3	3	3	Yes	3
	Fox	# trophic groups Diff. by location?	2 Yes	- -	2 Yes	- -	2 No	2 No	Yes	1
	Ishi	# trophic groups Diff. by location?	3 Yes	4 Yes	3 No	- -	3 No	3 No	Yes	1
	Price	# trophic groups Diff. by location?	3 Yes	- -	3 Yes	- -	1 Yes	3 Yes	Yes	2

We also examined differences in trophic position estimates by sampling location when applicable (two terrestrial and four aquatic datasets; Table 2.7), as several studies compared trophic structure in multiple different sites. The effect of location differed depending on methodology in four out of six datasets (two terrestrial and two aquatic).

For example, in the *Fox* dataset, trophic position differed significantly between sampling locations when using raw isotope values or a single source formula (autotroph as base), but trophic position did not differ by location when using a two source formula or baseline subtraction. Only one dataset included information for sampling location and two different baselines (the *Steffan* dataset). Interestingly, sampling location was not significant when autotrophs were used as a baseline, but location became significant when herbivores were used as a baseline instead (Table 2.7).

Overall, only three out of the ten total datasets resulted in the same conclusions regardless of methodology (*Pringle*, *Sanders*, and *Bilagay* datasets; Table 2.7). At least some of this may be due to sample size. Of the six datasets in which conclusions differed by methodology, a minimum of one to three samples was collected for at least one trophic group. By contrast, datasets in which conclusions did not differ by methodology collected a minimum of three (*Sanders*), 10 (*Bilagay*), and 20 (*Pringle*) samples per trophic group (Table 2.7).

2.4.4. Power and sample size analysis

Based on our review of a subset of the overall literature, approximately 41% of all studies collected only one sample in at least one of the trophic groups analyzed, and 26% of all studies did not specify how many samples were collected (Figure 2.8). The methodology used by authors was independent of the minimum number of samples authors collected per trophic group ($\chi^2 = 76.682$, df = 70, $P = 0.273$). Authors also

collected the same number of samples regardless of ecosystem type ($\chi^2 = 7.196$, df = 10, $P = 0.707$).

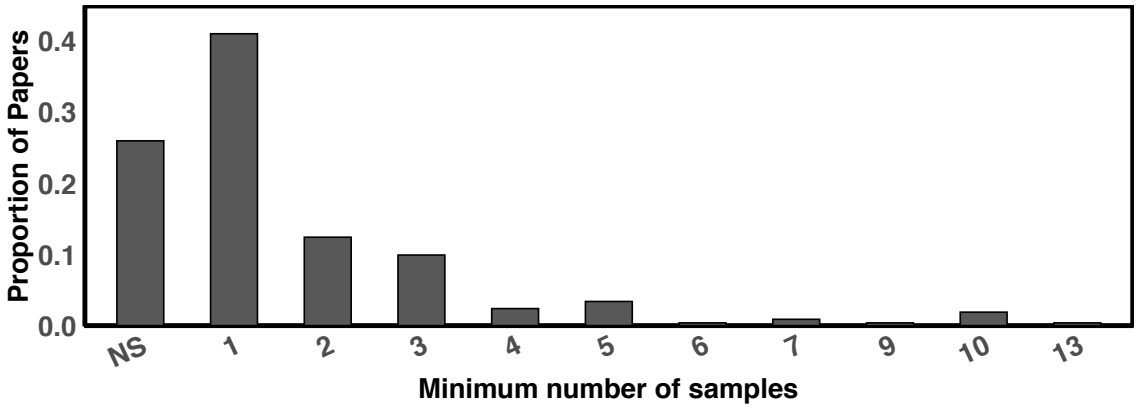


Figure 2.8 Proportion of papers (out of 200 total publications) and the minimum number of samples that were collected per trophic group.

Statistical power analyses revealed that CSIA required the fewest number of samples (1.3 ± 0.4) compared with any bulk-tissue isotope technique in order to achieve 95% confidence in trophic position (Figure 2.9). When only bulk-tissue isotope techniques were considered, raw values and baseline subtraction required the most samples (35.8 ± 11.1 and 34.7 ± 11.8, respectively) compared with single source (4.4 ± 0.8) or two source (14.4 ± 9.8) food web formulas (Figure 2.9).

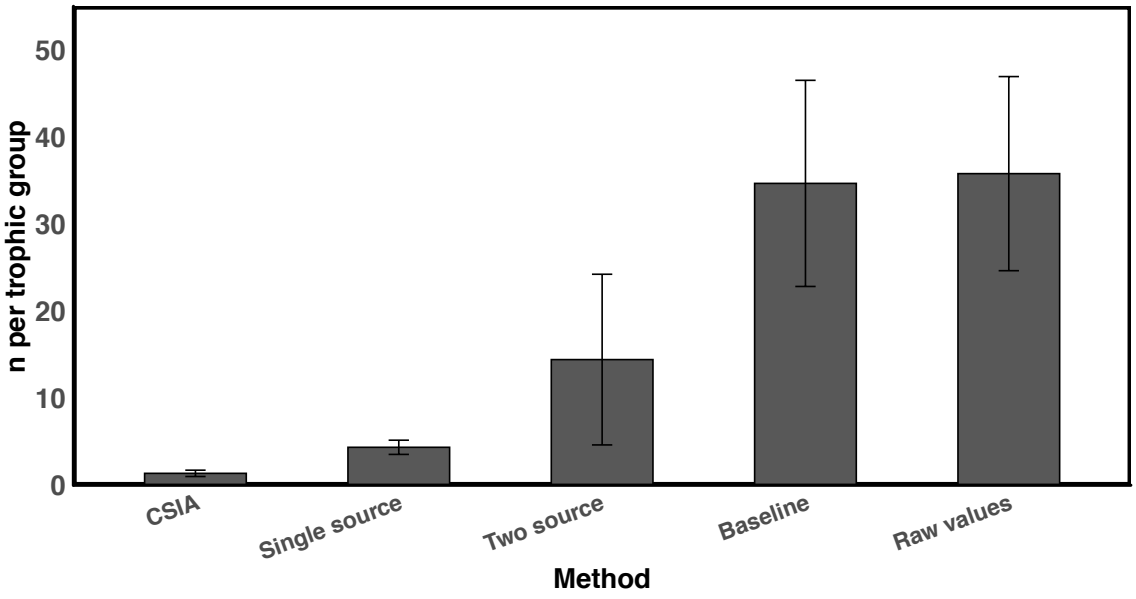


Figure 2.9 Power analysis results by methodology. Results are based on analysis of ten stable isotope datasets. An $\alpha = 0.05$ was used with a maximum difference between trophic positions of 1 and a power level of 0.80. Compound-specific isotope analysis abbreviated to "CSIA." Columns and error bars represent means ± SE.

2.5. Discussion

Our review of the stable isotope literature reveals that choosing the appropriate baseline and model to estimate trophic position is important, but there is currently large variation in which baselines and models are used in the literature. Authors are choosing not between one or two possible models and baselines, but instead from a number of baselines, techniques, and methodologies (Table 2.1, Figure 2.2 and 2.3). Additionally, information on what technique or model to use is conflicting or limited in most cases, especially in terrestrial studies (Figure 2.1). Moreover, we show that different baselines and methodologies can yield significantly different trophic position estimates for

individual species (Tables 2.3 and 2.4; Figure 2.5 and 2.6), as well as conclusions about overall food web structure (Table 2.7; Figure 2.7). Several other studies have compared the effects of two or three models on estimates of trophic position (Post 2002, Layman et al. 2012), but we believe we are the first to identify and examine the effects of most of the major methodologies currently in use. Considering the increasingly large number of studies that use stable isotopes to estimate trophic position (Figure 2.1), it is critical to establish more standardized practices to reduce potential biases in the literature. Information based on stable isotope data is being used to answer a variety of important basic and applied questions, including disease vector management (Rasgon 2008, Stapp and Salkeld 2009, Gilbreath et al. 2013, Heylen et al. 2019), conservation (Darimont and Reimchen 2002, Alves-Stanley et al. 2010, Merkle et al. 2011, Evans et al. 2012), and climate change assessment (Pomerleau et al. 2017), so inaccurate estimates due to variation in baseline and methodology could have far-reaching effects.

2.5.1. Patterns of variation in the literature

We found that authors focusing on aquatic ecosystems tended to use a single source formula, whereas authors focusing on terrestrial ecosystems used baseline subtraction or statistical analyses (which included a variety of Frequentist and Bayesian approaches; Figure 2.2). There are obvious differences between aquatic and terrestrial ecosystems (i.e., hydrology, variation in the size and nutritional quality of autotrophs, different trophic level-size relationships, stoichiometry, etc.), all of which influences food-web architecture and complexity (Cebrian 1999, Shurin et al. 2005). Despite these

differences, each methodology is theoretically appropriate for all ecosystem types. For example, in the original publication that formally described the single source formula, Post (2002) states the broad applicability of his approach to all ecosystems (although he focuses primarily on lakes throughout the paper). The discrepancy in methodology used between biomes, therefore, could be due to unequal representation in the literature. As other authors have noted (Boecklen et al. 2011), the stable isotope literature appears to be dominated by "wet things and wet places." Over 90% of all studies were conducted in aquatic ecosystems (Figure 2.1), with a primary focus on a few main taxonomic groups (Figure 2.4). By contrast, terrestrial ecosystem studies rarely shared the same species (Table 2.2).

Many aquatic ecosystems studies have verified the accuracy of stable isotope values by comparing with traditional techniques such as gut content analysis (Vander Zanden et al. 1997, Zambrano et al. 2010, Germain et al. 2013, Mancinelli et al. 2013, Cresson et al. 2014, Amezcua et al. 2015, Nielsen et al. 2015), but these comparisons are rare in terrestrial ecosystems (Sabat et al. 2013; but see Paszkowski et al. 2004, Campbell et al. 2017, Molina-Burgos et al. 2018). For example, Sabat et al. (2013) found that $\delta^{15}N$ values of South American passerine birds did not correspond with their observed diet. Although each approach is theoretically applicable to all food webs, variation among taxa and habitats may require a unique baseline or methodology for more accurate results (Jardine et al. 2014). Additional comparisons of stable isotope data with more traditional approaches (such as gut content analysis or direct observation), particularly in terrestrial habitats, will help identify the best methodology and baseline

moving forward. As many other authors have pointed out (Gannes et al. 1997, Martinez del Rio et al. 2009, Boecklen et al. 2011), experimental studies are lacking in this field (but see Menke et al. 2010, Steffan et al. 2013, Chikaraishi et al. 2014, Downs et al. 2014, Bowes and Thorp 2015), and these types of studies will be critical to ultimately determine the best method moving forward for each ecosystem type.

2.5.2. Are biological interpretations of absolute trophic positions useful?

Our review leads us to question whether or not biological interpretations of absolute trophic position estimates from stable isotope data are useful for ecology research. Many authors directly interpret absolute trophic positions in the context of a traditional food chain, in which 1 = primary producer, 2 = primary consumer, 3 = secondary consumer, etc. (i.e., Wilder et al. 2011, Campbell et al. 2017, Letvin et al. 2017, Molina-Burgos et al. 2018). Although this makes sense in theory, we show that these absolute estimates can change dramatically depending on the baseline and methodology used (Figures 2.5 and 2.6), which affects the biological interpretations of the same datasets. This may lead some authors to choose one model over another (Letvin et al. 2017), not necessarily because the model is more accurate, but because the model produces absolute trophic positions that fit within the traditional food chain scale. For example, using the same isotope dataset (*ChikT*), baseline subtraction yielded estimates that extended far beyond the traditional scale (i.e., 1-5 trophic levels) for the Japanese broad-winged katydid (*Holochlora japonica*; trophic position of 9.1) compared with estimates using CSIA (2.1), a single source formula with autotrophs as the baseline (2.7),

and a single source formula with herbivores as the baseline (3.9; Figure 2.6). When comparing across studies, it then becomes difficult to know if variation in absolute trophic positions are due to differences in diet or due to differences in the methodology or baseline used to generate these numbers. In our analysis of published trophic positions, absolute values differed in some cases by over two trophic levels depending on what methodology was used. For example, the trophic position of harbor seal (*Phoca vitulina*) was 2.2 (strict herbivore) when using CSIA compared with 4.2 and 4 (tertiary predator) when using a single source formula and traditional stomach content analysis respectively (Figure 2.5). Reviews that compile and average trophic positions of organisms from various studies (Pauly et al. 1998, Das et al. 2003, Potapov et al. 2019a) may not account for possible effects of methodology or baseline (but see Potapov et al. 2019a), which would lead to further biases in the literature.

Additionally, biological interpretations of absolute trophic positions lack the rigor of statistical analysis and can be easily altered (however unintentionally) to fit different narratives. For example, Chikaraishi et al. (2011) stated that CSIA was accurate because bee trophic position as indicated by CSIA (2.0 – 2.3) was consistent with their biologically expected trophic position as strict herbivores (2.0). Steffan et al. (2019), however, interpreted similar trophic positions (2.1 – 3.3) to mean that bees were actually omnivores because all trophic positions in their study were significantly higher than 2.0. We argue that we should be exceedingly cautious when interpreting individual and absolute trophic position estimates, as these estimates are prone to a number of biases. It may be useful to use trophic position estimates as a complement to more traditional

approaches such as gut content analysis or direct observation (Seminoff et al. 2012, Kloskowski and Trembaczowski 2015). By pairing both techniques, specific food items can be identified using gut content analysis or direct observation (a snap-shot of the diet), and then the relative importance of each food item can be assessed using stable isotope analysis (a more time-integrated evaluation of diet). Interpretations of absolute trophic positions may also become more robust when values are compared relative to multiple other organisms collected from the same habitat. For example, Hellmann et al. (2013) confirmed the omnivorous nature of freshwater shrimp by comparing their trophic positions to those of known predators and strict herbivores within the same stream.

2.5.3. Effects on food web structure

Comparing multiple trophic groups from the same habitat may be more robust than biological interpretations of absolute values; however, we found that conclusions about food web structure can also be affected by baseline and methodology (Table 2.7, Figure 2.7). In our analysis of the *Price* dataset, for example, there were three statistically distinct trophic groups when using raw isotope values, a single source formula with autotrophs as a baseline, and baseline subtraction, but only one trophic group when using a two source formula (Table 2.7, Figure 2.7a). Many studies quantify biomagnification of important contaminants in food webs based on stable isotope analysis of food web structure (Bowles et al. 2001, Di Beneditto et al. 2012, Pethybridge et al. 2012, Coelho et al. 2013, Schneider et al. 2015, Azevedo-Silva et al. 2016).

Inaccurate conclusions about the number of trophic groups would impact estimates of contaminant accumulation from one trophic level to the next. Coelho et al. (2013), for example, found a significantly positive linear relationship between trophic level and mercury biomagnification in a contaminated estuary. If there were fewer distinct trophic levels because an alternative methodology was used, however, there may be no statistically significant relationship between trophic level and biomagnification, thereby suggesting a more limited effect of contamination on the food web.

In addition to the number of trophic groups, variation in baseline and methodology impacted conclusions about differences by sampling location (Table 2.7). When we analyzed the effect of location on trophic position estimates in the *Steffan* dataset, for example, sampling location was not significant when autotrophs were used as a baseline, but location became significant when herbivores were used as a baseline instead (Table 2.7). Studies often correlate geographic variation in trophic levels with other factors to determine their effects on trophic interactions, many of which focus on changes due to anthropogenic disturbance (Bowes et al. 2020, Oczkowski et al. 2020, Pingram et al. 2020). Oczkowski et al. (2020), for example, found that geography, rather than anthropogenic disturbance, predicted the trophic levels of fish sampled over a 150-year period. Our analysis suggests, however, that if a different baseline or methodology was used to estimate those trophic levels, conclusions about the effect of geographic location may change and anthropogenic disturbance might have a greater impact instead. Additional experimental studies will be necessary to ultimately determine the best baseline and methodology, but increased sample size may mitigate some of the variation

caused by different baselines and methodologies. Datasets that collected over three samples per trophic group resulted in the same overall conclusions about food web structure (Table 2.7). Although these results are based on a small number of datasets, adequate sample size appears to be a pervasive problem in ecological research, and this likely impacts the reliability of conclusions from stable isotope data.

2.5.4. The issue of sample size

Based on our review of the literature, an alarmingly large proportion of studies either collected only one sample in at least one trophic group (usually the non-focal group) or did not report how many they collected at all (Figure 2.8), highlighting a critical need to increase stable isotope sample size in trophic ecology research. Low sample size decreases statistical power, which lowers the probability that a research finding reflects a true effect (type I error). To achieve recommended statistical power (80%), we found that studies needed to collect at least five to thirty-six samples on average per trophic group if using bulk isotope analysis and at least two samples on average if using compound-specific isotope analysis (Figure 2.9). Regardless of methodology, however, only ~8% of studies collected five or more samples for each trophic group (Figure 2.8). The vast majority instead collected only one sample for at least one trophic group (41%) or did not specify how many were collected (26%). It is important to note that many of these authors may have collected a sufficient number of samples for some trophic groups (i.e., their focal taxa) but used one sample (sometimes of several pooled individuals) to represent a non-target trophic group. For example,

Ishikawa et al. (2014) collected 4-8 replicates per site of periphyton (used as a baseline) but only 1-2 replicates per site of samples representing the "shredder" invertebrate functional group. Using a single sample to represent a trophic group can affect downstream analyses and reduces the statistical reliability of conclusions. In a study using stable isotopes to identify the food sources of consumers, Phillips and Gregg (2001) found that increasing the sample size from just one to four can reduce the uncertainty of source estimations by over half. The issue of appropriate sample size is a widespread problem in scientific research (Houle et al. 2011, Button et al. 2013, Grabowski and Porto 2017, Munafò et al. 2017) and has been identified as one of the primary factors preventing reproducibility in science (Baker 2016).

Based on our data, we believe it is critically important for trophic ecology researchers to collect at least five (but more than ten for best practices) individuals (or groups of individuals when pooled) per trophic group analyzed, regardless of methodology (Figure 2.9). When sample size is unavoidably constrained (i.e., many wildlife studies are limited in the numbers of samples that can be collected (Bissonette 1999)), we encourage authors to use compound-specific isotope analysis (CSIA) whenever possible. Although this approach requires more processing and is more expensive, it may require fewer samples to maintain appropriate statistical power and can provide more accurate estimates of trophic position (Steffan et al. 2013, Chikaraishi et al. 2014, Bowes and Thorp 2015). We stress the importance, however, of using a species- or taxon-specific trophic discrimination factor (i.e., the amount ^{15}N changes moving up the food chain) to ensure the most accurate results. Based on our review of

published trophic positions, CSIA produced estimates that were higher (Insecta) and lower (Actinopterygii and Mammalia) than estimates based on the use of other methodologies (Figure 2.5). The published trophic positions we extracted primarily used one standardized trophic discrimination factor for CSIA (7.6‰; Appendix A S2), but this number has been shown to differ by taxonomic groups (Popp et al. 2007, Germain et al. 2013, Steffan et al. 2013, Lorrain et al. 2015, Blanke et al. 2017, Pomerleau et al. 2017, Pollierer et al. 2019), which may be why we observed an interaction between CSIA and class. For example, Germain et al. (2013) found that the estimated trophic position of harbor seals (*P. vitulina*) was an entire trophic level higher when a trophic discrimination factor of 4.3‰ was used instead of 7.6‰ (trophic position of 3.2 and 2.2 respectively). In order to generate the most accurate and reliable conclusions from CSIA, we recommend additional experimental trials to establish species- or taxon-specific trophic discrimination factors (Steffan et al. 2013, Chikaraishi et al. 2014, Bowes and Thorp 2015).

2.6. Conclusions

Our review is a first step in identifying a lack of standardization in baseline and methodology for estimating trophic position in the stable isotope literature, and we show that both have a significant effect on conclusions drawn from stable isotope research. Trophic position estimates based on stable isotopes are currently being used to inform important management decisions (Merkle et al. 2011, Gilbreath et al. 2013, Senior et al. 2013, Heylen et al. 2019, James et al. 2020), so it is essential to standardize our approach

to generate more accurate and reliable conclusions. We recommend moving away from biological interpretations of absolute trophic positions, and instead comparing these values relative to other organisms collected from the same habitat. Adequate sample size is a pervasive problem in stable isotope trophic ecology research, and authors should collect a minimum of five samples per trophic group (but ten for best-practices) from as many trophic groups as possible to increase statistical power and redundancy in our comparisons. Compound-specific isotope analysis may require fewer samples to maintain appropriate statistical power, so we recommend using this technique with species- or taxon-specific trophic discrimination factors whenever possible, especially for studies that are limited by sample size (i.e., wildlife conservation, museum specimens, etc.). Additional experimental studies are lacking in this field (Gannes et al. 1997, Martinez del Rio et al. 2009, Boecklen et al. 2011), and these types of studies, especially in terrestrial habitats, will ultimately be necessary to determine the most appropriate method and baseline to use moving forward.

2.7. References

Adams, T. S., and R. W. Sterner. 2000. The effect of dietary nitrogen content on trophic level ^{15}N enrichment. Limnology and Oceanography **45**:601-607.

Alves-Stanley, C. D., G. A. Worthy, and R. K. Bonde. 2010. Feeding preferences of West Indian manatees in Florida, Belize, and Puerto Rico as indicated by stable isotope analysis. Marine Ecology Progress Series **402**:255-267.

Amezcua, F., V. Muro Torres, M. Soto Jiménez, and M. Soto Jimenez. 2015. Stable isotope analysis versus TROPH: a comparison of methods for estimating fish trophic positions in a subtropical estuarine system. Aquatic Ecology **49**:235-250.

Amundson, R., A. T. Austin, E. A. Schuur, K. Yoo, V. Matzek, C. Kendall, A. Uebersax, D. Brenner, and W. T. Baisden. 2003. Global patterns of the isotopic composition of soil and plant nitrogen. Global Biogeochemical Cycles **17**.

Arostegui, M. C., D. E. Schindler, and G. W. Holtgrieve. 2019. Does lipid-correction introduce biases into isotopic mixing models? Implications for diet reconstruction studies. Oecologia **191**:745-755.

Azevedo-Silva, C. E., R. Almeida, D. P. Carvalho, J. P. Ometto, P. B. de Camargo, P. R. Dorneles, A. Azeredo, W. R. Bastos, O. Malm, and J. P. Torres. 2016. Mercury biomagnification and the trophic structure of the ichthyofauna from a remote lake in the Brazilian Amazon. Environmental Research **151**:286-296.

Baker, M. 2016. Is there a reproducibility crisis? Nature **533**:353-366.

Bastos, R. F., F. Corrêa, K. O. Winemiller, and A. M. Garcia. 2017. Are you what you eat? Effects of trophic discrimination factors on estimates of food assimilation and trophic position with a new estimation method. Ecological Indicators **75**:234-241.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software **67**:1-48.

Benoit, K., A. Matsuo, and M. K. Benoit. 2018. R Package:'spacyr.'. London.

Bissonette, J. A. 1999. Small sample size problems in wildlife ecology: a contingent analytical approach. Wildlife Biology **5**:65-71.

Blaber, S. J., D. Cyrus, J.-J. Albaret, C. V. Ching, J. Day, M. Elliott, M. Fonseca, D. Hoss, J. Orensanz, and I. Potter. 2000. Effects of fishing on the structure and functioning of estuarine and nearshore ecosystems. ICES Journal of Marine Science **57**:590-602.

Blanke, C. M., Y. Chikaraishi, Y. Takizawa, S. A. Steffan, P. S. Dharampal, and M. J. Vander Zanden. 2017. Comparing compound-specific and bulk stable nitrogen isotope trophic discrimination factors across multiple freshwater fish species and diets. Canadian Journal of Fisheries and Aquatic Sciences **74**:1291-1297.

Boecklen, W. J., C. T. Yarnes, B. A. Cook, and A. C. James. 2011. On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution and Systematics **42**:411-440.

Bowes, R., J. Thorp, and M. Delong. 2020. Reweaving river food webs through time. Freshwater Biology **65**:390-402.

Bowes, R. E., and J. H. Thorp. 2015. Consequences of employing amino acid vs. bulk-tissue, stable isotope analysis: a laboratory trophic position experiment. Ecosphere **6**:1-12.

Bowles, K. C., S. C. Apte, W. A. Maher, M. Kawei, and R. Smith. 2001. Bioaccumulation and biomagnification of mercury in lake Murray, Papua New Guinea. Canadian Journal of Fisheries and Aquatic Sciences **58**:888-897.

Britton, J. R., G. Davies, and C. Harrod. 2010. Trophic interactions and consequent impacts of the invasive fish *Pseudorasbora parva* in a native aquatic foodweb: a field investigation in the UK. Biological Invasions **12**:1533-1542.

Button, K. S., J. P. Ioannidis, C. Mokrysz, B. A. Nosek, J. Flint, E. S. Robinson, and M. R. Munafò. 2013. Power failure: why small sample size undermines the reliability of neuroscience. Nature Reviews Neuroscience **14**:365-376.

Cabana, G., and J. B. Rasmussen. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature **372**:255.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences **93**:10844-10847.

Caddy, J. F., and L. Garibaldi. 2000. Apparent changes in the trophic composition of world marine harvests: the perspective from the FAO capture database. Ocean & Coastal Management **43**:615-655.

Cameron, S. J. K., M. Baltazar Soares, V. Stiebens, T. Reischig, S. Correia, C. Harrod, and C. Eizaguirre. 2019. Diversity of feeding strategies in loggerhead sea turtles from the Cape Verde archipelago. Marine Biology **166**:130.

Campbell, C. J., D. M. Nelson, N. O. Ogawa, Y. Chikaraishi, and N. Ohkouchi. 2017. Trophic position and dietary breadth of bats revealed by nitrogen isotopic composition of amino acids. Scientific Reports **7**:15932.

Caut, S., E. Angulo, and F. Courchamp. 2008. Dietary shift of an invasive predator: rats, seabirds and sea turtles. Journal of Applied Ecology **45**:428-437.

Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology **46**:443-453.

Cebrian, J. 1999. Patterns in the fate of production in plant communities. The American Naturalist 154:449-468.

Champely, S., C. Ekstrom, P. Dalgaard, and J. Gill. 2018. pwr: basic functions for power analysis. R Package Version 1.2.

Chikaraishi, Y., Y. Kashiyama, N. O. Ogawa, H. Kitazato, and N. Ohkouchi. 2007. Metabolic control of nitrogen isotope composition of amino acids in macroalgae and gastropods: implications for aquatic food web studies. Marine Ecology Progress Series **342**:85-90.

Chikaraishi, Y., N. O. Ogawa, H. Doi, and N. Ohkouchi. 2011. $^{15}N/^{14}N$ ratios of amino acids as a tool for studying terrestrial food webs: a case study of terrestrial insects (bees, wasps, and hornets). Ecological Research **26**:835-844.

Chikaraishi, Y., N. O. Ogawa, Y. Kashiyama, Y. Takano, H. Suga, A. Tomitani, H. Miyashita, H. Kitazato, and N. Ohkouchi. 2009. Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. Limnology and Oceanography: Methods **7**:740-750.

Chikaraishi, Y., S. Steffan, N. Ogawa, N. Ishikawa, Y. Sasaki, M. Tsuchiya, and N. Ohkouchi. 2014. High-resolution food webs based on nitrogen isotopic composition of amino acids. Ecology & Evolution **4**:2423-2449.

Coelho, J., C. Mieiro, E. Pereira, A. Duarte, and M. Pardal. 2013. Mercury biomagnification in a contaminated estuary food web: effects of age and trophic position using stable isotope analyses. Marine Pollution Bulletin **69**:110-115.

Cooke, S., and I. Cowx. 2006. Contrasting recreational and commercial fishing: Searching for common issues to promote unified conservation of fisheries resources and aquatic environments. Biological Conservation **128**:93-108.

Couey, F. M. 1935. Fish food studies of a number of northeastern Wisconsin lakes. Wisconsin Academy of Sciences, Arts and Letters **29**:131-172.

Cresson, P., S. Ruitton, M. Ourgaud, and M. Harmelin-Vivien. 2014. Contrasting perception of fish trophic level from stomach content and stable isotope analyses: a Mediterranean artificial reef experience. Journal of Experimental Marine Biology and Ecology **452**:54-62.

Croteau, M. N., S. N. Luoma, and A. R. Stewart. 2005. Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnology and Oceanography **50**:1511-1519.

Crotty, F. V., R. P. Blackshaw, S. M. Adl, R. Inger, and P. J. Murray. 2014. Divergence of feeding channels within the soil food web determined by ecosystem type. Ecology and Evolution **4**:1-13.

Da Silva, D. S., M. Lucotte, M. Roulet, H. Poirier, D. Mergler, E. O. Santos, and M. Crossa. 2005. Trophic structure and bioaccumulation of mercury in fish of three natural lakes of the Brazilian Amazon. Water, Air, and Soil Pollution **165**:77-94.

Darimont, C. T., and T. E. Reimchen. 2002. Intra-hair stable isotope analysis implies seasonal shift to salmon in gray wolf diet. Canadian Journal of Zoology **80**:1638-1642.

Darnell, R. M. 1961. Trophic spectrum of an estuarine community, based on studies of Lake Pontchartrain, Louisiana. Ecology **42**:553-568.

Das, K., G. Lepoint, Y. Leroy, and J.-M. Bouquegneau. 2003. Marine mammals from the southern North Sea: feeding ecology data from $\delta^{13}C$ and $\delta^{15}N$ measurements. Marine Ecology Progress Series **263**:287-298.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta **45**:341-351.

Des Roches, S., L. J. Harmon, and E. B. Rosenblum. 2016. Colonization of a novel depauperate habitat leads to trophic niche shifts in three desert lizard species. Oikos **125**:343-353.

Di Beneditto, A. P. M., V. T. Bittar, P. B. Camargo, C. E. Rezende, and H. A. Kehrig. 2012. Mercury and nitrogen isotope in a marine species from a tropical coastal food web. Archives of Environmental Contamination and Toxicology **62**:264-271.

Docmac, F., M. Araya, I. A. Hinojosa, C. Dorador, and C. Harrod. 2017. Habitat coupling writ large: pelagic-derived materials fuel benthivorous macroalgal reef fishes in an upwelling zone. Ecology **98**:2267-2272.

Downs, E. E., B. N. Popp, and C. M. Holl. 2014. Nitrogen isotope fractionation and amino acid turnover rates in the Pacific white shrimp *Litopenaeus vannamei*. Marine Ecology Progress Series **516**:239-250.

Evangelista, C., A. Lecerf, J. R. Britton, and J. Cucherousset. 2017. Resource composition mediates the effects of intraspecific variability in nutrient recycling on ecosystem processes. Oikos **126**:1439-1450.

Evans, K. L., J. Newton, J. W. Mallord, and S. Markman. 2012. Stable isotope analysis provides new information on winter habitat use of declining avian migrants that is relevant to their conservation. PLoS One **7**:e34542.

Feranec, R., and B. MacFadden. 2000. Evolution of the grazing niche in Pleistocene mammals from Florida: evidence from stable isotopes. Palaeogeography, palaeoclimatology, palaeoecology **162**:155-169.

Fetcher, N., S. J. Agosta, J. C. Moore, J. A. Stratford, and M. A. Steele. 2015. The food web of a severely contaminated site following reclamation with warm season grasses. Restoration Ecology **23**:421-429.

Fox, M. D., E. A. Elliott Smith, J. E. Smith, and S. D. Newsome. 2019. Trophic plasticity in a common reef-building coral: Insights from $\delta^{13}C$ analysis of essential amino acids. Functional Ecology **33**:2203-2214.

Frisch, A. J., M. Ireland, J. R. Rizzari, O. M. Lönnstedt, K. A. Magnenat, C. E. Mirbach, and J.-P. A. Hobbs. 2016. Reassessing the trophic role of reef sharks as apex predators on coral reefs. Coral Reefs **35**:459-472.

Froese, R., and D. Pauly. 2000. FishBase 2000: concepts designs and data sources. WorldFish.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnology and Oceanography **33**:1182-1190.

Fry, B. 2006. Stable isotope ecology. Springer.

Gannes, L. Z., D. M. O'Brien, and C. M. Del Rio. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology **78**:1271-1276.

Geiger, W., P. Alcorlo, A. Baltanás, C. Montes, and A. Baltanas. 2005. Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands. Biological Invasions **7**:49-73.

Germain, L. R., P. L. Koch, J. Harvey, and M. D. McCarthy. 2013. Nitrogen isotope fractionation in amino acids from harbor seals: implications for compound-specific trophic position calculations. Marine Ecology Progress Series **482**:265-277.

Gibb, H., and S. A. Cunningham. 2011. Habitat contrasts reveal a shift in the trophic position of ant assemblages. Journal of Animal Ecology **80**:119-127.

Gibb, H., J. Stoklosa, D. I. Warton, A. M. Brown, N. R. Andrew, and S. A. Cunningham. 2015. Does morphology predict trophic position and habitat use of ant species and assemblages? Oecologia **177**:519-531.

Gilbreath, T. M., E. J. Kweka, Y. A. Afrane, A. K. Githeko, and G. Yan. 2013. Evaluating larval mosquito resource partitioning in western Kenya using stable isotopes of carbon and nitrogen. Parasites & Vectors **6**:353.

Grabowski, M., and A. Porto. 2017. How many more? Sample size determination in studies of morphological integration and evolvability. Methods in Ecology and Evolution **8**:592-603.

Gratton, C., and R. F. Denno. 2006. Arthropod food web restoration following removal of an invasive wetland plant. Ecological Applications **16**:622-631.

Guzzo, M. M., G. D. Haffner, S. Sorge, S. A. Rush, and A. T. Fisk. 2011. Spatial and temporal variabilities of $\delta^{13}C$ and $\delta^{15}N$ within lower trophic levels of a large lake: implications for estimating trophic relationships of consumers. Hydrobiologia **675**:41-53.

Hamer, K. C., R. J. Newton, F. A. Edwards, S. Benedick, S. H. Bottrell, and D. P. Edwards. 2015. Impacts of selective logging on insectivorous birds in Borneo: the importance of trophic position, body size and foraging height. Biological Conservation **188**:82-88.

Haynert, K., M. Kiggen, B. Klarner, M. Maraun, and S. Scheu. 2017. The structure of salt marsh soil mesofauna food webs–The prevalence of disturbance. PLoS One **12**:e0189645.

Hebert, C. E., D. C. Weseloh, L. T. Gauthier, M. T. Arts, and R. J. Letcher. 2009. Biochemical tracers reveal intra-specific differences in the food webs utilized by individual seabirds. Oecologia **160**:15-23.

Hellmann, C., B. Wissel, and C. Winkelmann. 2013. Omnivores as seasonally important predators in a stream food web. Freshwater Science **32**:548-562.

Hette-Tronquart, N., T. Oberdorff, E. Tales, A. Zahm, and J. Belliard. 2018. Biological impacts of local vs. regional land use on a small tributary of the Seine River (France): insights from a food web approach based on stable isotopes. Environmental Science and Pollution Research **25**:23583-23594.

Heylen, D., O. Schmidt, H. Dautel, L. Gern, H. Kampen, J. Newton, and J. Gray. 2019. Host identification in unfed ticks from stable isotope compositions ($\delta^{13}C$ and $\delta^{15}N$). Medical and Veterinary Entomology **33**:360-366.

Houle, D., C. Pélabon, G. P. Wagner, and T. F. Hansen. 2011. Measurement and meaning in biology. The Quarterly Review of Biology **86**:3-34.

Hussey, N. E., M. A. MacNeil, B. C. McMeans, J. A. Olin, S. F. Dudley, G. Cliff, S. P. Wintner, S. T. Fennessy, and A. T. Fisk. 2014. Rescaling the trophic structure of marine food webs. Ecology Letters **17**:239-250.

Iken, K., B. Bluhm, and K. Dunton. 2010. Benthic food-web structure under differing water mass properties in the southern Chukchi Sea. Deep Sea Research Part II: Topical Studies in Oceanography **57**:71-85.

Ingimarsdóttir, M., A. Michelsen, J. Ripa, and K. Hedlund. 2014. Food sources of early colonising arthropods: the importance of allochthonous input. Pedobiologia **57**:21-26.

Ishikawa, N., Y. Kato, H. Togashi, M. Yoshimura, C. Yoshimizu, N. Okuda, and I. Tayasu. 2014. Stable nitrogen isotopic composition of amino acids reveals food web structure in stream ecosystems. Oecologia **175**:911-922.

Ishikawa, N. F. 2018. Use of compound-specific nitrogen isotope analysis of amino acids in trophic ecology: assumptions, applications, and implications. Ecological Research **33**:1-13.

James, W. R., J. S. Lesser, S. Y. Litvin, and J. A. Nelson. 2020. Assessment of food web recovery following restoration using resource niche metrics. Science of the Total Environment **711**:134801.

Jardine, T. 2016. A top predator forages low on species-rich tropical food chains. Freshwater Science **35**:666-675.

Jardine, T. D., W. L. Hadwen, S. K. Hamilton, S. Hladyz, and S. M. Mitrovic. 2014. Understanding and overcoming baseline isotopic variability in running waters. River Research and Applications **30**:155-165.

Kim, T., S. Bartel, and C. Gratton. 2019. Grassland harvesting alters ant community trophic structure: an isotopic study in tallgrass prairies. Ecology and Evolution **9**:9815-9826.

Kitchell, J. F., S. P. Cox, C. J. Harvey, T. B. Johnson, D. M. Mason, K. K. Schoen, K. Aydin, C. Bronte, M. Ebener, and M. Hansen. 2000. Sustainability of the Lake Superior fish community: interactions in a food web context. Ecosystems **3**:545-560.

Kloskowski, J., and A. Trembaczowski. 2015. Fish reduce habitat coupling by a waterbird: evidence from combined stable isotope and conventional dietary approaches. Aquatic Ecology **49**:21-31.

Kohzu, A., T. Iwata, M. Kato, J. Nishikawa, E. Wada, N. Amartuvshin, B. Namkhaidorj, and N. Fujita. 2009. Food webs in Mongolian grasslands: the analysis of ^{13}C and ^{15}N natural abundances. Isotopes in Environmental and Health Studies **45**:208-219.

König, T., R. Kaufmann, and S. Scheu. 2011. The formation of terrestrial food webs in glacier foreland: evidence for the pivotal role of decomposer prey and intraguild predation. Pedobiologia **54**:147-152.

Korobushkin, D. I., K. B. Gongalsky, and A. V. Tiunov. 2014. Isotopic niche ($\delta^{13}C$ and $\delta^{15}N$ values) of soil macrofauna in temperate forests. Rapid Communications in Mass Spectrometry **28**:1303-1311.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest package: tests in linear mixed effects models. Journal of Statistical Software **82**:1-26.

Ladygina, N., T. Caruso, and K. Hedlund. 2008. Dietary switching of collembola in grassland soil food webs. Soil Biology & Biochemistry **40**:2898-2903.

Lavoie, R. A., T. D. Jardine, M. M. Chumchal, K. A. Kidd, and L. M. Campbell. 2013. Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. Environmental Science & Technology **47**:13385-13394.

Layman, C., M. Araujo, R. Boucek, C. Hammerschlag Peyer, E. Harrison, Z. Jud, P. Matich, A. Rosenblatt, J. Vaudo, L. Yeager, D. Post, and S. Bearhop. 2012.

Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biological Reviews **87**:545-562.

Letvin, A., M. Brown, K. Bertrand, and M. Weber. 2017. Effects of common carp on trophic dynamics of sport fishes in shallow South Dakota water bodies. Transactions of the American Fisheries Society **146**:331-340.

Lorrain, A., B. S. Graham, B. N. Popp, V. Allain, R. J. Olson, B. P. Hunt, M. Potier, B. Fry, F. Galván-Magaña, and C. E. Menkes. 2015. Nitrogen isotopic baselines and implications for estimating foraging habitat and trophic position of yellowfin tuna in the Indian and Pacific Oceans. Deep Sea Research Part II: Topical Studies in Oceanography **113**:188-198.

Mancinelli, G., S. Vizzini, A. Mazzola, S. Maci, and A. Basset. 2013. Cross-validation of $\delta^{15}N$ and FishBase estimates of fish trophic position in a Mediterranean lagoon: the importance of the isotopic baseline. Estuarine, Coastal and Shelf Science **135**:77-85.

Martinez del Rio, C., N. Wolf, S. Carleton, and L. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews **84**:91-111.

Mavraki, N., S. Degraer, T. Moens, and J. Vanaverbeke. 2019. Functional differences in trophic structure of offshore wind farm communities: A stable isotope study. Marine Environmental Research:104868.

McCutchan, J., W. Lewis, C. Kendall, and C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos **102**:378-390.

McMeans, B. C., T. Kadoya, T. K. Pool, G. W. Holtgrieve, S. Lek, H. Kong, K. Winemiller, V. Elliott, N. Rooney, and P. Laffaille. 2019. Consumer trophic positions respond variably to seasonally fluctuating environments. Ecology:e02570.

Ménard, F., A. Lorrain, M. Potier, and F. Marsac. 2007. Isotopic evidence of distinct feeding ecologies and movement patterns in two migratory predators (yellowfin tuna and swordfish) of the western Indian Ocean. Marine Biology **153**:141-152.

Menke, S. B., A. V. Suarez, C. V. Tillberg, C. T. Chou, and D. A. Holway. 2010. Trophic ecology of the invasive argentine ant: spatio-temporal variation in resource assimilation and isotopic enrichment. Oecologia **164**:763-771.

Merkle, J. A., J. J. Derbridge, and P. R. Krausman. 2011. Using stable isotope analysis to quantify anthropogenic foraging in black bears. Human-Wildlife Interactions **5**:159-167.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta **48**:1135-1140.

Molina-Burgos, B. E., A. Valenzuela-Sánchez, M. Alvarado-Rybak, S. Klarian, and C. Soto-Azat. 2018. Trophic ecology of the Endangered Darwin's frog inferred by stable isotopes. Endangered Species Research **36**:269-278.

Munafò, M. R., B. A. Nosek, D. V. Bishop, K. S. Button, C. D. Chambers, N. P. Du Sert, U. Simonsohn, E.-J. Wagenmakers, J. J. Ware, and J. P. Ioannidis. 2017. A manifesto for reproducible science. Nature Human Behaviour **1**:1-9.

Nielsen, J. M., B. N. Popp, and M. Winder. 2015. Meta-analysis of amino acid stable nitrogen isotope ratios for estimating trophic position in marine organisms. Oecologia **178**:631-642.

Oczkowski, A., B. Kreakie, M. N. Gutierrez, M. Pelletier, M. Charpentier, E. Santos, and J. Kiddon. 2020. Geography, not human impact, is the predominant predictor in a 150-year stable isotope fish record from the coastal United States. Ecological Indicators **111**:106022.

Oelbermann, K., and S. Scheu. 2002. Stable isotope enrichment ($\delta^{15}N$ and $\delta^{13}C$) in a generalist predator (*Pardosa lugubris*, Araneae: Lycosidae): effects of prey quality. Oecologia **130**:337-344.

Ogle, D. H., P. Wheeler, and A. Dinno. 2019. FSA: Fisheries Stock Analysis.

Paine, R. T. 1980. Food webs: linkage, interaction strength and community infrastructure. Journal of Animal Ecology **49**:667-685.

Paszkowski, C., B. Gingras, K. Wilcox, P. Klatt, and W. Tonn. 2004. Trophic relations of the red-necked grebe on lakes in the western boreal forest: a stable-isotope analysis. The Condor **106**:638-651.

Pauly, D., A. Trites, E. Capuli, and V. Christensen. 1998. Diet composition and trophic levels of marine mammals. ICES Journal of Marine Science **55**:467-481.

Pethybridge, H., E. Butler, D. Cossa, R. Daley, and A. Boudou. 2012. Trophic structure and biomagnification of mercury in an assemblage of deepwater chondrichthyans from southeastern Australia. Marine Ecology Progress Series **451**:163-174.

Pethybridge, H., C. A. Choy, J. Logan, V. Allain, A. Lorrain, N. Bodin, C. Somes, J. Young, F. Ménard, C. Langlais, L. Duffy, A. Hobday, P. Kuhnert, B. Fry, C. Menkes, and R. Olson. 2018. A global meta-analysis of marine predator nitrogen stable isotopes: Relationships between trophic structure and environmental conditions. Global Ecology and Biogeography **27**:1043-1055.

Pfennig, D. W., and P. J. Murphy. 2000. Character displacement in polyphenic tadpoles. Evolution **54**:1738-1749.

Phillips, D. L., and J. W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia **127**:171-179.

Pingram, M. A., K. J. Collier, D. Özkundakci, J. Garrett Walker, and D. Ozkundakci. 2020. Food web characteristics of fish communities across degraded lakes provide insights for management in multi-stressor environments. Aquatic Ecology **54**:401-419.

Polis, G., and D. Strong. 1996. Food web complexity and community dynamics. The American Naturalist **147**:813-846.

Pollierer, M., T. Larsen, A. Potapov, A. Brückner, M. Heethoff, J. Dyckmans, S. Scheu, and A. Brueckner. 2019. Compound-specific isotope analysis of amino acids as a new tool to uncover trophic chains in soil food webs. Ecological Monographs **89**:e01384.

Pomerleau, C., M. Peter Heide-Jørgensen, S. H. Ferguson, H. L. Stern, J. L. Høyer, and G. A. Stern. 2017. Reconstructing variability in West Greenland ocean

biogeochemistry and bowhead whale (*Balaena mysticetus*) food web structure using amino acid isotope ratios. Polar Biology **40**:2225-2238.

Ponsard, S., and R. Arditi. 2000. What can stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) tell about the food web of soil micro-invertebrates? Ecology **81**:852-864.

Popp, B. N., B. S. Graham, R. J. Olson, C. C. Hannides, M. J. Lott, G. A. López-Ibarra, F. Galván-Magaña, and B. Fry. 2007. Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids. Terrestrial Ecology **1**:173-190.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**:703-718.

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montana. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia **152**:179-189.

Potapov, A. M., U. Brose, S. Scheu, and A. V. Tiunov. 2019a. Trophic position of consumers and size structure of food webs across aquatic and terrestrial ecosystems. The American Naturalist **194**:823-839.

Potapov, A. M., A. V. Tiunov, and S. Scheu. 2019b. Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biological Reviews **94**:37-59.

Price, E. L., M. Sertić Perić, G. Q. Romero, and P. Kratina. 2019. Land use alters trophic redundancy and resource flow through stream food webs. Journal of Animal Ecology **88**:677-689.

Pringle, R. M., and K. Fox-Dobbs. 2008. Coupling of canopy and understory food webs by ground-dwelling predators. Ecology Letters **11**:1328-1337.

Quezada-Romegialli, C., A. L. Jackson, B. Hayden, K. K. Kahilainen, C. Lopes, and C. Harrod. 2018. tRophicPosition, an R package for the Bayesian estimation of trophic position from consumer stable isotope ratios. Methods in Ecology and Evolution **9**:1592-1599.

Quiroga, E., D. Gerdes, A. Montiel, R. Knust, and U. Jacob. 2014. Normalized biomass size spectra in high Antarctic macrobenthic communities: linking trophic position and body size. Marine Ecology Progress Series **506**:99-113.

R Core Team 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rasgon, J. L. 2008. Stable isotope analysis can potentially identify completely-digested bloodmeals in mosquitoes. PLoS One **3**:e2198.

Renaud, P. E., M. Tessmann, A. Evenset, and G. N. Christensen. 2011. Benthic food-web structure of an Arctic fjord (Kongsfjorden, Svalbard). Marine Biology Research **7**:13-26.

Reum, J. C., and K. N. Marshall. 2013. Evaluating δ^{15}N–body size relationships across taxonomic levels using hierarchical models. Oecologia **173**:1159-1168.

Roell, M. J., and D. J. Orth. 1998. Indirect effects of fishery exploitation and pest control in a riverine food web. North American Journal of Fisheries Management **18**:337-346.

Roskov, Y., G. Ower, T. Orrell, D. Nicolson, N. Bailly, P. M. Kirk, T. Bourgoin, R. E. DeWalt, W. Decock, E. v. Nieukerken, and L. Penev. 2020. Species 2000 & ITIS Catalogue of Life. Species 2000: Naturalis, Leiden, the Netherlands.

Rounick, J., and M. Winterbourn. 1986. Stable carbon isotopes and carbon flow in ecosystems. Bioscience **36**:171-177.

Rowell, K., D. L. Dettman, and R. Dietz. 2010. Nitrogen isotopes in otoliths reconstruct ancient trophic position. Environmental Biology of Fishes **89**:415-425.

Sabat, P., N. Ramirez-Otarola, F. Bozinovic, and C. M. Del Rio. 2013. The isotopic composition and insect content of diet predict tissue isotopic values in a South American passerine assemblage. Journal of Comparative Physiology B **183**:419-430.

Sampedro, L., and J. Domínguez. 2008. Stable isotope natural abundances ($\delta^{13}C$ and $\delta^{15}N$) of the earthworm *Eisenia fetida* and other soil fauna living in two different vermicomposting environments. Applied Soil Ecology **38**:91-99.

Sanders, D., and C. Platner. 2007. Intraguild interactions between spiders and ants and top-down control in a grassland food web. Oecologia **150**:611-624.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods **9**:671-675.

Schneider, K., S. Migge, R. A. Norton, S. Scheu, R. Langel, A. Reineking, and M. Maraun. 2004. Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios ($^{15}N/^{14}N$). Soil Biology and Biochemistry **36**:1769-1774.

Schneider, L., W. A. Maher, J. Potts, A. M. Taylor, G. E. Batley, F. Krikowa, A. A. Chariton, and B. Gruber. 2015. Modeling food web structure and selenium biomagnification in lake macquarie, New South Wales, Australia, using stable carbon and nitrogen isotopes. Environmental Toxicology and Chemistry **34**:608-617.

Seminoff, J. A., S. R. Benson, K. E. Arthur, T. Eguchi, P. H. Dutton, R. F. Tapilatu, and B. N. Popp. 2012. Stable isotope tracking of endangered sea turtles: validation with satellite telemetry and $\delta^{15}N$ analysis of amino acids. PLoS One **7**:e37403.

Senior, M. J. M., K. Hamer, S. Bottrell, D. Edwards, T. Fayle, J. Lucey, P. Mayhew, R. Newton, K. S. H. Peh, F. Sheldon, C. Stewart, A. Styring, M. D. F. Thom, P. Woodcock, and J. Hill. 2013. Trait-dependent declines of species following conversion of rain forest to oil palm plantations. Biodiversity & Conservation **22**:253-268.

Shurin, J. B., D. S. Gruner, and H. Hillebrand. 2005. All wet or dried up? Real differences between aquatic and terrestrial food webs. Proceedings of the Royal Society B: Biological Sciences **273**:1-9.

Silge, J., and D. Robinson. 2016. tidytext: Text mining and analysis using tidy data principles in R. Journal of Open Source Software **1**:37.

Spence, K. O., and J. A. Rosenheim. 2005. Isotopic enrichment in herbivorous insects: a comparative field-based study of variation. Oecologia **146**:89-97.

Stapp, P., and D. J. Salkeld. 2009. Inferring host–parasite relationships using stable isotopes: implications for disease transmission and host specificity. Ecology **90**:3268-3273.

Starrfelt, J., K. Borgå, A. Ruus, and E. Fjeld. 2013. Estimating trophic levels and trophic magnification factors using Bayesian inference. Environmental Science & Technology **47**:11599-11606.

Steffan, S., P. Dharampal, B. Danforth, H. Gaines Day, Y. Takizawa, and Y. Chikaraishi. 2019. Omnivory in bees: elevated trophic positions among all major bee families. The American Naturalist **194**:414-421.

Steffan, S. A., Y. Chikaraishi, D. R. Horton, N. Ohkouchi, M. E. Singleton, E. Miliczky, D. B. Hogg, and V. P. Jones. 2013. Trophic hierarchies illuminated via amino acid isotopic analysis. PLoS One **8**:e76152.

Tillberg, C., D. Holway, E. LeBrun, and A. Suarez. 2007. Trophic ecology of invasive Argentine ants in their native and introduced ranges. Proceedings of the National Academy of Sciences of the United States of America **104**:20856-20861.

Traugott, M., S. Kamenova, L. Ruess, J. Seeber, and M. Plantegenest. 2013. Empirically characterising trophic networks: what emerging DNA-based methods, stable isotope and fatty acid analyses can offer. Advances in Ecological Research **49**:177-224.

Vander Zanden, J. M., G. Cabana, and J. B. Rasmussen. 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios ($\delta^{15}N$) and literature dietary data. Canadian Journal of Fisheries and Aquatic Sciences **54**:1142-1158.

Vander Zanden, J. M., and J. B. Rasmussen. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology **80**:1395-1404.

Vander Zanden, M. J., J. M. Casselman, and J. B. Rasmussen. 1999a. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature **401**:464.

Vander Zanden, M. J., and J. B. Rasmussen. 2001. Variation in delta N-15 and delta C-13 trophic fractionation: Implications for aquatic food web studies. Limnology and Oceanography **46**:2061-2066.

Vander Zanden, M. J., B. J. Shuter, N. Lester, and J. B. Rasmussen. 1999b. Patterns of food chain length in lakes: a stable isotope study. The American Naturalist **154**:406-416.

Vanderklift, M., and S. Ponsard. 2003. Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. Oecologia **136**:169-182.

Vinagre, C., C. Máguas, H. Cabral, and M. Costa. 2012. Food web structure of the coastal area adjacent to the Tagus estuary revealed by stable isotope analysis. Journal of Sea Research **67**:21-26.

Wheeler, D. L., T. Barrett, D. A. Benson, S. H. Bryant, K. Canese, V. Chetvernin, D. M. Church, M. DiCuccio, R. Edgar, and S. Federhen. 2007. Database resources of

the national center for biotechnology information. Nucleic Acids Research **36**:D13-D21.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer.

Wilder, S., D. Holway, A. Suarez, E. LeBrun, and M. Eubanks. 2011. Intercontinental differences in resource use reveal the importance of mutualisms in fire ant invasions. Proceedings of the National Academy of Sciences of the United States of America **108**:20639-20644.

Wilson, A. C. C., L. d. S. L. Sternberg, and K. Hurley. 2011. Aphids alter host-plant nitrogen isotope fractionation. Proceedings of the National Academy of Sciences of the United States of America **108**:10220-10224.

Woodcock, P., D. Edwards, R. Newton, F. Edwards, C. Khen, S. Bottrell, and K. Hamer. 2012. Assessing trophic position from nitrogen isotope ratios: effective calibration against spatially varying baselines. Naturwissenschaften **99**:275-283.

Zambrano, L., E. Valiente, and M. J. Vander Zanden. 2010. Food web overlap among native axolotl (*Ambystoma mexicanum*) and two exotic fishes: carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*) in Xochimilco, Mexico City. Biological Invasions **12**:3061-3069.

Zohary, T., J. Erez, M. Gophen, I. Berman-Frank, and M. Stiller. 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. Limnology and Oceanography **39**:1030-1043.

3. SUGAR IS AN ANT'S BEST FRIEND? : TESTING FOOD WEB THEORY PREDICTIONS IN AN INVASIVE ANT (*Nylanderia fulva*)

3.1. Overview

Classical food web theory predicts that individuals at the base of the food web will be more abundant than those at the top (i.e., herbivores are more abundant than predators). The relationship between the abundance and trophic position of many invasive ant species reflects this prediction, as colony densities and dominance over resources are often highest when feeding as herbivores (i.e., on high amounts of carbohydrate resources). We tested the relationship between diet, colony densities, and resource dominance using the invasive tawny crazy ant (*Nylanderia fulva*). The tawny crazy ant is a major invasive pest that can reach densities of nearly one million ants per hectare. We used stable isotope analysis, attraction of ants to baits, and pitfall sampling to investigate tawny crazy ant trophic position, density, and competition with other ant species over five months at sites across Texas. We predicted that tawny crazy ant colonies that feed as herbivores would consequently have higher colony densities than those that feed as predators. We also predicted that populations of tawny crazy ants that fed at lower trophic positions would be more competitively dominant in the ant community due to higher abundance and enhanced worker aggression. Counter to our expectations, tawny crazy ant densities at baits and overall resource dominance were positively correlated with trophic position. Tawny crazy ant densities and dominance were highest when colonies fed on a more predaceous diet compared with colonies that

fed on a more herbivorous diet. Moreover, when compared with known predators and herbivores in the community, tawny crazy ants were much more predaceous than traditional food web theory predicts. Tawny crazy ants were highly omnivorous. Our direct observations provide further support that tawny crazy ants take advantage of a broad range of food items. We observed workers foraging on plant nectaries, tending aphids on a variety of plants, and consuming arthropod prey (dead and alive). Our results suggest that tawny crazy ants reach high densities not by feeding as herbivores, as classical food web theory predicts, but by feeding flexibly on many different resources. Past research has proposed controlling tawny crazy ant numbers by excluding workers from insect mutualists, but our results imply that this may be an ineffective strategy due to the substantial dietary flexibility of tawny crazy ants in the field.

3.2. Introduction

Ants are extremely abundant in many ecological systems, sometimes comprising over half of the total dry weight of arthropods within a single habitat (Hölldobler and Wilson 1990, Tobin 1991). Initially, it was thought that most ant species were predators, so their incredible abundance seemed to run counter to the traditional 'pyramid of numbers' hypothesis in ecology, which states that predators should be less abundant than the prey they consume (Elton 1927). Tobin (1991) was one of the first to propose that abundant ant species may actually feed as herbivores, which would fit with traditional ecology theory and mean that ants were taking advantage of a more abundant resource (i.e., plants) than prey. In the following years, research emerged in support of this idea

and suggested that abundant arboreal ants rely heavily on plant and homopteran exudates (i.e., honeydew; Davidson and Patrell-Kim 1996, Davidson 1997, Davidson et al. 2003). For example, Davidson et al. (2003) found that many abundant and dominant tropical ant species in the subfamilies Formicinae and Dolichoderinae functioned as herbivores, whereas less abundant species in the same habitats were more predaceous. They proposed that the high levels of carbohydrates found in plant and insect exudates 'fuel' ant worker activity, which allows for higher numbers of workers and increased overall activity in the form of foraging or colony defense.

This idea found additional support in invasive ants. The consumption of plant- and insect-produced carbohydrate resources is often implicated in the population explosions and increased range expansion of many invasive ant species (Holway et al. 2002, Helms 2013), including the red imported fire ant (*Solenopsis invicta*; Wilder et al. 2011b), the Argentine ant (*Linepithema humile*; Tillberg et al. 2007), the yellow crazy ant (*Anoplolepis gracilipes*; Abbott 2005), and the big-headed ant (*Pheidole megacephala*; Wetterer et al. 2006). Many invasive ant species tend honeydew-producing insects in their invaded range (Benois et al. 1973, Aldana et al. 1995, Davidson 1997, Holway et al. 2002, Menke et al. 2010), and they often more aggressively dominate these resources than colonies in their native range (Tillberg et al. 2007, Wilder et al. 2011b). For example, colonies of *S. invicta* in their invaded range in the USA dominated over 75% of observed hemipteran aggregations compared with only 2% in their native Argentina (Wilder et al. 2011b). The increased dominance over and consumption of insect-produced honeydew may be one reason why *S. invicta* is 5-7

times more abundant in the USA than in their native range (Porter et al. 1997). Increased consumption of carbohydrate resources typically corresponds with greater colony sizes and increased worker survival and activity. For example, access to carbohydrate resources in the laboratory increased brood production and worker foraging activity in the red imported fire ant (Helms and Vinson 2008, Wilder et al. 2011a), the Argentine ant (Grover et al. 2007, Kay et al. 2010), and the yellow crazy ant (Wittman et al. 2018). Invasive ant populations that rely on greater amounts of plant-based resources typically occupy lower trophic positions (i.e., are more herbivorous) and achieve greater densities in the field than their more predaceous counterparts (Tillberg et al. 2007, Wilder et al. 2011b, Wittman et al. 2018). Yellow crazy ants, for example, were 20 times more abundant when they fed on greater amounts of plant-based resources than when they were more predaceous (Wittman et al. 2018).

We chose to test the relationship between ant abundance and diet using the invasive tawny crazy ant (*Nylanderia fulva*). Native to Argentina and Brazil, the tawny crazy ant has invaded many parts of the world and caused considerable damage to the native fauna (Zenner-Polania 1994, Aldana et al. 1995, LeBrun et al. 2013, Wetterer et al. 2014, Lester and Gruber 2016). For example, initial invasion of the tawny crazy ant reduced arthropod species richness by approximately 74% at a nature reserve in Colombia (Aldana et al. 1995). The tawny crazy ant has become a major pest in most of the Gulf Coast states, with ant worker numbers that often exceed the combined biomass of all other ants by nearly two-fold (LeBrun et al. 2013). At high abundance, tawny crazy ants displace the previously dominant red imported fire ant (*S. invicta*) and reduce

arthropod diversity in grassland ecosystems (LeBrun et al. 2013). Tawny crazy ants regularly undergo local population explosions and crashes (Wetterer et al. 2014), which may be due to changes in the availability of plant- and insect-produced honeydew. Tawny crazy ants have been seen tending honeydew-producing insects (Zenner-Polania 1990, Aldana et al. 1995, Sharma et al. 2013), and worker activity is reduced on plants where honeydew-producing hemipterans have been removed (Sharma et al. 2019). However, the relationship between tawny crazy ant diet and abundance in the field has not been examined.

Our primary objectives for this study were to 1) quantify tawny crazy ant density and resource dominance (i.e., ability to outcompete other ant species for a resource) on the ground and in trees at multiple sites in Texas, and 2) correlate density and resource dominance with tawny crazy ant trophic position. We predicted that tawny crazy ant density would be negatively correlated with ant trophic position, indicating a greater reliance on plant- and insect-produced honeydew (i.e., more herbivorous) at higher colony sizes. We also predicted that tawny crazy ant resource dominance against competing species would be negatively correlated with their trophic position. To estimate tawny crazy ant trophic position, we used stable isotope analysis. This technique has been used extensively in the past to evaluate the relationship between ant diet and abundance (Davidson et al. 2003, Tillberg et al. 2007, Menke et al. 2010, Gibb and Cunningham 2011, Wilder et al. 2011b, Wittman et al. 2018) due to the increase in the ratio of heavy to light nitrogen stable isotopes ($\delta^{15}N$) from one trophic level to the next (DeNiro and Epstein 1981, Minagawa and Wada 1984, Fry 1988). In general,

consumers are enriched in the heavier isotope (i.e., have higher $\delta^{15}N$) relative to their food resource, and lower $\delta^{15}N$ values typically correspond to a more herbivorous diet (i.e., increased consumption of carbohydrate resources).

3.3. Methods

3.3.1. Study sites

We sampled at three locations with an ongoing tawny crazy ant invasion in Texas, USA: Bryan: 30.72˚N, 96.32˚W, Austin: 30.46˚N, 97.83˚W, and Weslaco: 26.12˚N, 97.95˚W. Whenever possible, we sampled from multiple sites within each location. In total, we sampled from five sites across the three locations, with two sites in Bryan (Bryan – 1 and Bryan – 2), one site within a private property in Austin (Austin), and two sites in a protected state park in Weslaco (Estero Llano Grande State Park; Weslaco – 1 and Weslaco – 2). Sites within the same location were separated by at least 100m to avoid sampling ants that may have shared resources with each other and to ensure that any differences across sites could be detected. Habitats ranged from frequently disturbed, grassy areas near human habitation (Bryan – 1 and Bryan – 2) to relatively undisturbed grassy (Weslaco – 2) and wooded areas (Weslaco – 1 and Austin). Locations also differed in the time since invasion; tawny crazy ants had been present for at least one year prior to sampling at the Austin location (B. Howell, *pers. comm.*), over two years at the Bryan location (Kjeldgaard, *unpubl. data*), and at least six years at the Weslaco location (J. de León, *pers. comm.*).

3.3.2. Sampling

We sampled from each site at the Weslaco and Bryan locations every month from April to August 2018. We sampled at the Austin location starting in May to August 2018. Sampling took place over a two-day period. To assess tawny crazy ant density at each site, we dug five pitfall traps in a quincunx formation that was approximately 10m wide by 40m long. The two traps on the outside edges of the quincunx were separated by 10m, and the middle trap was 20m from each outside edge. Pitfall traps consisted of 50mL polypropylene centrifuge tubes (VWR International, Radnor, PA, USA) charged with ~20mL of a solution of water, odorless dish soap, and salt. Pitfall traps were placed flush with the ground in early April 2018 and left closed for at least one week prior to the first sampling period to avoid the digging-in effect (Greenslade 1973, Lasmar et al. 2017). Traps were opened for 24 hours and then replaced with fresh traps that remained closed between sampling periods.

To assess ground and arboreal ant foraging, we placed five bait stations on the ground and five in nearby trees at breast height. Weslaco – 2 was primarily grassy habitat and contained only two trees, so we ran only two arboreal bait stations at this site. Bait stations consisted of a single hot dog piece (~4g wet weight; Bar-S Franks, Walmart, Bentonville, AR, USA) and an equal-sized droplet of honey on a polystyrene hexagonal weigh boat (51mm base; VWR International, Radnor, PA, USA). Two sides of each ground bait station were removed to allow ants to more easily access the bait, and stations were placed flush with the soil approximately 2m from pitfall traps to avoid influencing pitfall catches. For arboreal bait stations, we selected trees that were closest

to pitfall traps, most of which were honey mesquite (*Prosopis glandulosa*), post oak (*Quercus stellata*), and ashe juniper (*Juniperus ashei*). We used duct tape to secure the upper edge of arboreal bait stations to branches at breast height (~1.3m from the ground), which kept baits horizontal and allowed ants to access the baits by walking from branches onto the weigh boat. We ran bait stations for approximately one hour in the morning between 8am-11am before the hottest part of the day when ants were less active (Kjeldgaard, *pers. obs.*). After one hour, we recorded the number and species of ants foraging at each bait, as well as the surface temperature next to the bait using an infrared non-contact digital thermometer (ANGGO, Amazon, Seattle, WA, USA). An ant species was considered to have "won" (i.e., dominated) a bait station when more than five workers were foraging at the bait after one hour. If more than five workers of multiple ant species were observed at the bait, it was considered a tie.

To measure a species' ability to outcompete others for a resource, each ant species that was present at a site for a given month was assigned a behavioral dominance score. First, we calculated the raw proportion of baits "won" out of the total baits to which the ant species had access (based on whether or not the ant was detected in the nearby pitfall or at the bait itself). Next, we quantified the behavioral dominance of each species by entering the raw proportions into a Colley matrix (Colley 2002). The Colley matrix provides for an improved measure of dominance by adjusting the value of each win and loss of a species by the dominance of their competitors (Feener 1981, Colley 2002, Lebrun and Feener 2007). A higher behavioral dominance score indicates that

more baits were won against competitors, whereas a lower score indicates that more baits were lost.

Before recollecting the baits, we aspirated 20-30 workers from each bait station for stable isotope analysis. We also collected three to five samples of the most common plant species, insects that are known to feed as herbivores (i.e., planthoppers and caterpillars), and arthropods that are known to feed as predators (i.e., spiders) at each site every month (when possible). Whenever possible, we collected plant material from the same plants and from the same part of the tree every month at each site to minimize variation due to different individuals or different parts of the plant. All arthropods were frozen at -10˚C after returning to the laboratory. Samples for stable isotope analysis were never stored in EtOH to avoid possible effects of EtOH on isotopic signatures (Tillberg et al. 2006).

3.3.3. Stable isotope analysis

All arthropods and plants were dried in an oven at 60˚C for 24-48 hours and then stored in airtight vials (arthropods) or bags (plants) prior to processing. All plants were ground to a homogeneous powder using a Retsch Oscillating Mixer Mill MM 400 (Verder Scientific, Inc., Newtown, PA, USA). The abdomens of all ants were removed prior to weighing to avoid the effects of stomach contents on isotopic signatures (Tillberg et al. 2006). Whenever possible, we used the legs and/or thoraxes of arthropods, but whole bodies were used for especially small samples. To achieve appropriate weights for each sample, five to ten workers per sample were pooled for all

ant samples and two to three individuals were pooled for especially small arthropod samples. Approximately 0.400mg of each arthropod sample and 2.200mg of each plant sample were weighed into tin capsules (Costech Analytical Technologies Inc., Valencia, CA, USA) using a microbalance (Mettler Toledo, Columbus, OH, USA). All samples were analyzed at the Texas A&M University Stable Isotopes for Biosphere Science Laboratory (https://sibs.tamu.edu/) using a Delta V Advantage Isotope Ratio Mass Spectrometer coupled with Costech Elemental Analyzer and Thermo ConFlo IV Universal Interface (Thermo Fisher Scientific, Waltham, MA, USA).

Stable isotope values are reported as "δX" (i.e., δ^{15}N) and expressed in "per mil" or "‰" notation. Values were calculated by comparing the ratio of heavy to light stable isotopes in the sample to that in a standard (air for nitrogen, and Vienna Pee Dee Belemnite for carbon) using the following equation (Coplen 2011):

$$\delta X = [(R_{sample}/ R_{standard}) - 1] \times 1{,}000$$

3.3.4. Data analysis

All analyses were conducted in R v3.6.1 (R Core Team 2019), and all plots were generated using *ggplot2* (Wickham 2016). We estimated trophic position from stable isotope data using the following highly-cited formula via Post (2002):

$$\text{Trophic Position}_{\text{consumer}} = 1 + \frac{\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{plants}}}{\Delta\text{N}}$$

We chose to use plants as a baseline because of the lower rates of tissue turnover compared with arthropod herbivores in terrestrial ecosystems (Shurin et al. 2005). We also used a ΔN (i.e., trophic enrichment factor) of 3.4‰ (Post 2002), which can vary largely by species (McCutchan et al. 2003, Vanderklift and Ponsard 2003); however, because we compared the trophic position of the same species (i.e., tawny crazy ants) across sites and not the trophic position of different species with potentially different ΔN values, the ΔN value should not affect our conclusions. We constructed linear models using the *lm* function in base R software. Tawny crazy ant trophic position was treated as the dependent variable. We treated site, month, log-transformed tawny crazy ant densities in pitfalls, ground baits, and arboreal baits as fixed effects. We tested data for heterogeneity of variances and normality to ensure all assumptions were met.

Because of the challenges in interpreting absolute estimates of trophic position from stable isotope data (Kjeldgaard et al., *in prep.*), we also statistically compared tawny crazy ant δ^{15}N with those of plants, herbivores, and predators collected from the same site to determine tawny crazy ant diet relative to other trophic levels. Due to the non-normal distribution of the data, we conducted a non-parametric Friedman test blocking by site. We averaged δ^{15}N values across sampling months and treated this average as the dependent variable. The material collected (i.e., plants, arthropod herbivores, tawny crazy ants, and arthropod predators) was treated as the independent variable. We conducted a Conover test as a post-hoc to determine pairwise differences between groups using the *PMCMR* package (Pohlert 2014).

To analyze tawny crazy ant pitfall and foraging densities, we constructed generalized linear mixed models using a Poisson distribution with the *glmer* function in the *lme4* package (Bates et al. 2015). We downloaded monthly temperature and precipitation data from April to August 2018 for each location from the National Weather Service's Climate Prediction Center (https://www.ncdc.noaa.gov/cdo-web/) to examine the effects of certain abiotic conditions on tawny crazy ant densities. Specifically, we tested for the effects of DX90 (the number of days in the month with a maximum temperature exceeding or equal to 32.2˚C) and DP10 (the number of days in the month with greater than or equal to 25.4mm of precipitation). We treated pitfall/ bait station nested within site as a random factor because we repeatedly measured from the same location at each site. We treated month, surface temperature, DX90, and DP10 as fixed effects. The number of tawny crazy ants in pitfalls, tawny crazy ant foragers on baits on the ground, and tawny crazy ant foragers on arboreal baits were treated as dependent variables and analyzed separately. We used Type II Wald Chi-square tests to generate analysis of deviance tables using the *Anova* function in the *car* package (Fox and Weisberg 2018). We also performed a principal components analysis to determine the relationship between tawny crazy ant densities in pitfalls, ground baits, and arboreal baits, as well as the abiotic conditions DX90 and DP10.

Because the behavioral dominance score relies on both bait and pitfall trap data, this number is highly correlated with the number of ants in pitfall traps and at bait stations. Therefore, to avoid collinearity in our linear models, we separately examined

the relationship between trophic position and behavioral dominance, as well as the relationship between behavioral dominance and the number of workers at baits.

3.4. Results

3.4.1. Trophic position

Counter to our expectations, tawny crazy ant trophic position was positively correlated with the number of foragers at baits (Figure 3.1). Moreover, tawny crazy ant trophic position was positively correlated with their behavioral dominance, so ants are also more likely to outcompete other species at baits when more predaceous (Figure 3.2). This relationship is likely due to a higher number of foragers at baits, as tawny crazy ants were more dominant at higher densities (Figure 3.3). Tawny crazy ants were much more predaceous than expected, because workers were omnivores, and not strict herbivores, when compared with other organisms collected from the same habitat (Figure 3.4; Appendix B S1). When tawny crazy ant δ^{15}N values were compared with those of plants, arthropod herbivores, and arthropod predators collected from the same site, there were significant differences depending on the material ($\chi^2 = 14.04$, df = 3, $P = 0.003$). Plants and herbivores were not significantly different on average from each other, tawny crazy ants were significantly higher than plants and herbivores but lower than predators, and predators were significantly higher than all other groups (Figure 3.4), indicating that tawny crazy ants have an omnivorous diet.

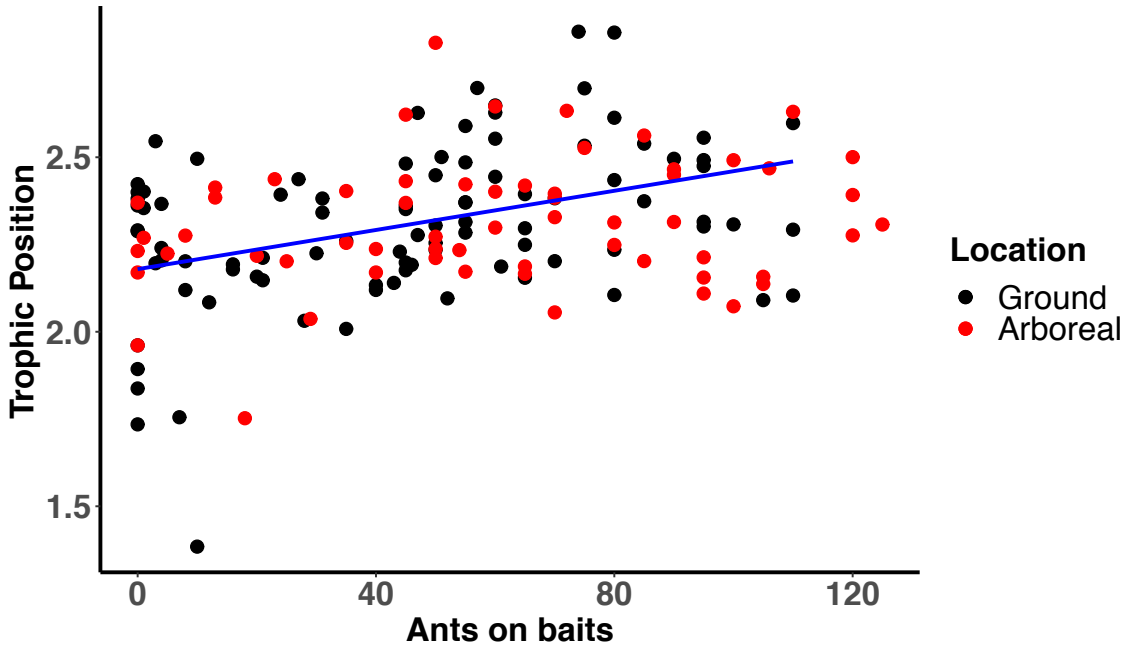


Figure 3.1 Tawny crazy ant trophic position is positively correlated with the number of foragers on baits. Best-fit line is in blue: y = 0.0028063x + 2.1794013; *P* < 0.001, Adjusted R^2 = 0.1447.

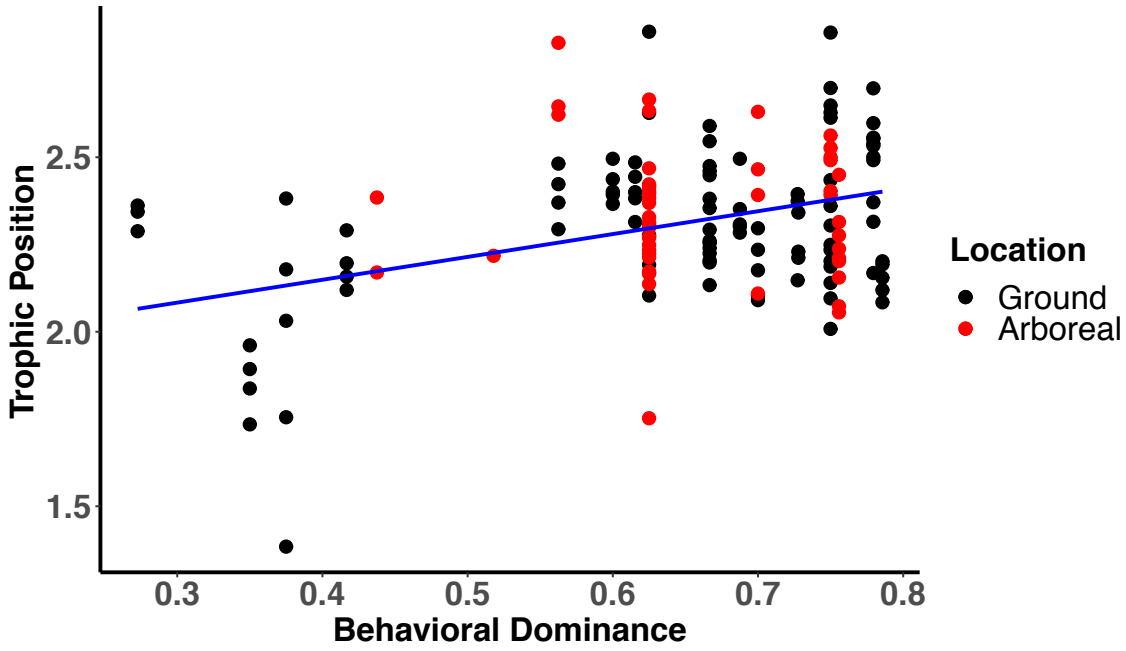


Figure 3.2 Tawny crazy ant trophic position is positively correlated with their behavioral dominance (i.e., adjusted proportion of competitive encounters won). Best-fit line is in blue: y = 0.65466x + 1.88711; $P < 0.001$, Adjusted $R^2 = 0.156$.

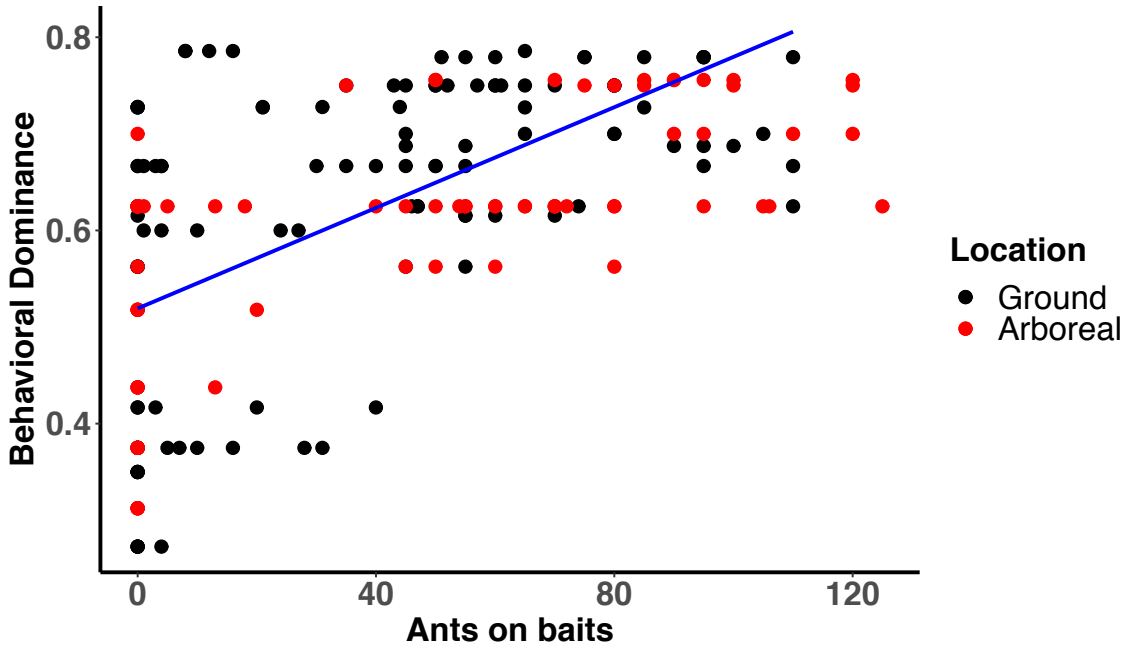


Figure 3.3 Tawny crazy ant behavioral dominance (i.e., adjusted proportion of competitive encounters won) is positively correlated with the number of foragers on baits. Best-fit line is in blue: y = 123.79x – 38.18; $P < 0.001$, Adjusted R^2 =0.3161.

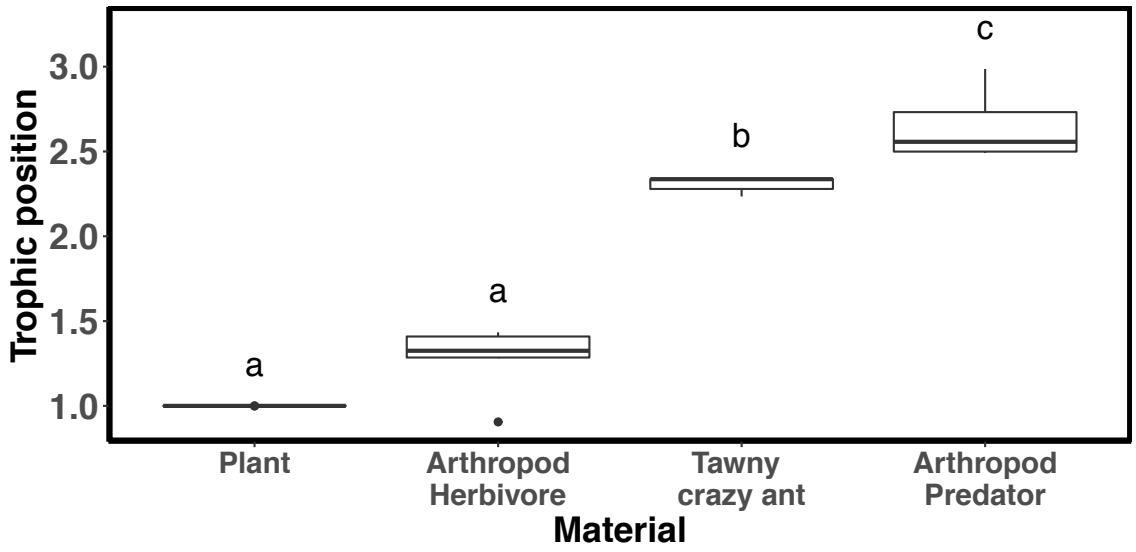


Figure 3.4 Trophic position of material collected from the same site as tawny crazy ants. Center lines within box plots represent medians, hinges represent upper and lower quartiles, whiskers represent 1.5 times above or below the interquartile range, and dots represent outliers. Letters indicate significant differences between groups according to a Conover test (α = 0.05).

Our direct observations provide further support that tawny crazy ants are omnivorous. We observed tawny crazy ants foraging on a broad range of resources, including plant nectaries, aphid honeydew, and arthropod prey (both dead and living). For example, tawny crazy ants were observed at the extrafloral nectaries of partridge pea (*Chamaecrista fasciculata*; Appendix B Supplementary Video 1) and may have fed on the extrafloral nectaries of honey mesquite (*P. glandulosa*). Similar to other authors, we also observed the ants tending aphids, including those on post oak (*Quercus stellata*; Appendix B Supplementary Videos 2 & 3) and wild petunia (*Reullia spp*.; Appendix B Supplementary Video 4). Finally, ants were also seen on arthropod prey, including a

dead grasshopper caught on a sticky trap (Appendix B Supplementary Video 5) and a live cockroach subdued by workers (Appendix B Supplementary Videos 6 & 7). Although we did not quantify their feeding on these specific resources, these observations provide further evidence that tawny crazy ants are an opportunistic and highly omnivorous species.

Tawny crazy ant trophic position changed significantly over time, but average trophic position did not differ by site (Table 3.1). Although trophic position was related to the density of foragers at baits, tawny crazy ant trophic position was not significantly related to the number of ants in pitfall traps (Table 3.1, Figure 3.5).

Table 3.1 Influence of various factors on tawny crazy ant trophic position based on linear model results.

	df	Sum of squares	Mean square	F-value	*P*
Month	4	0.579	0.145	4.522	0.002
Site	4	0.181	0.045	1.412	0.238
Log-transformed Ground Pitfalls	1	0.007	0.007	0.216	0.643
Log-transformed Ground Baits	1	0.318	0.318	9.920	0.002
Log-transformed Arboreal Baits	1	0.205	0.205	6.408	0.013
Residuals	74	2.369	0.032		

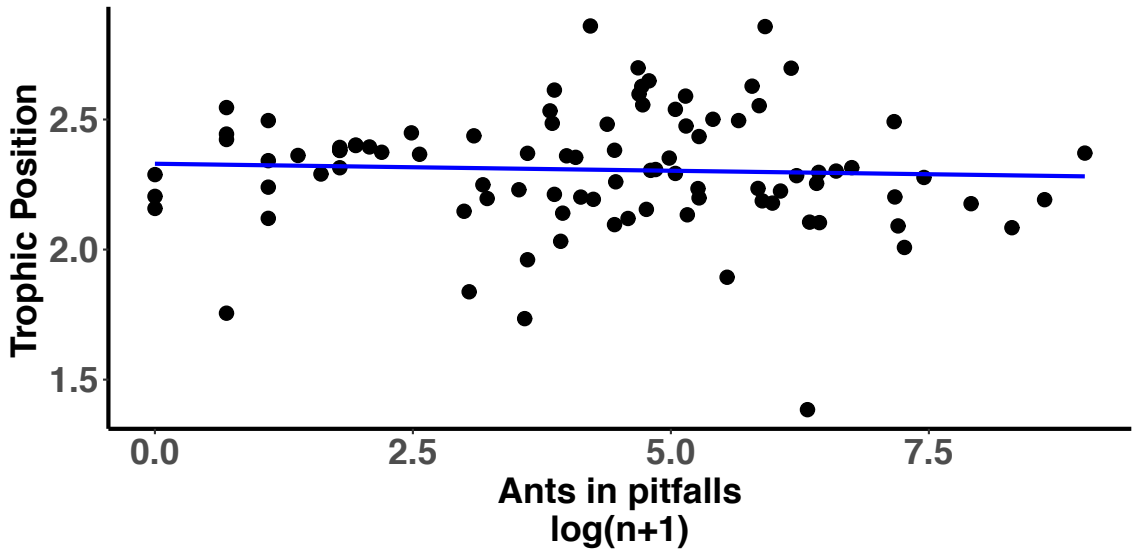


Figure 3.5 No significant relationship between tawny crazy ant trophic position and the number of ants in pitfall traps. Best-fit line is in blue: y = -0.005373x + 2.329875; *P* = 0.6405, Adjusted R^2 = -0.008556.

3.4.2. Density of tawny crazy ant workers

Tawny crazy ant densities in pitfall traps, ground baits, and arboreal baits varied largely by month and site (Table 3.2; Appendix B S2). Densities remained lower at the Austin site and varied much less over time compared with the other locations (Appendix B S2). For example, the average number ± SE of tawny crazy ants per pitfall trap at the Austin site changed less than two-fold from 16.4 ± 9.89 in May to 23.2 ± 15.57 in August. By contrast, the average number of tawny crazy ants per pitfall trap increased from 2.0 ± 0.83 and 1.4 ± 0.51 in April to 1,118.6 ± 433.84 and 1,996.8 ± 1,553.72 in August at Bryan – 1 and Bryan – 2, respectively. Sites in Weslaco experienced a decline in the average number of ants per pitfall trap over time; ants at the Weslaco – 1 site decreased rapidly from 628.8 ± 302.61 at their peak in July to just 7.8 ± 4.32 in August. This number decreased even earlier at Weslaco – 2, with 437.0 ± 158.0 ants per pitfall trap on average in April to just 1.5 ± 0.5 in June and zero ants by August.

Table 3.2 Influence of various factors on the number of tawny crazy ants in pitfall traps, on baits on the ground, and on baits in nearby trees. DX90 and DP10 were downloaded for each sampling month at each location from the National Weather Service's Climate Prediction Center (https://www.ncdc.noaa.gov/cdo-web/). DX90 represents the number of days in the month with a maximum temperature exceeding or equal to 32.2˚C, and DP10 represents the number of days in the month with greater than or equal to 25.4mm of precipitation.

Ground pitfalls

	χ^2	df	*P*
Month	55.429	4	<0.001
Site	72.63	4	<0.001
DX90	73.731	1	<0.001
DP10	15.166	1	<0.001

Ground baits

	χ^2	df	*P*
Month	32.118	4	<0.001
Site	43.083	4	<0.001
Ground surface temperature	4.952	1	0.026
DX90	23.332	1	<0.001
DP10	5.216	1	0.022

Arboreal baits

	χ^2	df	*P*
Month	31.677	4	<0.001
Site	26.455	4	<0.001
Tree species	5.788	7	0.565
Arboreal surface temperature	0.021	1	0.886
DX90	51.513	1	<0.001
DP10	22.731	1	<0.001

As expected, the number of tawny crazy ants in pitfall traps was positively correlated with the number of tawny crazy ant foragers at baits on the ground and in trees (Figure 3.6, Appendix B S2). When densities in pitfall traps were high, the average

number of tawny crazy ants on ground and arboreal baits regularly exceeded 80 workers per bait (Appendix B S2). The densities of ants in pitfall traps and on both types of bait were significantly affected by the number of days in the month with a maximum temperature exceeding or equal to 32.2˚C (DX90) and by the number of days in the month with greater than or equal to 25.4mm of precipitation (DP10; Table 3.2). Ant foraging on the ground was also significantly affected by ground surface temperature (Table 3.2); ants were generally found in high densities at baits when the surface temperature was between 20-35˚C, but the number of workers decreased rapidly as temperatures exceeded 40˚C (Appendix B S3). There was no significant effect of arboreal surface temperature on the number of foragers found on arboreal baits (Table 3.2), but this may be because the temperature was lower in the trees than on the soil surface and never exceeded 40˚C (Appendix B S3). There was no significant effect of tree species on the number of tawny crazy ants foraging on arboreal bait stations (Table 3.2); however, not all tree species were sampled at each site, so this should be interpreted with caution.

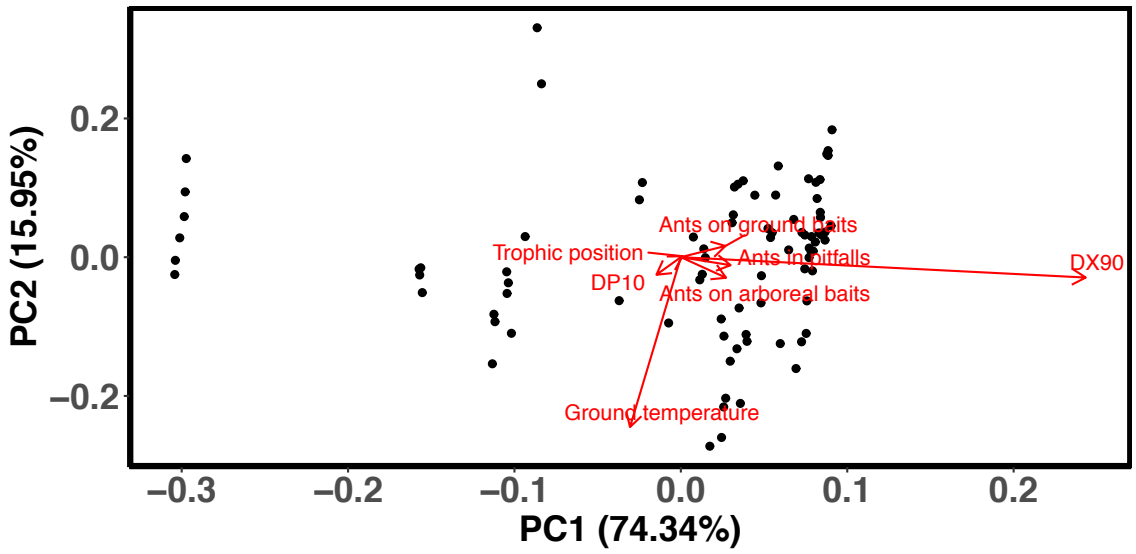


Figure 3.6 Principal Components Analysis showing the relationship between the number of ants in pitfalls and at baits, tawny crazy ant trophic position, surface temperature, DX90, and DP10. All ant counts (in pitfalls and on baits) were log-transformed. DX90 and DP10 were downloaded for each sampling month at each location from the National Weather Service's Climate Prediction Center (https://www.ncdc.noaa.gov/cdo-web/). DX90 represents the number of days in the month with a maximum temperature exceeding or equal to 32.2˚C, and DP10 represents the number of days in the month with greater than or equal to 25.4mm of precipitation.

3.5. Discussion

Counter to our expectations, tawny crazy ant trophic position was positively correlated with densities at baits (Figure 3.1) and with their overall dominance over resources (Figure 3.2). Moreover, tawny crazy ants were much more predaceous overall than predicted (Figure 3.4), suggesting a highly omnivorous diet. We observed workers feeding on plant nectaries, tending aphids on several plant species (including in trees and on small herbaceous plants), and consuming arthropod prey (Appendix B Supplementary

video files), which provides further support that this species is highly opportunistic and omnivorous. Past research on other abundant ant species proposed that colonies achieve high densities by feeding as strict herbivores (Tobin 1991, Davidson and Patrell-Kim 1996, Davidson et al. 2003). For example, arboreal ant species that were highly abundant in Peru had $\delta^{15}N$ values that were lower than those of known insect herbivores; in fact, their $\delta^{15}N$ values actually overlapped with those of plants collected from the same habitat (Davidson et al. 2003). Based on our results, tawny crazy ants do not follow the same trend, as workers had $\delta^{15}N$ values that were much higher than plants or known insect herbivores (Figure 3.4), and worker densities at baits increased with a more predaceous diet (Figure 3.1). As density increased, so too did their ability to outcompete other ant species for resources (Figure 3.3). There are several possible explanations for this relationship, as $\delta^{15}N$ values can be raised by feeding on higher amounts of prey or by feeding on lower amounts of carbohydrate resources. Workers were often observed walking rapidly up and down vegetation (i.e., on ashe juniper in Appendix B Supplementary Video 8), so greater foraging activity from carbohydrate consumption may increase the likelihood of workers encountering prey, thereby raising their trophic position. Many ant-hemipteran mutualisms correlate with reduced arthropod herbivore and predator abundance on plants (Styrsky and Eubanks 2007), often because the ants actively predate other arthropods occurring on or near tended hemipterans (Kaplan and Eubanks 2005). Conversely, tawny crazy ants may be more active when feeding on lower amounts of carbohydrates, which would also increase their trophic position. Tawny crazy ants fed low-sugar diets are more aggressive and less likely to be killed in

competitive interactions with red imported fire ants in the laboratory (Horn et al. 2013). Carbohydrate deprivation has also been shown to increase worker aggression and colony foraging in other ant species (Grangier and Lester 2014, Felden et al. 2018, Henry et al. 2018). Ultimately, future studies should quantify more specifically what tawny crazy ants are consuming in the field to tease apart the relationship between ant trophic position and foraging activity.

Several studies of other ant species have also found contrasting evidence to the hypothesis that ants typically reach higher abundance by behaving as herbivores (Tobin 1991, Davidson and Patrell-Kim 1996, Davidson et al. 2003). For example, Gibb and Cunningham (2011) found that none of the 42 ant species in their study fed as herbivores despite high abundance of several species. Carbohydrate supplementation did not increase brood production or alter worker foraging behavior in the obligate plant-ant *Crematogaster nigriceps* (Rudolph and Palmer 2013). Likewise, the behavior of two abundant North American ant species (*Camponotus chromaoides* and *Formica neogagates*) did not change in the presence of their honeydew-producing mutualist (Clark and Singer 2018). This suggests that the link between ant abundance, foraging activity, and herbivory may not be as straightforward as originally thought. In fact, many other invasive ant species are known to be opportunistic and highly omnivorous (Holway et al. 2002), which is part of what allows them to establish in many different environments. For example, foraging fire ant workers (*S. invicta*) were found returning with as many as 94 different prey items in a single hour (Rashid et al. 2013). Fire ant colonies also grow fastest with access to both carbohydrate resources and insect prey

(Helms and Vinson 2008). So-called 'top-heavy' food webs, in which predators achieve a greater biomass than herbivores, are not uncommon in nature (Snyder and Evans 2006, Hall 2011), especially when predators are actually omnivorous (McCauley et al. 2018, Woodson et al. 2018). Besides the obvious benefits of increased dietary flexibility in the face of limited resource availability, omnivores are also thought to more effectively route energy up a food web by avoiding less efficient feeding pathways (Utne-Palm et al. 2010, McCauley et al. 2018). We propose that extreme omnivory, rather than strict herbivory, may drive invasive ant abundance, especially in the case of tawny crazy ants.

Tawny crazy ant trophic position was surprisingly constant across sites despite dramatic changes in ant pitfall trap density (Figure 3.5). Increased dietary flexibility may be one way that this species maintains a relatively constant diet even in very different habitat types. The dietary flexibility of tawny crazy ants also indicates that management of ant densities may not be mitigated solely by reducing hemipteran abundance in trees or other plants, especially if floral and extrafloral nectaries are available. Several studies have shown decreased foraging activity when workers are excluded from hemipteran mutualists, including in yellow crazy ants (Green et al. 2013), Argentine ants (Brightwell et al. 2010), and tawny crazy ants (Sharma et al. 2019), but it is not known how these treatments affect worker mortality. Unfortunately, if workers are able to shift to another resource, this may make control of hemipteran insects ineffective in reducing ant densities. This treatment may be more effective at times of the year when plant-produced resources are scarce and climatic conditions have reduced colony size. For example, precipitation and temperature significantly affected ant densities at our field sites (Table

3.2, Figure 3.6). Additionally, pairing the control of hemipteran mutualists with the microsporidian pathogen *Myrmecomorba nylanderiae* may help in controlling tawny crazy ant numbers (Plowes et al. 2015, LeBrun et al. 2018). Carbohydrate-deprived colonies treated with the microsporidian pathogen suffered greater losses than colonies with access to carbohydrates (LeBrun et al. 2018), so colonies with reduced access to plant- or insect-produced honeydew may be more susceptible to the pathogen in the field. Because of their dietary flexibility, climatic conditions, rather than resource use, may have a greater influence on tawny crazy ant densities. Climate was also suggested as an overriding factor influencing Argentine ant abundance, despite some influence of resource availability (Rowles and Silverman 2009). Abiotic conditions are obviously critically important in determining the establishment and spread of invasive ant species. For example, cold temperatures and reduced soil moisture inhibit the spread of *S. invicta* in North America (Callcott et al. 2000, LeBrun et al. 2012). Our results suggest that temperature and precipitation also influence the abundance of tawny crazy ants, but it will be important to further examine the abiotic factors that limit or promote colony size in the field.

3.6. Conclusions

Overall, we found that tawny crazy ant trophic position was positively correlated with worker densities at baits and with resource dominance, suggesting that worker foraging is higher on a more predaceous diet. Greater densities allowed tawny crazy ants to more effectively outcompete other species for resources. Tawny crazy ants were much

more predaceous than traditional food web theory would predict, as colonies were highly omnivorous and opportunistic based on stable isotope analysis and direct observation. Due to their dietary flexibility, control of hemipteran mutualists alone may be ineffective at reducing tawny crazy ant abundance, especially in sites with alternative plant-based resources. Combining control of hemipteran mutualists with a microsporidian pathogen at times when abiotic conditions have already reduced colony sizes may be a more effective integrated pest management strategy for this species. Further research will be necessary to tease apart the effects of resource use on tawny crazy ant abundance. However, our results contribute to a growing body of literature suggesting that increased ant abundance and foraging activity does not always correlate with a more herbivorous diet. We propose that omnivory, rather than herbivory, may drive tawny crazy ant densities throughout their invaded range.

3.7. References

Abbott, K. L. 2005. Supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on an oceanic island: forager activity patterns, density and biomass. Insectes Sociaux **52**:266-273.

Aldana, R. C., M. L. Baena, and P. Chacón de Ulloa. 1995. Introducción de la hormiga loca (*Paratrechina fulva*) a la Reserva Natural Laguna de Sonso (Valle del Cauca, Colombia). Boletin del Museo de Entomología de la Universidad del Valle **3**:15-28.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software **67**:1-48.

Benois, A., J. Lafont, and J. Marro. 1973. Incidence des facteurs écologiques sur le cycle annuel et l'activité saisonnière de la fourmi d'Argentine, *Iridomyrmex humilis* Mayr (Hymenoptera, Formicidae), dans la région d'Antibes. Insectes Sociaux **20**:267-295.

Brightwell, R., S. Bambara, and J. Silverman. 2010. Combined effect of hemipteran control and liquid bait on Argentine ant populations. Journal of Economic Entomology **103**:1790-1796.

Callcott, A.-M. A., D. H. Oi, H. L. Collins, D. F. Williams, and T. C. Lockley. 2000. Seasonal studies of an isolated red imported fire ant (Hymenoptera: Formicidae) population in eastern Tennessee. Environmental Entomology **29**:788-794.

Clark, R. E., and M. S. Singer. 2018. Differences in aggressive behaviors between two ant species determine the ecological consequences of a facultative food-for-protection mutualism. Journal of Insect Behavior **31**:510-522.

Colley, W. N. 2002. Colley's bias free college football ranking method: the Colley matrix explained. Princeton University, Princeton.

Coplen, T. B. 2011. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Communications in Mass Spectrometry **25**:2538-2560.

Davidson, D. W. 1997. The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. Biological Journal of the Linnean Society **61**:153-181.

Davidson, D. W., S. C. Cook, R. R. Snelling, and T. H. Chua. 2003. Explaining the abundance of ants in lowland tropical rainforest canopies. Science **300**:969-972.

Davidson, D. W., and L. Patrell-Kim. 1996. Tropical arboreal ants: why so abundant? Pages 127-140 Neotropical biodiversity and conservation. Mildred E. Mathias Botanical Garden.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta **45**:341-351.

Elton, C. 1927. Animal ecology. The Macmillan Company, New York.

Feener, D. H. 1981. Competition between ant species: outcome controlled by parasitic flies. Science **214**:815-817.

Felden, A., C. I. Paris, D. G. Chapple, J. Haywood, A. V. Suarez, N. D. Tsutsui, P. J. Lester, and M. A. Gruber. 2018. Behavioural variation and plasticity along an invasive ant introduction pathway. Journal of Animal Ecology **87**:1653-1666.

Fox, J., and S. Weisberg. 2018. An R companion to applied regression. Sage Publications.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnology and Oceanography **33**:1182-1190.

Gibb, H., and S. A. Cunningham. 2011. Habitat contrasts reveal a shift in the trophic position of ant assemblages. Journal of Animal Ecology **80**:119-127.

Grangier, J., and P. J. Lester. 2014. Carbohydrate scarcity increases foraging activities and aggressiveness in the ant *Prolasius advenus* (Hymenoptera: Formicidae). Ecological Entomology **39**:684-692.

Green, P. T., D. J. O'Dowd, G. Neumann, and S. Wittman. 2013. Research and development of biological control for scale insects: indirect control of the yellow crazy ant on Christmas Island, 2009-2013. Bulletin of Entomological Research **14**:147-212.

Greenslade, P. 1973. Sampling ants with pitfall traps: digging-in effects. Insectes Sociaux **20**:343-353.

Grover, C. D., A. D. Kay, J. A. Monson, T. C. Marsh, and D. A. Holway. 2007. Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. Proceedings of the Royal Society of London B: Biological Sciences **274**:2951-2957.

Hall, R. J. 2011. Eating the competition speeds up invasions. Biology Letters:rsbl20100766.

Helms, K. R. 2013. Mutualisms between ants (Hymenoptera: Formicidae) and honeydew-producing insects: are they important in ant invasions. Myrmecological News **18**:61-71.

Helms, K. R., and S. B. Vinson. 2008. Plant resources and colony growth in an invasive ant: the importance of honeydew-producing hemiptera in carbohydrate transfer across trophic levels. Environmental Entomology **37**:487-493.

Henry, L. P., C. K. Tokita, M. Misra, A. B. Forrow, and D. I. Rubenstein. 2018. Mutualistic acacia ants exhibit reduced aggression and more frequent off-tree movements near termite mounds. Biotropica **50**:559-562.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Harvard University Press.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, and T. J. Case. 2002. The causes and consequences of ant invasions. Annual Review of Ecology and Systematics **33**:181-233.

Horn, K. C., M. D. Eubanks, and E. Siemann. 2013. The effect of diet and opponent size on aggressive interactions involving caribbean crazy ants (*Nylanderia fulva*). PloS One **8**:e66912.

Kaplan, I., and M. D. Eubanks. 2005. Aphids alter the community-wide impact of fire ants. Ecology **86**:1640-1649.

Kay, A. D., T. Zumbusch, J. L. Heinen, T. C. Marsh, and D. A. Holway. 2010. Nutrition and interference competition have interactive effects on the behavior and performance of Argentine ants. Ecology **91**:57-64.

Kjeldgaard, M.K., J. Hewlett, M.D. Eubanks. 2020. Widespread variation in stable isotope trophic position estimates: patterns, causes, and potential consequences. *Manuscript in preparation.*

Lasmar, C., A. Queiroz, A. Rabello, R. Feitosa, E. Canedo-Júnior, F. Schmidt, R. Cuissi, and C. Ribas. 2017. Testing the effect of pitfall-trap installation on ant sampling. Insectes Sociaux **64**:445-451.

LeBrun, E., R. Plowes, and L. Gilbert. 2012. Imported fire ants near the edge of their range: disturbance and moisture determine prevalence and impact of an invasive social insect. Journal of Animal Ecology **81**:884-895.

LeBrun, E. G., J. Abbott, and L. E. Gilbert. 2013. Imported crazy ant displaces imported fire ant, reduces and homogenizes grassland ant and arthropod assemblages. Biological Invasions **15**:2429-2442.

Lebrun, E. G., and D. H. Feener. 2007. When trade-offs interact: balance of terror enforces dominance discovery trade-off in a local ant assemblage. Journal of Animal Ecology **76**:58-64.

LeBrun, E. G., K. J. Ottens, and L. E. Gilbert. 2018. The microsporidian pathogen *Myrmecomorba nylanderiae*: Intracolony transmission and impact upon tawny crazy ant (*Nylanderia fulva*) colonies. Journal of Applied Entomology **142**:114-124.

Lester, P. J. 2008. Integrated pest management: an under-utilized tool for conservation and the management of invasive ants and their mutualistic Hemiptera in the Pacific. Pacific Conservation Biology **14**:246-247.

Lester, P. J., and M. A. Gruber. 2016. Booms, busts and population collapses in invasive ants. Biological Invasions **18**:1-11.

McCauley, D. J., G. Gellner, N. D. Martinez, R. J. Williams, S. A. Sandin, F. Micheli, P. J. Mumby, and K. S. McCann. 2018. On the prevalence and dynamics of inverted trophic pyramids and otherwise top-heavy communities. Ecology Letters **21**:439-454.

McCutchan, J., W. Lewis, C. Kendall, and C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos **102**:378-390.

Menke, S. B., A. V. Suarez, C. V. Tillberg, C. T. Chou, and D. A. Holway. 2010. Trophic ecology of the invasive argentine ant: spatio-temporal variation in resource assimilation and isotopic enrichment. Oecologia **164**:763-771.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta **48**:1135-1140.

Plowes, R. M., J. J. Becnel, E. G. LeBrun, D. H. Oi, S. M. Valles, N. T. Jones, and L. E. Gilbert. 2015. *Myrmecomorba nylanderiae* gen. et sp. nov., a microsporidian parasite of the tawny crazy ant *Nylanderia fulva*. Journal of Invertebrate Pathology **129**:45-56.

Pohlert, T. 2014. The pairwise multiple comparison of mean ranks package (PMCMR). R package **27**:9.

Porter, S. D., D. F. Williams, R. S. Patterson, and H. G. Fowler. 1997. Intercontinental differences in the abundance of *Solenopsis* fire ants (Hymenoptera: Formicidae): escape from natural enemies? Environmental Entomology **26**:373-384.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**:703-718.

R Core Team 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rashid, T., J. Chen, J. T. Vogt, and P. J. McLeod. 2013. Arthropod prey of imported fire ants (Hymenoptera: Formicidae) in Mississippi sweetpotato fields. Insect Science **20**:467-471.

Rowles, A. D., and J. Silverman. 2009. Carbohydrate supply limits invasion of natural communities by Argentine ants. Oecologia **161**:161-171.

Rudolph, K. P., and T. M. Palmer. 2013. Carbohydrate as fuel for foraging, resource defense and colony growth–a long-term experiment with the plant-ant *Crematogaster nigriceps*. Biotropica **45**:620-627.

Sharma, S., E. A. Buss, G. S. Hodges, and D. H. Oi. 2019. Effect of soil treatments for cottony cushion scale (Hemiptera: Monophlebidae) control on *Nylanderia fulva* (Hymenoptera: Formidicae) survival and trailing activity. Florida Entomologist **102**:202-206.

Sharma, S., D. Oi, and E. Buss. 2013. Honeydew-producing hemipterans in Florida associated with *Nylanderia fulva* (Hymenoptera: Formicidae), an invasive crazy ant. The Florida Entomologist **96**:538-547.

Shurin, J. B., D. S. Gruner, and H. Hillebrand. 2005. All wet or dried up? Real differences between aquatic and terrestrial food webs. Proceedings of the Royal Society B: Biological Sciences **273**:1-9.

Snyder, W., and E. Evans. 2006. Ecological effects of invasive arthropod generalist predators. Annual Review of Ecology, Evolution, and Systematics **37**:95-122.

Styrsky, J. D., and M. D. Eubanks. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. Proceedings of the Royal Society of London B: Biological Sciences **274**:151-164.

Tillberg, C., D. Holway, E. LeBrun, and A. Suarez. 2007. Trophic ecology of invasive Argentine ants in their native and introduced ranges. Proceedings of the National Academy of Sciences of the United States of America **104**:20856-20861.

Tillberg, C. V., D. P. McCarthy, A. G. Dolezal, and A. V. Suarez. 2006. Measuring the trophic ecology of ants using stable isotopes. Insectes Sociaux **53**:65-69.

Tobin, J. E. 1991. A neotropical rainforest canopy, ant community: some ecological considerations. Pages 536-538 *in* C. Huxley and D. Cutler, editors. Ant-plant interactions. Oxford University Press.

Utne-Palm, A. C., A. G. Salvanes, B. Currie, S. Kaartvedt, G. E. Nilsson, V. A. Braithwaite, J. A. Stecyk, M. Hundt, M. Van der Bank, and B. Flynn. 2010. Trophic structure and community stability in an overfished ecosystem. Science **329**:333-336.

Vanderklift, M., and S. Ponsard. 2003. Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. Oecologia **136**:169-182.

Wetterer, J., O. Davis, and J. Williamson. 2014. Boom and bust of the tawny crazy ant, *Nylanderia fulva* (Hymenoptera: Formicidae), on St. Croix, US Virgin Islands. The Florida Entomologist **97**:1099-1103.

Wetterer, J. K., X. Espadaler, A. L. Wetterer, D. Aguin-Pombo, and A. M. Franquinho-Aguiar. 2006. Long-term impact of exotic ants on the native ants of Madeira. Ecological Entomology **31**:358-368.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer.

Wilder, S., D. Holway, A. Suarez, and M. Eubanks. 2011a. Macronutrient content of plant-based food affects growth of a carnivorous arthropod. Ecology **92**:325-332.

Wilder, S., D. Holway, A. Suarez, E. LeBrun, and M. Eubanks. 2011b. Intercontinental differences in resource use reveal the importance of mutualisms in fire ant invasions. Proceedings of the National Academy of Sciences of the United States of America **108**:20639-20644.

Wittman, S. E., D. J. O'Dowd, and P. T. Green. 2018. Carbohydrate supply drives colony size, aggression, and impacts of an invasive ant. Ecosphere **9**:e02403.

Woodson, C. B., J. R. Schramski, and S. B. Joye. 2018. A unifying theory for top-heavy ecosystem structure in the ocean. Nature Communications **9**:23.

Zenner-Polania. 1990. Biological aspects of the hormiga-loca, *Paratrechina-(Nylanderia)-fulva* (Mayr), in Colombia. Pages 290-297 *in* R. K. Vander Meer, K. Jaffe, and A. Cedeno, editors. Applied myrmecology: a world perspective. Westview Press.

Zenner-Polania, I. 1994. Impact of *Paratrechina fulva* on other ant species. Pages 121-132 *in* D. F. Williams, editor. Exotic ants. Biology, impact, and control of introduced species. Westview Press.

4. SPIKING THE ANT PUNCH: EVALUATION OF STABLE ISOTOPE TRACERS FOR STUDYING THE TROPHIC ECOLOGY OF INVASIVE ANTS

4.1. Overview

Labeling food using stable isotope tracers is an effective way to measure trophic interactions, but it has rarely been applied to ants. Isotope tracers could be used to answer a variety of questions in ant ecology research, including quantifying colony sharing, determining the importance of specific food resources, and mapping the flow of nutrients between ant colonies and other trophic levels. We applied this technique to laboratory and field colonies of the red imported fire ant (*Solenopsis invicta*; hereafter fire ant). Our primary objectives were to: 1) determine the amount of isotope necessary to sufficiently enrich ant worker isotope values in the laboratory, 2) characterize how worker isotope values change over time, and 3) verify our techniques in the field. We also examined the applicability of our technique to other ant species using the tawny crazy ant (*Nylanderia fulva*). We fed standardized experimental colonies of fire ants a total of 33.8µg of ^{15}N-labeled glycine and 270.2µg of ^{13}C-labeled glucose in the laboratory. We collected workers before feeding and then at 24, 48, and 72 hours post-feeding. To verify our methods, we also fed a total of 160mg of ^{15}N-labeled glycine over a 14-day period to active fire ant mounds in the field. We collected workers from each mound before feeding and then at 7 and 14 days while feeding. We applied the same methods to a population of tawny crazy ants. Both tracers were sufficient to detect highly enriched isotope values in laboratory colonies of fire ants after 24 hours, and

these signals remained detectable for up to 48 hours for carbon and 72 hours for nitrogen. Our methods were also successful in the field for both ant species, especially after 14 days of feeding. Due to the larger size of field colonies, we fed each colony a greater amount of ^{15}N over a longer period of time compared with laboratory colonies, so the maximum values of $\delta^{15}N$ were much higher in the field than in our laboratory experiment (fire ant = 1403‰; tawny crazy ant = 2018‰). We recommend analyzing background natural abundance samples in a separate mass spectrometry run than the labeled samples to eliminate potential 'memory effects' from highly ^{15}N (or ^{13}C) enriched samples. Isotope tracers allow for a more direct measurement of nutrient fluxes and are a promising tool to unravel fundamental questions in ant trophic ecology.

4.2. Introduction

The abundance and distribution of ants is strongly impacted by their trophic interactions. Ant species that feed on higher levels of carbohydrate resources, for example, are often more abundant than predaceous species (Tobin 1991, Davidson 1997, Davidson et al. 2003). Invasion-associated changes in diet can also fuel the invasion of certain ant species by increasing colony growth, worker survival, aggression, and foraging activity (Holway et al. 2002, Tillberg et al. 2007, Savage et al. 2011, Wilder et al. 2011b). This appears to be the case for the red imported fire ant (*Solenopsis invicta*; hereafter fire ant). Fire ant densities are 4-7 times higher in their invaded range in North America compared with their native range in South America (Porter et al. 1997), which may be due in part to their increased consumption of carbohydrate resources in North

America (Wilder et al. 2011b). Fire ant workers dominated carbohydrate baits in the field seven times more frequently in their invaded range than in their native range (Wilder et al. 2011b), and colonies with access to aphid honeydew produced 50% more workers than colonies without access to aphid honeydew (Wilder et al. 2011a).

Although diet often determines the density and spatial distribution of ant colonies, trophic interactions are difficult to assess in ants. Many ants spend most of their lives underground, within decaying wood, or inside a nest, so direct observation of their diet is challenging, if not impossible (Hölldobler & Wilson 1990). Additionally, the food that is consumed by a colony is typically collected by dozens, hundreds, or even thousands of workers, further complicating attempts to characterize their diets. Labeling food using dye or radioactive elements is one effective way to quantify resource use within and among ant colonies (Vinson 1968, Howard & Tschinkel 1981, Hagler & Jackson 2001, Catalani et al. 2019). However, most dyes are retained for a relatively short amount of time in insects (Oi 2000, Hagler & Jackson 2001), so longer–term studies are typically not possible. For example, Miller (1993) detected only very small amounts of a fluorescent dye fed to termites after two weeks. Additionally, dyes are usually lost between trophic levels, so this method is not suitable for ecologists interested in the flow of nutrients between multiple trophic levels (Hagler & Jackson 2001). Radioactive elements may be detectable for a longer period of time, but stricter environmental laws have constrained the use of radioactive elements in the field.

The use of stable isotope tracers to label food is a suitable alternative to dye or radioactive elements. Stable isotope tracers employ naturally occurring, stable (non-

radioactive) forms of biologically relevant elements, such as nitrogen and carbon. The heavier isotopes of nitrogen and carbon (^{15}N and ^{13}C) occur rarely in nature, so by artificially 'spiking' a food with an appropriate concentration of a heavy isotope, ecologists can trace the movement of this isotope through consumers and identify the flow of nutrients through an ecosystem (Fry 2006). Grabmaier et al. (2014), for example, used isotopically spiked earthworms to quantify the flow of nitrogen between earthworms, plants, and aphids on the plants. Isotopes are incorporated into the tissue of the consumer, so an isotope tracer can be detected much longer after feeding. For example, after feeding ant colonies of *Petalomyrmex phylax* an isotope tracer mixed in honey, workers showed spiked isotope levels for up to 660 days (Defossez et al. 2010).

Despite the utility of isotope tracers, there are several challenges. First, it is important to introduce the right amount of a tracer into a system. Too little and it will not enrich consumer isotope values enough to be detected (Fry 2006). Too much and the cost of materials quickly becomes prohibitively expensive. High levels of the heavier isotope in one sample can also influence the mass spectrometer measurements of subsequent samples in what is known as the 'memory effect' (Mannerheim et al. 2019), so extremely enriched samples may result in less accurate measurements. In one case, Mannerheim et al. (2019) found that samples highly enriched in ^{15}N influenced the accuracy of mass spectrometer measurements by an average of 6.7%. Second, the length of time that a tracer remains detectable in an organism varies depending on the consumer, the ecosystem, and the turnover rate of the tracer in the labeled organism (Tieszen et al. 1983, Overmyer et al. 2008). For example, although ant workers showed

spiked nitrogen values for up to 660 days post-feeding (Defossez et al. 2010), the nitrogen values in spiked earthworms decreased dramatically after just 16 days (Dyckmans et al. 2005). It is therefore useful to establish best practices with a given species before introducing a tracer into the field.

Although the methods for isotope tracers are relatively well described in other taxa (Mulholland et al. 2000, Van den Meersche et al. 2004, Dyckmans et al. 2005, Fry 2006), this technique has rarely been applied to ants (Fischer et al. 2003, Defossez et al. 2010). Myrmecologists have used stable isotope analysis of natural abundance values of ^{15}N and ^{13}C to characterize colony trophic position and describe food webs (Blüthgen et al. 2003, Tillberg et al. 2007, Feldhaar et al. 2010, Wilder et al. 2011b), but isotope tracers allow for a more direct measurement of nutrient fluxes between organisms. When an isotope label is detected in an organism that was not fed the labeled resource, this serves as direct evidence of nutrient exchange (Feldhaar et al. 2010). Isotope tracers, therefore, allow myrmecologists to quantify nutrient fluxes between nestmates, conspecifics, and other trophic levels. For example, after feeding a nitrogen tracer to ant colonies of *Pheidole bicornis*, Fischer et al. (2003) found highly enriched values in the myrmecophytic *Piper* plants that housed the colonies, indicating nutrient exchange between ant colonies and their host plant mutualists.

Past research using isotope tracers in ants has primarily focused on ant-plant mutualisms, using ant species limited to a single plant with relatively small colony sizes (i.e., 100 - 10,000 workers; Fischer et al. 2003, Dalecky et al. 2005). Because ant-plant mutualists continually exchange nutrients with each other (Fischer et al. 2003, Defossez

et al. 2010), the tracer likely remains much longer in the colony compared with other ant species that forage more broadly. The methods, therefore, may differ when applying isotope tracers to large colonies of ground-nesting ant species. More of the tracer will likely be required to detect enriched values in larger colonies because more workers will be feeding on the labeled food. The isotope values of workers may also change differently over time compared with ant-plant mutualists because workers are often foraging across a larger landscape instead of on a single plant.

As a first step in developing methods for isotope tracers in ground-nesting ant species, we applied this technique to laboratory and field colonies of fire ants (*S. invicta*). Fire ant colonies nest in distinctive mounds in the soil and often contain 200,000 - 300,000 workers per mound (Vinson 1997, Tschinkel 2006). Fire ants are an invasive pest in many parts of the world (CABI 2019), and their trophic interactions can vary significantly across their biogeographic range (Wilder et al. 2011b), season (Claborn & Phillips 1986, Porter & Tschinkel 1987, Cook et al. 2011), and social form (Weeks Jr et al. 2004, Tschinkel 2006). Understanding fire ant resource use has important implications for management and ecological impact assessment, so it is essential to develop techniques to more easily assess their trophic interactions in the field.

We also examined the applicability of our technique to another tramp ant species using the tawny crazy ant (*Nylanderia fulva*). The tawny crazy ant is a highly destructive invader with a very different biology from the fire ant (Zenner-Polania 1994, Aldana et al. 1995, LeBrun et al. 2013, Wetterer et al. 2014). Tawny crazy ant colonies are also

very large and can sometimes reach densities of more than one million ants per hectare (Kjeldgaard et al., *unpubl. data*). Instead of distinct mounds, however, tawny crazy ants form vast supercolonies throughout their invaded range, in which workers regularly share collected resources with each other and occupy transitory nests (Zenner-Polania 1990, Wang et al. 2016, Eyer et al. 2018). As a consequence, the amount of tracer required to detect enriched isotope signatures in tawny crazy ants may differ from that of fire ants. By applying an isotope tracer to both fire ants and tawny crazy ants in the field, we hoped to determine how broadly this technique can be applied to different ant species.

Our experiment had three primary objectives: 1) determine the amount of isotope necessary to detect a sufficient change in ant worker isotope values in a controlled laboratory setting, 2) characterize how worker isotope values change over time, and 3) verify our techniques in the field.

4.3. Methods

4.3.1. Laboratory experiment

We collected four fire ant colonies in July 2019 from a pasture near College Station, Texas (Texas A&M Field Laboratory, Burleson Co., TX; 30° 33' 14"N, 96° 25' 41"W; permission granted by Texas A&M AgriLife Research). Colonies were extracted from the soil using drip flotation (Banks et al. 1981), and each field colony was divided into three standardized experimental "colonies" of 40 workers, resulting in a total of 12 experimental colonies.

Experimental colonies were maintained in individual 8-oz. deli containers lined with Fluon® (Insect-a-slip Insect Barrier, BioQuip Products, 2321 Gladwick St., Rancho Dominguez, CA, USA) with a nesting tube (15mL plastic centrifuge tube) filled one-third with water and stoppered with cotton. All colonies were maintained in standardized laboratory conditions throughout the experiment (12:12 hr light/dark cycle, 24-32˚C temperature, 40-70% relative humidity).

A solution with 15mM of ^{15}N-labeled glycine (98 atom%, Sigma-Aldrich, Inc., St. Louis, MO, USA) and 50mM of ^{13}C-labeled glucose (D-$^{13}C_6$, 99 atom%, Sigma-Aldrich, Inc., St. Louis, MO, USA) was created using distilled water. We placed a 30µL droplet of solution on the bottom of each colony container using a pipette and allowed workers to feed completely on the droplet. Each experimental colony was fed a total of 33.8µg of ^{15}N-labeled glycine (~0.845µg per worker) and 270.2µg of ^{13}C-labeled glucose (~6.755µg per worker). These concentrations were established by calculating the amount of tracer necessary to detect changes in each of the 40 workers within an experimental colony.

Ten workers were removed from each experimental colony at the beginning of the experiment within a few hours of creating experimental colonies (time point 0) to serve as a baseline measurement. After pipetting the solution into each colony, we then removed 10 workers per colony at 24, 48, and 72 hours until no workers remained. All workers were frozen at -10˚C prior to processing for stable isotope analysis.

4.3.2. Fire ant field validation

To verify our methods, we also fed a nitrogen tracer to fire ant colonies in the field in August 2019. We used only a nitrogen tracer for our field experiment based on results from our laboratory experiment and due to the lower cost of ^{15}N-labeled glycine compared with ^{13}C-labeled glucose. We identified active fire ant mounds in two field sites near College Station, Texas, USA (Texas A&M Ecology and Natural Resource Teaching Area, Brazos Co., TX: 30˚ 34' 30.3486" N, 96˚ 22' 0.048" W; Coulter Airfield, Brazos Co., TX: 30˚ 42' 59.1156" N, 96˚ 20' 1.95" W). We selected 3-4 mounds at each site separated by at least 50m to avoid possible contamination between mounds. We treated a total of seven mounds across two field sites. A solution with 102mM of ^{15}N-labeled glycine (98 atom%, Sigma-Aldrich, Inc., St. Louis, MO, USA) and 61.5mM of unlabeled sucrose was created using distilled water. We chose to use a higher concentration of ^{15}N-labeled glycine due to the large number of fire ants often found within field colonies, which can exceed 250,000 worker ants (Tschinkel 2006). The solution was mixed in bulk at the beginning of the field experiment and frozen between uses to avoid mold growth. We filled 1-mL microcentrifuge tubes with 1mL of the solution and stoppered each with cotton. Three of these vials were left on the surface of each mound and replaced every other day for 14 days. Each field colony was fed a total of 160mg of ^{15}N-labeled glycine in 21mL of sugar solution over a 14-day period. Fire ant workers were observed feeding on the solution, and there was evidence of mound building over the vial opening, indicating worker foraging (Figure 4.1).

We disturbed each mound and collected 10 workers from each field colony at the beginning of the experiment (time point 0) to serve as baseline measurements. While feeding colonies the tracer solution, we then removed 10 workers per colony at 7 days (in the middle of the experiment) and 14 days (at the end of the experiment). All workers were frozen at -10˚C prior to processing for stable isotope analysis.

Figure 4.1 Evidence of fire ant feeding and mound building over the opening of a 1-mL vial containing a nitrogen tracer in sugar solution (photo by MacKenzie K. Kjeldgaard).

4.3.3. Tawny crazy ant field validation

To verify the applicability of our technique with other ant species, we also fed a tracer solution (using the same amounts and methods as described above for fire ant colonies in the field) to a population of tawny crazy ants (*N. fulva*) near College Station, TX, USA (Silver Horse Ranch, Brazos Co., TX: 30˚ 43' 38.6184" N, 96˚ 19' 26.097" W). The density of tawny crazy ants at this site is very high, and there are no longer any fire ants at this location (Kjeldgaard et al., *unpubl. data*).

We marked three points separated by at least 50m to serve as replicates. Because tawny crazy ants do not form mounds, we sampled ants at each point using baits that consisted of a single hotdog piece (~4g wet weight) and honey on a laminated card. Baits were left out for five minutes and then approximately 40 workers were aspirated from each bait card. We left three 1-mL vials of the tracer mixed with sugar water (as described above) at each point and sampled ants at 0 (for baseline measurements), 7, and 14 days from these same sampling points. All ants were frozen at -10˚C prior to processing for stable isotope analysis.

4.3.4. Stable isotope analysis

Workers were dried in an oven at 60˚C for 48 hours and then stored in airtight vials. The abdomens of all ants were removed and discarded prior to weighing to avoid possible effects of crop contents on isotopic signatures (Tillberg et al. 2006). Approximately five workers per sample were pooled, and chopped in glass vials to fine homogeneous powders using small scissors. Approximately 0.400mg of each sample

was weighed into tin capsules (Costech Analytical Technologies Inc., Valencia, CA, USA) using a microbalance (Mettler Toledo, Columbus, OH, USA). All samples were analyzed at the Texas A&M University Stable Isotopes for Biosphere Science Laboratory (https://sibs.tamu.edu/) using a Delta V Advantage Isotope Ratio Mass Spectrometer coupled with Costech Elemental Analyzer and Thermo ConFlo IV Universal Interface (Thermo Fisher Scientific, Waltham, MA, USA). All baseline natural abundance samples were run before any post-feeding samples to ensure that natural abundance values were not influenced by memory effects from the high levels of ^{15}N in spiked samples, which can sometimes impact the measurements.

Stable isotope values are reported as "δX" (i.e., $\delta^{15}N$) and expressed in "per mil" or "‰" notation. Values were calculated by comparing the ratio of heavy to light stable isotopes in the sample to that in a standard (air for nitrogen, and Vienna Pee Dee Belemnite for carbon) using the following equation (Coplen 2011):

$$\delta X = [(R_{sample}/ R_{standard}) - 1] \times 1{,}000$$

4.3.5. Data analysis

All statistical analyses were conducted in R v3.6.1 (R Core Team 2019). We conducted repeated-measures ANOVAs using restricted maximum likelihood (REML) in the *nlme* package (Pinheiro et al. 2019). For our laboratory experiment, $\delta^{15}N$ and $\delta^{13}C$ were the response variables, time point was a fixed effect, and colony of origin was a random effect. For our fire ant field experiment, $\delta^{15}N$ was the response variable, time

point and site were fixed effects, and mound was a random effect. Similarly, for our tawny crazy ant field experiment, $\delta^{15}N$ was the response variable, time point was a fixed effect, and sampling point was a random effect. We used Tukey's HSD as a post-hoc test with a significance level of $\alpha = 0.05$. We tested data for heterogeneity of variances and normality to ensure all assumptions were met. All plots were generated using *ggplot2* (Wickham 2016).

4.4. Results

4.4.1. Laboratory experiment

Our feeding trial was sufficient to see spiked isotopic values for both nitrogen and carbon at just 24 hours after feeding, and values remained significantly spiked after 48 hours (Figures 4.2 and 4.3). At 72 hours, $\delta^{15}N$ remained significantly more enriched than natural abundance levels (Figure 4.2); however, $\delta^{13}C$ values were not statistically significantly different from either natural abundance values or values at 48 hours (Figure 4.3). We found no significant effect of colony on either $\delta^{15}N$ values ($F_{11,33} = 1.700$, $P = 0.117$) or $\delta^{13}C$ values ($F_{11,33} = 1.657$, $P = 0.128$). Isotope levels changed significantly over time for both nitrogen (Figure 4.2; $F_{3,33} = 15.860$, $P < 0.001$) and carbon (Figure 4.3; $F_{3,33} = 16.902$, $P < 0.001$), with 24 hours post-feeding showing the highest average levels of both tracers ($\delta^{15}N = 428 \pm 70‰$; $\delta^{13}C = 438 \pm 73‰$) and 72 hours showing the lowest ($\delta^{15}N = 191 \pm 26‰$; $\delta^{13}C = 131 \pm 29‰$). Values at 48 hours were intermediate for both tracers ($\delta^{15}N = 327 \pm 66‰$; $\delta^{13}C = 247 \pm 65‰$). The baseline isotope levels before feeding colonies the tracers were $6.9 \pm 0.2‰$ for $\delta^{15}N$ and $-20.4 \pm 0.7‰$ for $\delta^{13}C$.

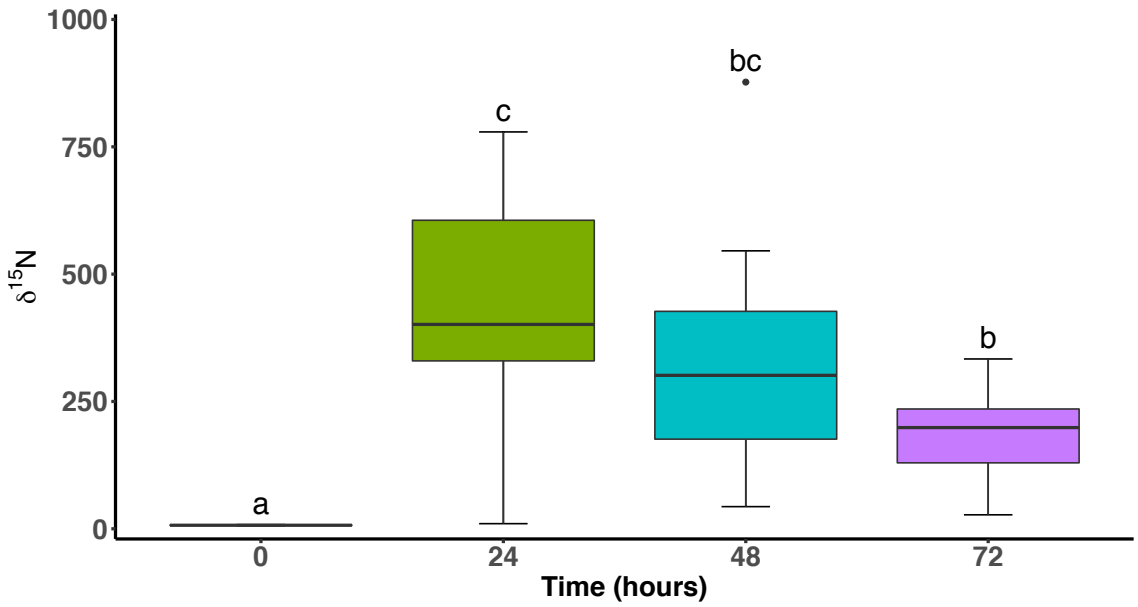


Figure 4.2 $\delta^{15}N$ values of ant workers before feeding the tracers (time = 0) and 24, 48, and 72 hours after feeding. Center lines within box plots represent medians, hinges represent upper and lower quartiles, whiskers represent 1.5 times above or below the interquartile range, and dots represent outliers. Letters indicate significant differences according to Tukey's HSD at $\alpha = 0.05$.

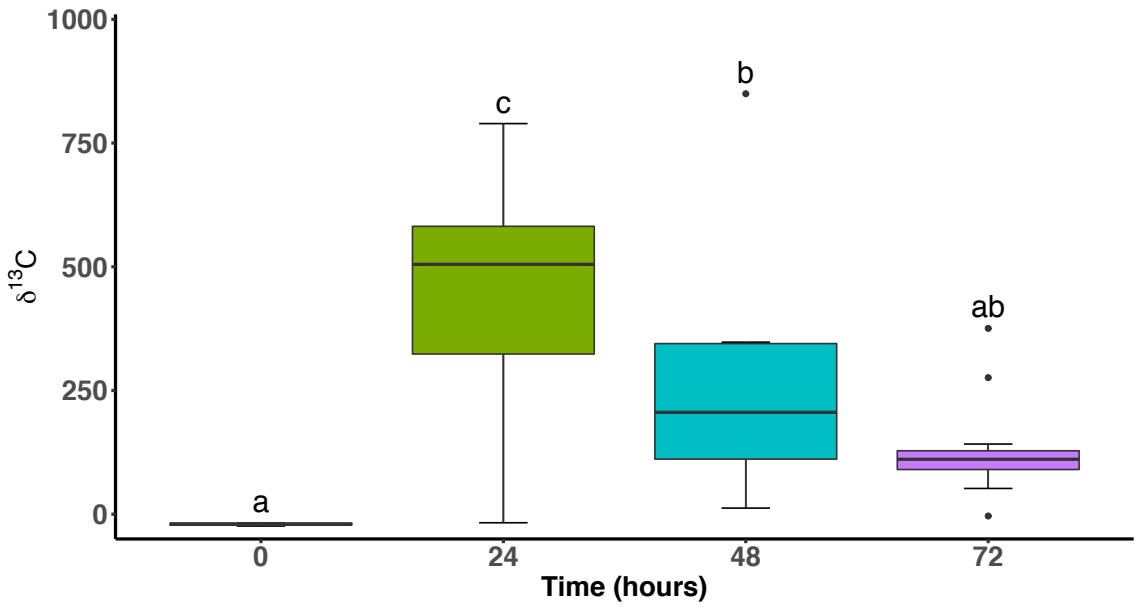


Figure 4.3 $\delta^{13}C$ values of ant workers before feeding the tracers (time = 0) and 24, 48, and 72 hours after feeding. Center lines within box plots represent medians, hinges represent upper and lower quartiles, whiskers represent 1.5 times above or below the interquartile range, and dots represent outliers. Letters indicate significant differences according to Tukey's HSD at α = 0.05.

At 24 hours post-feeding, the $\delta^{15}N$ values ranged from 10‰ to 779‰, and the $\delta^{13}C$ values ranged from -17‰ (which was the same sample with the lowest $\delta^{15}N$ value) to 789‰. At 48 hours post-feeding, the $\delta^{15}N$ values ranged from 44‰ to 877‰, and the $\delta^{13}C$ values ranged from 12‰ to 850‰. Finally, at 72 hours post-feeding, the $\delta^{15}N$ values ranged from 27‰ to 333‰, and the $\delta^{13}C$ values ranged from -4‰ to 375‰.

4.4.2. Field validation

Our methods also worked well in both fire ants and tawny crazy ants in the field. As expected, there was a significant effect of time on worker $\delta^{15}N$ values for both fire

ants ($F_{2,17} = 11.611$, $P < 0.001$) and tawny crazy ants ($F_{2,6} = 8.378$, $P = 0.018$). Baseline $\delta^{15}N$ levels before feeding field colonies were 5.4 ± 0.6‰ for fire ants and 5.0 ± 0.4‰ for tawny crazy ants. Worker values were significantly enriched after 7 days of tracer feeding (fire ant: $\delta^{15}N = 430 \pm 130$‰; tawny crazy ant: $\delta^{15}N = 1407 \pm 413$‰), and these values did not change significantly after 14 days of tracer feeding (Figures 4.4 and 4.5; fire ant: $\delta^{15}N = 791 \pm 147$‰; tawny crazy ant: $\delta^{15}N = 1305 \pm 219$‰). This trend was consistent across both fire ant sites, as there was no significant effect of site on fire ant $\delta^{15}N$ values ($F_{1,17} = 0.419$, $P = 0.526$). Tawny crazy ants had significantly higher $\delta^{15}N$ values compared with fire ants ($F_{1,26} = 10.266$, $P = 0.0036$), which may be due to a difference in collection method (i.e., disturbing the mound vs. collecting from baits).

For fire ants, worker $\delta^{15}N$ values ranged from 9‰ to 929‰ after 7 days of tracer feeding and from 341‰ to 1403‰ after 14 days of tracer feeding. The low minimum $\delta^{15}N$ at 7 days (9‰) was from one mound that was not observed feeding on the tracer solution at the beginning of the experiment. Workers from this colony began feeding during the remainder of the experiment, however, and showed spiked $\delta^{15}N$ values at 14 days (341‰). Without this mound, the minimum $\delta^{15}N$ for fire ants at 7 days was 36‰. For tawny crazy ants worker $\delta^{15}N$ ranged from 619‰ to 2018‰ after 7 days of tracer feeding and from 341‰ to 1403‰ after 14 days of tracer feeding.

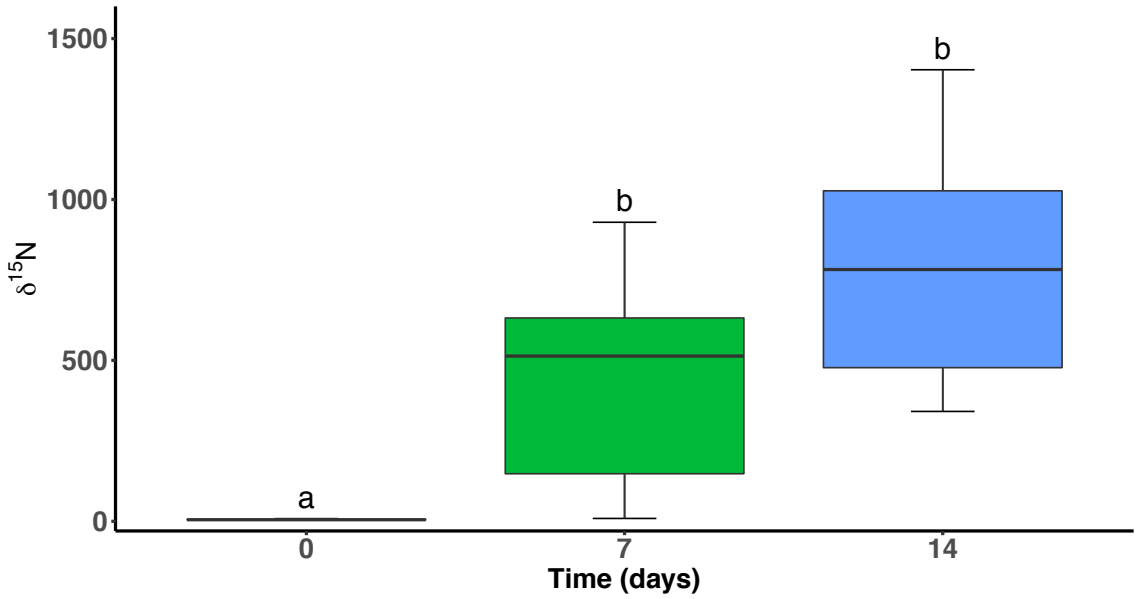


Figure 4.4 $\delta^{15}N$ values of fire ant workers from two field sites before feeding a nitrogen tracer (time = 0), and 7 and 14 days during tracer feeding. Center lines within box plots represent medians, hinges represent upper and lower quartiles, whiskers represent 1.5 times above or below the interquartile range, and dots represent outliers. Letters indicate significant differences according to Tukey's HSD at $\alpha = 0.05$.

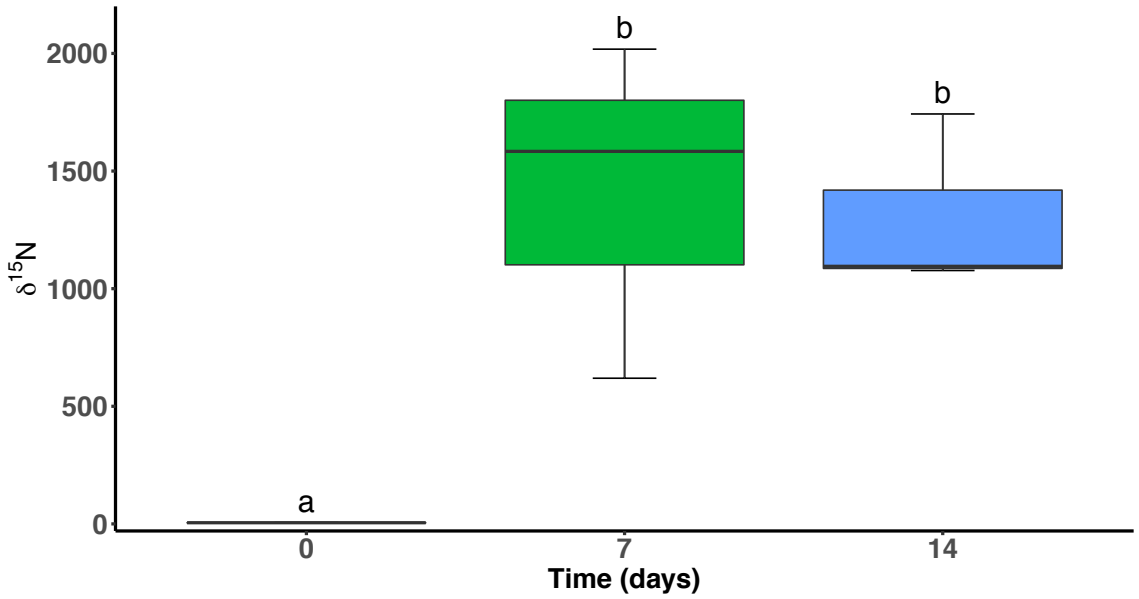


Figure 4.5 $\delta^{15}N$ values of tawny crazy ant workers from one field site before feeding a nitrogen tracer (time = 0), and 7 and 14 days during tracer feeding. Center lines within box plots represent medians, hinges represent upper and lower quartiles, whiskers represent 1.5 times above or below the interquartile range, and dots represent outliers. Letters indicate significant differences according to Tukey's HSD at $\alpha = 0.05$.

4.4.3. Memory effect

The memory effect was checked by analyzing natural abundance standards whose isotope values are known immediately after analyzing highly ^{13}C- and ^{15}N-enriched samples. There was no memory effect found for carbon. However, memory effects were evident for $\delta^{15}N$, with 1-4‰ of an effect after a 500‰ sample and 1-3‰ after a 200‰ sample. Although a difference of 1-4‰ would affect analysis of natural abundance isotope samples, this has little effect on conclusions from spiked values, most of which were several orders of magnitude higher than natural abundance values.

4.5. Discussion

We show that relatively small amounts of both ^{15}N and ^{13}C tracers sufficiently spike ant worker isotope values in the laboratory and that these techniques are applicable in the field for two ant species. As far as we know, relatively few studies have used isotope tracers in ants (Fischer et al. 2003, Defossez et al. 2010), and these studies have primarily focused on ant-plant mutualisms. Our study is one of the first to examine isotope tracer methods in ground-nesting ant species and should inform future research on how much tracer is required to label food resources.

We found that both ^{15}N and ^{13}C tracers enrich ant worker isotope values within just 24 hours after feeding, but the nitrogen tracer may last longer in colonies compared with that of carbon. In our laboratory experiment, ant worker isotope values increased dramatically after 24 hours post-feeding, and this spike persisted for both nitrogen and carbon at 48 hours and at 72 hours for nitrogen (Figures 4.2 and 4.3). Although even the lowest carbon isotope values at 72 hours were still enriched relative to baseline levels (-4‰ compared with an average of -20.4‰), these values were not statistically significantly different from pre-feeding values (Figure 4.3). Our results are comparable to other studies with ants. Defossez et al. (2010), for example, reported substantial changes in both $\delta^{15}N$ and $\delta^{13}C$ values in ant workers within 24 hours after administration of the tracer, but ^{13}C disappeared faster over time than ^{15}N in spiked ant colonies. This may mean that nitrogen isotope tracers persist in ants longer than carbon isotope tracers. Interestingly, the opposite was found in earthworms: ^{13}C tracers persisted more than twice as long in treated earthworms compared with ^{15}N tracers (Dyckmans et al. 2005).

This demonstrates the importance of documenting changes in multiple tracers over time in different taxa prior to choosing the most appropriate tracer for the organism of interest. During isotope analysis, we found no memory effect for carbon and a relatively small memory effect for nitrogen based on analyzing natural abundance standards after ^{15}N- (or ^{13}C-) enriched samples. Our results are similar to previous studies documenting the memory effect of highly enriched samples (Reineking et al. 1993, Mannerheim et al. 2019), and we agree with past studies that suggest running natural abundance samples first before enriched samples, particularly in the case of nitrogen, to reduce the effects of enriched samples on subsequent measurements.

Similar to Defossez et al. (2010), worker isotope values from laboratory colonies remained enriched but declined steadily over time. Because we removed abdomens before analysis, worker isotope values were not influenced by crop contents; however, the decline in values indicates that at least some of the heavy isotopes were being excreted rather than incorporated into the tissues. Metabolic activity, growth, and restructuring of tissues influences the incorporation rate of heavy isotopes into the tissue of consumers (Tieszen et al. 1983, Vanderklift & Ponsard 2003, Fry 2006), so ant workers, which are no longer growing, may incorporate less heavy isotope compared with other members of the colony. For example, after feeding isotope tracers to ant colonies, Defossez et al. (2010) found that while worker isotope values decreased, larval isotope values actually increased over a four-day period. This is perhaps part of the reason why they detected spiked values in workers from these colonies up to 660 days post-feeding (these workers were likely fed the tracers during larval development).

Because workers are often more easily accessible than the rest of the colony for many ant species, we focused primarily on workers over a shorter period of time than Defossez et al. (2010). The isotope values of ant brood, however, likely change differently than adults when fed isotope tracers, so it will be interesting for future studies to examine the effects of tracers on the rest of the colony over a longer period of time.

We show that our methods can also be applied to two ant species in the field. Previous studies using isotope tracers in ants have focused on species with relatively small colony sizes (i.e., 100-10,000 workers) that nest within a single plant (Fischer et al. 2003, Defossez et al. 2010). Larger colonies that feed on a broader range of resources may, therefore, require different levels of an isotope tracer to sufficiently spike ant isotope values. Fire ant colonies can contain more than 200,000 workers within a single nest (Vinson 1997, Tschinkel 2006), and tawny crazy ants can reach densities of more than one million ants per hectare (Kjeldgaard et al., *unpubl. data*). The amount of ^{15}N-labeled glycine used in our field experiment was sufficient to detect enriched isotope values well above natural abundance levels in ant workers of both species (Figures 4.4 and 4.5). The highest δ^{15}N value that we detected in our fire ant samples was 1403‰, and the highest δ^{15}N value that we detected in our tawny crazy ant samples was 2018‰. Tawny crazy ants showed significantly higher nitrogen values compared with fire ants, which may be due to differences in colony densities and/ or the collection method (i.e., disturbing the mound vs. collecting foragers from baits). Greater colony densities would require more isotope tracer to spike the colony to the same degree as a smaller colony. Additionally, foragers that directly fed on the isotope tracer may have more enriched

isotope values compared with nestmates that received the tracer via trophallaxis. Further research will be necessary to determine the possible effects of colony size and collection method on isotope values.

We detected enriched nitrogen values in most ant workers of both species after 7 days of tracer feeding and in all workers after 14 days (Figures 4.4 and 4.5). We only observed one fire ant mound that did not feed on the tracer for the first 7 days, but this colony fed on the tracer for the remainder of the experiment and showed spiked values after 14 days. For this reason, we recommend verifying that ants are actually feeding on the tracer solution. Additionally, although feeding colonies for 7 days is likely sufficient to detect spiked isotope values in field colonies, it may be beneficial to feed colonies for a longer period of time to ensure that all colonies are consuming the tracer solution. Similar to our methods, Fischer et al. (2003) fed ant colonies a tracer seven times within a 15-day period, but Defossez et al. (2010) fed colonies tracers for just one day. The amount of tracer feeding necessary to sufficiently spike field colonies likely depends on the size and biology of the ant species and the goals of the study.

Although our values are much lower than those reported in Defossez et al. (2010; >15,000‰), our results are similar to those in other taxa (earthworms: 400-500‰ (Grabmaier et al. 2014); mosquitos: 500-800‰ (Hamer et al. 2012)). The amount of enrichment will necessarily depend on the questions being investigated. For example, if the isotope tracers are being used to detect the movement of nutrients through multiple trophic levels (like in Defossez et al. (2010)), it may be necessary to add more tracer to create a larger enrichment that can be traced through the food web. Our techniques,

however, are sufficient to detect resource use in individual colonies and could be expanded to answer a broad variety of questions in ant research. For example, labeling food with isotope tracers could be used to quantify resource sharing between conspecifics, identify the number of workers in a colony, and evaluate the retention of certain nutrients within different members of the colony. Stable isotope analysis using the natural abundance of ^{15}N and ^{13}C has yielded important insights into ant trophic interactions (Blüthgen et al. 2003, Tillberg et al. 2007, Feldhaar et al. 2010, Wilder et al. 2011b), but it is limited in specificity. Isotope tracers allow for a more direct measurement of nutrient fluxes and are a promising tool to unravel additional questions in ant trophic ecology.

4.6. References

Aldana, R. C., M. L. Baena, and P. Chacón de Ulloa. 1995. Introducción de la hormiga loca (*Paratrechina fulva*) a la Reserva Natural Laguna de Sonso (Valle del Cauca, Colombia). Boletin del Museo de Entomología de la Universidad del Valle **3**:15-28.

Blüthgen, N., G. Gebauer, and K. Fiedler. 2003. Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. Oecologia **137**:426-435.

CABI. 2019. *Solenopsis invicta* (red imported fire ant). In: Invasive Species Compendium. Wallingford, UK: CAB Inernational.

Catalani, G., R. Camargo, K. Sousa, N. Caldato, A. Silva, and L. Forti. 2019. Fat-soluble substance flow during symbiotic fungus cultivation by leaf-cutter ants. Neotropical Entomology **49**:1-8.

Claborn, D. M., and S. A. Phillips. 1986. Temporal foraging activities of *Solenopsis invicta* (Hymenoptera: Formicidae) and other predominant ants of central Texas. The Southwestern Naturalist **31**:555-557.

Cook, S. C., M. D. Eubanks, R. E. Gold, and S. T. Behmer. 2011. Seasonality directs contrasting food collection behavior and nutrient regulation strategies in ants. PLoS One **6**:e25407.

Coplen, T. B. 2011. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Communications In Mass Spectrometry **25**:2538-2560.

Dalecky, A., L. Gaume, B. Schatz, D. Mckey, and F. Kjellberg. 2005. Facultative polygyny in the plant-ant *Petalomyrmex phylax* (Hymenoptera: Formicinae): sociogenetic and ecological determinants of queen number. Biological Journal of the Linnean Society **86**:133-151.

Davidson, D. W. 1997. The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. Biological Journal of the Linnean Society **61**:153-181.

Davidson, D. W., S. C. Cook, R. R. Snelling, and T. H. Chua. 2003. Explaining the abundance of ants in lowland tropical rainforest canopies. Science **300**:969-972.

Defossez, E., C. Djiéto-Lordon, D. McKey, M.-A. Selosse, and R. Blatrix. 2010. Plant-ants feed their host plant, but above all a fungal symbiont to recycle nitrogen. Proceedings of the Royal Society B: Biological Sciences **278**:1419-1426.

Dyckmans, J., C. M. Scrimgeour, and O. Schmidt. 2005. A simple and rapid method for labelling earthworms with ^{15}N and ^{13}C. Soil Biology and Biochemistry **37**:989-993.

Eyer, P.-A., B. McDowell, L. N. Johnson, L. A. Calcaterra, M. B. Fernandez, D. Shoemaker, R. T. Puckett, and E. L. Vargo. 2018. Supercolonial structure of invasive populations of the tawny crazy ant *Nylanderia fulva* in the US. BMC Evolutionary Biology **18**:209.

Feldhaar, H., G. Gebauer, and N. Blüthgen. 2010. Stable isotopes: past and future in exposing secrets of ant nutrition (Hymenoptera: Formicidae). Myrmecological News **13**:3-13.

Fischer, R. C., W. Wanek, A. Richter, and V. Mayer. 2003. Do ants feed plants? A ^{15}N labelling study of nitrogen fluxes from ants to plants in the mutualism of *Pheidole* and *Piper*. Journal of Ecology **91**:126-134.

Fry, B. 2006. Stable isotope ecology. Springer.

Grabmaier, A., F. Heigl, N. Eisenhauer, M. G. van der Heijden, and J. G. Zaller. 2014. Stable isotope labelling of earthworms can help deciphering belowground–aboveground interactions involving earthworms, mycorrhizal fungi, plants and aphids. Pedobiologia **57**:197-203.

Hagler, J. R., and C. G. Jackson. 2001. Methods for marking insects: current techniques and future prospects. Annual Review of Entomology **46**:511-543.

Hamer, G. L., D. J. Donovan, R. Hood-Nowotny, M. G. Kaufman, T. L. Goldberg, and E. D. Walker. 2012. Evaluation of a stable isotope method to mark naturally-breeding larval mosquitoes for adult dispersal studies. Journal of Medical Entomology **49**:61-70.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Harvard University Press.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, and T. J. Case. 2002. The causes and consequences of ant invasions. Annual Review of Ecology and Systematics **33**:181-233.

Howard, D. F., and W. R. Tschinkel. 1981. The flow of food in colonies of the fire ant, *Solenopsis invicta*: a multifactorial study. Physiological Entomology **6**:297-306.

LeBrun, E. G., J. Abbott, and L. E. Gilbert. 2013. Imported crazy ant displaces imported fire ant, reduces and homogenizes grassland ant and arthropod assemblages. Biological Invasions **15**:2429-2442.

Mannerheim, N., R. Werner, and N. Buchmann. 2019. Measurement precision and accuracy of high artificial enrichment ^{15}N and ^{13}C tracer samples. Rapid Communications in Mass Spectrometry **33**:1153-1163.

Miller, L. R. 1993. Fluorescent dyes as markers in studies of foraging biology of termite colonies (Isoptera). Sociobiology **23**:127-134.

Mulholland, P. J., J. L. Tank, D. M. Sanzone, W. M. Wollheim, B. J. Peterson, J. R. Webster, and J. L. Meyer. 2000. Food resources of stream macroinvertebrates

determined by natural-abundance stable C and N isotopes and a ^{15}N tracer addition. Journal of the North American Benthological Society **19**:145-157.

Oi, F. 2000. Purple dye-marker for *Reticulitermes spp.* (Isoptera: Rhinotermitidae). Florida Entomologist **83**:112.

Overmyer, J. P., M. A. MacNeil, and A. T. Fisk. 2008. Fractionation and metabolic turnover of carbon and nitrogen stable isotopes in black fly larvae. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry **22**:694-700.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2019. nlme: linear and nonlinear mixed effects models. R package version 3.1-140.

Porter, S. D., and W. R. Tschinkel. 1987. Foraging in *Solenopsis invicta* (Hymenoptera, Formicidae) effects of weather and season. Environmental Entomology **16**:802-808.

Porter, S. D., D. F. Williams, R. S. Patterson, and H. G. Fowler. 1997. Intercontinental differences in the abundance of *Solenopsis* fire ants (Hymenoptera: Formicidae): escape from natural enemies? Environmental Entomology **26**:373-384.

R Core Team 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reineking, A., R. Langel, and J. Schikowski. 1993. ^{15}N, ^{13}C-on-line measurements with an elemental analyser (Carlo Erba, NA 1500), a modified trapping box and a gas

isotope mass spectrometer (Finnigan, MAT 251). Isotopes in Environmental and Health Studies **29**:169-174.

Savage, A. M., S. D. Johnson, K. D. Whitney, and J. A. Rudgers. 2011. Do invasive ants respond more strongly to carbohydrate availability than co-occurring non-invasive ants? A test along an active *Anoplolepis gracilipes* invasion front. Austral Ecology **36**:310-319.

Tieszen, L. L., T. W. Boutton, K. G. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia **57**:32-37.

Tillberg, C., D. Holway, E. LeBrun, and A. Suarez. 2007. Trophic ecology of invasive Argentine ants in their native and introduced ranges. Proceedings of the National Academy of Sciences of the United States of America **104**:20856-20861.

Tillberg, C. V., D. P. McCarthy, A. G. Dolezal, and A. V. Suarez. 2006. Measuring the trophic ecology of ants using stable isotopes. Insectes Sociaux **53**:65-69.

Tobin, J. E. 1991. A neotropical rainforest canopy, ant community: some ecological considerations. Pages 536-538 *in* C. Huxley and D. Cutler, editors. Ant-plant interactions. Oxford University Press.

Tschinkel, W. R. 2006. The fire ants. Harvard University Press.

Van den Meersche, K., J. J. Middelburg, K. Soetaert, P. Van Rijswijk, H. T. Boschker, and C. H. Heip. 2004. Carbon-nitrogen coupling and algal-bacterial interactions during an experimental bloom: modeling a ^{13}C tracer experiment. Limnology and Oceanography **49**:862-878.

Vanderklift, M., and S. Ponsard. 2003. Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. Oecologia **136**:169-182.

Vinson, S. B. 1968. The Distribution of an Oil, Carbohydrate, and Protein Food Source to Members of the Imported Fire Ant Colony. Journal of Economic Entomology **61**:712-714.

Vinson, S. B. 1997. Invasion of the red imported fire ant (Hymenoptera: Formicidae): spread, biology, and impact. American Entomologist **43**:23-39.

Wang, Z., L. Moshman, E. Kraus, B. Wilson, N. Acharya, and R. Diaz. 2016. A review of the tawny crazy ant, *Nylanderia fulva*, an emergent ant invader in the southern United States: is biological control a feasible management option? Insects **7**:77.

Weeks, R. D., L. Wilson, and S. Vinson. 2004. Resource partitioning among colonies of polygyne red imported fire ants (Hymenoptera: Formicidae). Environmental Entomology **33**:1602-1608.

Wetterer, J., O. Davis, and J. Williamson. 2014. Boom and bust of the tawny crazy ant, *Nylanderia fulva* (Hymenoptera: Formicidae), on St. Croix, US Virgin Islands. The Florida Entomologist **97**:1099-1103.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer.

Wilder, S., D. Holway, A. Suarez, and M. Eubanks. 2011a. Macronutrient content of plant-based food affects growth of a carnivorous arthropod. Ecology **92**:325-332.

Wilder, S., D. Holway, A. Suarez, E. LeBrun, and M. Eubanks. 2011b. Intercontinental differences in resource use reveal the importance of mutualisms in fire ant

invasions. Proceedings of the National Academy of Sciences of the United States of America **108**:20639-20644.

Zenner-Polania. 1990. Biological aspects of the hormiga-loca, *Paratrechina-(Nylanderia)-fulva* (Mayr), in Colombia. Pages 290-297 *in* R. K. Vander Meer, K. Jaffe, and A. Cedeno, editors. Applied myrmecology: a world perspective. Westview Press.

Zenner-Polania, I. 1994. Impact of *Paratrechina fulva* on other ant species. Pages 121-132 *in* D. F. Williams, editor. Exotic ants. Biology, impact, and control of introduced species. Westview Press.

5. POLYGYNE NESTS ARE NOT COOPERATING: COLONY BOUNDARIES AND LARVAL DISCRIMINATION IN MULTIPLE-QUEEN COLONIES OF THE RED IMPORTED FIRE ANT (*Solenopsis invicta*)

5.1. Overview

The red imported fire ant (*Solenopsis invicta*; hereafter fire ant) occurs as two social forms; monogyne (i.e., colonies with a single egg-laying queen) and polygyne (i.e., colonies with multiple egg-laying queens), the latter of which is commonly referred to as unicolonial throughout its invaded range. Unicoloniality confers a number of ecological benefits, which may be one reason why polygyne fire ant colonies are often more abundant and ecologically dominant in invaded habitats than monogyne colonies. However, interconnectedness between polygyne colonies in the field is poorly documented. Moreover, polygyne fire ant workers may increase their inclusive fitness by discerning and preferentially sharing with more related individuals. In this study, we tested fundamental assumptions about inter- and intracolonial behavior in polygyne fire ant colonies. Our objectives were to: 1) correlate colony genetic structure and social form with between-colony sharing/ boundaries in fire ants in Texas, and 2) evaluate within-colony nepotism using worker-brood interactions. We quantified sharing between nests by treating individual mounds with an isotope tracer. Counter to our expectations, treated nests shared very little with neighboring nests, regardless of social form and within-colony relatedness between workers. Within-colony relatedness between workers was significantly higher in monogyne nests (mean ± SE: 0.644 ± 0.024) than in

polygyne nests (0.269 ± 0.037), but relatedness in polygyne nests was much higher than those reported in previous studies in the USA. To determine within-colony nepotism, we established single-lineage colonies of polygyne fire ants in the laboratory and then created experimental colonies using workers and a combination of brood from the same natal colony and brood from another colony. We used food coloring to distinguish the two types of brood (nestmates and non-nestmates) and fluorescent dye to quantify larval feeding by workers. Workers fed significantly more nestmate than non-nestmate larvae. Additionally, survival of larvae from the same natal colony as the workers was significantly higher than larvae from other colonies, indicating that workers may preferentially cannibalize less related individuals. Our results suggest that polygyne fire ant colonies are actually multicolonial and may engage in high levels of intraspecific competition with neighboring colonies and may exhibit low levels of nepotism within the colony. Polygyne colonies from our study were similar in some ways to colonies in the native range, where there is strong intraspecific competition and higher within-colony relatedness between workers. Future research should examine polygyne colony behavior in other parts of their invaded range where within-colony relatedness is lower.

5.2. Introduction

The classic example of a colony of eusocial Hymenoptera is that of a single family functioning as a unit to satisfy the needs of the colony. This simple, haplodiploid family maximizes inclusive fitness among workers as they rear reproductive sisters (Hamilton 1964). However, many eusocial Hymenoptera species do not fit this model, as

intraspecific cooperation between unrelated individuals occurs relatively commonly. Many species practice polygyny, in which multiple queens cooperate and lay eggs within the same nest, a phenomenon that is particularly widespread in the ants (Hölldobler and Wilson 1977, Ross and Carpenter 1991, Keller 1995, Boulay et al. 2014). Cooperation between queens conveys a number of ecological benefits. Polygyne colonies are associated with larger colony sizes and greater ecological dominance (Boulay et al. 2014). Many polygyne species still compete strongly, however, with neighboring conspecifics, which is often more costly than competition with other species (Hölldobler and Wilson 1990). At the extreme end of cooperation are unicolonial ant species, which are characterized by reduced intraspecific aggression and an absence of behavioral boundaries between nests (Helanterä et al. 2009, Krushelnycky et al. 2010). By cooperating instead of competing with conspecifics, unicolonial ants avoid the costs of intraspecific competition, which allows them to reach higher densities (Porter et al. 1992, Giraud et al. 2002) and achieve greater ecological dominance by more effectively outcompeting other species (Holway et al. 2002, Holway and Suarez 2004, LeBrun et al. 2013). The benefits of unicoloniality may explain the ecological success of many (but not all i.e., Eyer et al. 2020) invasive ant species (Passera 1994, Holway et al. 2002). For example, the number of Argentine ant workers (*Linepithema humile*) was approximately 50-fold higher in sites where colonies cooperated compared with sites where colonies competed (Holway and Suarez 2004). High densities of Argentine ant workers corresponded with a 50% reduction in ant species richness, as Argentine ant colonies exploitatively outcompeted other ant species (Holway and Suarez 2004).

Despite the ecological benefits, when many unrelated queens cooperate and produce workers within a single colony, relatedness between nestmates can decrease to effectively zero (Keller 1995, Crozier and Pamilo 1996, Queller and Strassmann 1998). As a consequence, polygyny and especially unicoloniality increase the likelihood of workers interacting with and caring for less related individuals, which in turn reduces their inclusive fitness (Schmid-Hempel 1997, Schmid-Hempel and Crozier 1999, Helanterä et al. 2009). Inclusive fitness may be maintained, however, if workers discern and preferentially direct care towards kin over non-kin (Hamilton 1964, Keller 1995). For example, although Argentine ant supercolonies are characterized by cooperative, interconnected nests (Tsutsui et al. 2000, Giraud et al. 2002), workers spend more time investigating (i.e., antennating) non-nestmates than nestmates from the same supercolony (Björkman-Chiswell et al. 2008), indicating nestmate recognition despite a lack of aggression in this species. Nestmate recognition may reduce or eliminate indiscriminate sharing between Argentine ant nests, as sharing was consistently limited to distinct clusters of nests within a single supercolony over a three-year-period (Heller et al. 2008). Kin recognition can also impact sharing between individuals within the nest in what is known as nepotism. For example, in polygynous ant colonies of *Formica argentea*, workers preferentially care for brood that are more closely related to them (Snyder 1993). By discriminately sharing food resources with more related individuals from neighboring nests or from within the same nest, workers may increase their inclusive fitness in polygynous colonies (Hamilton 1964, Helanterä et al. 2009). Despite the evolutionary benefits of preferential care towards kin over non-kin, it has only rarely

been documented in polygynous or unicolonial ant species and may be less common than previously thought (Keller 1997, Boomsma and d'Ettorre 2013).

We tested for the influence of kinship on between- and within-colony interactions with related individuals using the red imported fire ant, *Solenopsis invicta* (hereafter fire ant). Fire ants are an excellent model system to investigate the relationship between intraspecific cooperation and kinship in ant colonies. Fire ants occur as two social forms: the polygyne form (i.e., colonies with multiple egg-laying queens) and the monogyne form (i.e., colonies with only a single egg-laying queen; Ross 1993, Ross et al. 1996, Tschinkel 2006, Gotzek et al. 2007). Polygyne fire ant colonies are considered highly cooperative, and they are often referred to as unicolonial throughout their invaded range (e.g., Morel et al. 1990, Vander Meer et al. 1990, Greenberg et al. 1992, Porter et al. 1992, Holway et al. 2002, Plowes et al. 2007). However, there is also evidence that boundaries may exist at least on some level between polygyne colonies (Weeks et al. 2004, Goodisman et al. 2007, Krushelnycky et al. 2010). For example, polygyne nests in Georgia, USA, showed distinct genotypic frequencies and worker mass profiles, suggesting that workers and queens are not moving freely between nests (Goodisman et al. 2007). Moreover, although polygyne workers do not aggressively attack non-nestmates like their monogyne counterparts (Vander Meer et al. 1990), workers will antennate and occasionally bite non-nestmates in the laboratory, indicating well-developed nestmate recognition (Obin et al. 1993). There is also evidence of exploitative competition between neighboring polygyne nests in the field (Weeks et al. 2004).

Unfortunately, the physical exchange of workers and resources between polygyne nests in the field is poorly documented. Bhatkar and Vinson (1987) is often cited as evidence that polygyne nests are highly interconnected, but this study is limited and does not provide data supporting this hypothesis. Moreover, although genetic relatedness may influence between-colony interactions, the genetic structure of polygyne and monogyne fire ant colonies has not been correlated with resource sharing in the field. As for within-colony interactions, workers from polygyne colonies do not preferentially tend their own mother or closely-related alate queens (DeHeer and Ross 1997). However, nepotism has not been investigated using worker-brood interactions, which are also critical to colony dynamics and can differ from worker-queen interactions. For example, although *F. argentea* workers preferentially care for more related brood in polygynous colonies, they show no preference towards related or unrelated queens within the colony (Snyder 1993).

Our objectives for this study were to 1) correlate colony genetic structure and social form with between-colony sharing/ boundaries in fire ants in Texas, and 2) evaluate within-colony nepotism using worker-brood interactions. To delimit sharing between nests, we quantified the exchange of a labeled carbohydrate resource. This resource could be shared among nests by either trophallaxis between non-nestmates and/or workers moving freely from one nest to another. Using a labeled resource in combination with population genetics allows for two different ways to define colony boundaries (Ellis et al. 2017). If sharing occurs both between and within polygyne nests regardless of kinship, polygyne fire ants in Texas could be considered unicolonial.

Unicolonial species often have a much greater ecological impact than their multicolonial counterparts (Helanterä et al. 2009, Krushelnycky et al. 2010), and it is thought that polygyne fire ants may have stronger impacts on invaded communities than monogyne fire ants (Porter and Savignano 1990). If polygyne colonies are competing with each other, however, their effects on invaded communities may be weaker than expected.

Results from this study will be useful for fire ant management, as increased interconnectedness and sharing between polygyne colonies would increase the dispersal of insecticidal bait throughout a field site (i.e., horizontal transfer), which has been shown to be effective in the unicolonial Argentine ants (*L. humile*; Buczkowski and Wossler 2019). Finally, our study also tests fundamental questions in kin selection theory. If nests are completely interconnected and there is little evidence of within-colony conflict, then the inclusive fitness of workers will be low, which is considered evolutionarily disadvantageous (Helanterä et al. 2009). Clarifying the balance between selfish and altruistic behaviors that facilitate the maintenance of genetically diverse colonies will provide further insight into the ongoing debate over the evolution of eusociality and the evolutionary consequences of unicoloniality.

5.3. Methods

5.3.1. Population genetics of fire ants in Texas

5.3.1.1. Genetic analysis

We identified active fire ant mounds in six field sites in Texas, USA (Appendix C S1). Mounds were used as a proxy for individual nests. Mounds were identified in

clusters to quantify functional boundaries (see *Between-nest sharing* section below for complete description). Habitats ranged from restored grasslands (sites O, A, and B) to mowed fields (sites C, T, and T2). Sampling was conducted between August and October 2019. Once mounds were identified and flagged, we disturbed a small section of each mound and collected 40-50 workers for genetic and isotopic analyses (see *Between-nest sharing* section below). Workers were frozen at -10˚C. We then transferred 10-30 workers per mound to 95% EtOH for storage prior to DNA extraction. In total, we sampled from 73 fire ant mounds across six sites, with 12 mounds in Site A, 13 in Site B, 12 in site C, 11 in Site O, 13 in Site T, and 12 in Site T2.

Genetic analyses were used to infer the social structure of each fire ant colony analyzed (i.e., monogyne or polygyne), and to determine whether different mounds belonged to the same polydomous colony. For each mound, eight randomly chosen workers were directly stored in pure ethanol at -20 ºC until DNA extraction. DNA was extracted from individuals workers following a modified Gentra-PureGene protocol (Gentra Systems, Inc. Minneapolis, MN, USA).

To determine the social form of each nest, we pooled the DNA extracted from each of the eight workers per mound and screened this pooled sample for the presence of the *Gp-9*^{b} allele, which is exclusively present in polygyne colonies (Ross and Keller 1998, Krieger and Ross 2002). A PCR reaction was performed on each pooled sample using the specific primer pair 24bS and 25bAS (Valles and Porter 2003). This primer pair amplifies a 423 bp amplicon, and a successful amplification denotes the presence of the *Gp-9*^{b} allele, thereby characterizing the workers as polygyne. Amplifications were

performed according to the protocol described in Valles and Porter (2003) and visualized on a 1% agarose gel.

In addition, five microsatellite markers previously developed for *S. invicta* (*Sol11, Sol20*, *Sol42*, *Sol49* and *Sol55*; Krieger and Keller 1997) were amplified for each of the eight workers per nest. The allelic polymorphism of these five microsatellites was previously shown to be relevant to delimit colonies of *S. invicta* and infer their colony structure (Krieger and Keller 1997). The microsatellites were genotyped using the M13-tailed primer method (Boutin-Ganache et al. 2001), consisting of 5'-fluorescently labeled tails with 6-FAM, VIC, PET or NED dyes to facilitate multiplexing. DNA amplifications were performed in a volume of 15 µL including 0.25-1.0 U of MyTaq™ HS DNA polymerase (Bioline), 2 µL of MyTaq™ 5x reaction buffer (Bioline), 0.08 µL of each primers, 0.08 of each M13 dye and 1 µL of the DNA template. PCR reactions were carried out using a Bio-Rad thermocycler T100 (Bio-Rad, Pleasanton, CA, USA). PCR products were sized against LIZ500 internal standard on an ABI 3500 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Allele calling was performed using Geneious software v.9.1 (Kearse et al. 2012).

For every mound, the social structure result obtained with the *Gp-9*^{b} method was confirmed using microsatellite markers, inferring whether all workers from a mound could be assigned to a single queen (carrying one of the two alleles of the mother queen at each microsatellite marker studied). Polygyny was deduced when more than one worker per colony could not be unambiguously assigned to a single queen (see Appendix C S2 for results). In addition, we compared the relatedness coefficients (*r*) between

monogyne and polygyne nests (as identified using *Gp-9*) using analysis of variance (ANOVA) to verify that relatedness coefficients were significantly lower in polygyne versus monogyne nests (i.e., suggesting the reproduction of several unrelated queens) and to determine any differences by site. We also used t-tests to establish if relatedness coefficients were significantly greater than 0 for both social forms. Relatedness coefficients were calculated using the program COANCESTRY v.1.0 (Wang 2011), according to the algorithm described by Queller and Goodnight (1989). Relatedness coefficients were weighted equally and standard errors (SE) were obtained by jackknifing over colonies.

Colony structure was investigated for the six sites to determine whether distinct mounds of *S. invicta,* especially those collected within 5m of each other, consisted of a single colony (i.e., polydomy) or separate colonies. To answer this question, genotypic frequencies at all mounds were compared using a log-likelihood (G)-based test of differentiation using GENEPOP ON THE WEB (Rousset 2008). Bonferroni's correction was applied to account for multiple comparisons of all pairs (adjusted P-value < 0.0008). Significance was determined using a Fisher's combined probability test.

Colony clustering was visualized for each site by plotting individuals on a principal component analysis (PCA) using the *adegenet* R package (Jombart 2008). The clustering of mounds into distinct colonies was also represented by Bayesian assignments of individuals into genetic clusters (i.e., colonies; K) using STRUCTURE v.2.3.4 (Pritchard et al. 2000). For each site, STRUCTURE simulations were run with values of K from 1 to the total number of mounds encountered in each site and repeated

10 times for each value of K. Each run included a 5×10^4 burn-in period followed by 1×10^5 iterations of the MCMC. The most likely number of groupings was evaluated using the ΔK method (Evanno et al. 2005) implemented in Structure Harvester v.0.6.8 (Earl 2012). Additional details and results for clustering analysis can be found in Appendix C S2 and S3.

5.3.1.2. Within-colony relatedness of fire ants in the literature

We also compiled published coefficients of within-colony relatedness between workers in the red imported fire ant to compare our results with those in the literature. We searched the *Web of Science* using the following search terms: "*Solenopsis invicta*" OR "*red imported fire ant"* AND *population* AND *microsatellite*. Our search generated 87 records, but a large number of these studies focused on a different ant or social insect species. We only included studies that examined relatedness between workers of *Solenopsis invicta*. We excluded any study that contained only queen-queen relatedness coefficients as well as any study of a different species of ant or social insect. We reviewed each record and extracted within-colony relatedness coefficients between workers (means and standard error whenever available) and recorded the sampling location. In total, we extracted information from eight studies.

5.3.2. Between-nest sharing

To quantify sharing between nests in the field, we treated select mounds in each site with a stable isotope tracer and quantified its movement into neighboring nests.

Stable isotope tracers employ naturally occurring, stable (non-radioactive) forms of biologically relevant elements, such as nitrogen. The heavier isotope of nitrogen (^{15}N) occurs rarely in nature, so by artificially 'spiking' a food with an appropriate concentration of the heavy isotope, ecologists can trace the movement of this isotope through consumers and identify the flow of nutrients through an ecosystem (Fry 2006). We ensured that only the treated mounds had access to the isotope tracer, so if a neighboring untreated nest showed unnaturally high levels of ^{15}N, this would indicate an exchange of either workers or resources between the treated and untreated nests (i.e., no colony boundaries).

Out of the 73 fire ant mounds from the six sites that we analyzed in Objective 1, we identified three clusters of four to five mounds at each site. Clusters were separated by at least 50m within each site to avoid potential sharing between clusters. One mound within each cluster was selected as the treatment mound. To determine any effect of distance on resource sharing, untreated mounds within each cluster were between 0.4m – 29.07m from the treated mound, with an average distance of 7.65m ± 0.72m. Similar to other studies (Goodisman et al. 2007), several mounds disappeared or moved over the course of the sampling period. As a consequence, we were unable to find three mounds (one mound from site T, one from O, and one from T2) after the treatment period. Each of these were untreated mounds within different clusters, so their removal did not affect the number of clusters analyzed in each location.

5.3.2.1. Treatment with the tracer

We fed each treated mound a nitrogen tracer mixed in sugar water. A solution with 102mM of ^{15}N-labeled glycine (98 atom%, Sigma-Aldrich, Inc., St. Louis, MO, USA) and 61.5mM of unlabeled sucrose was created using distilled water. This concentration was determined based on a preliminary laboratory experiment with a small number of fire ant workers and an approximation of colony sizes in the field, which can exceed 250,000 workers within a single mound (Tschinkel 2006). The solution was mixed in bulk at the beginning of the field experiment and frozen between uses to avoid mold growth. We filled 1-mL microcentrifuge tubes with 1mL of the solution and stoppered each with cotton. Three of these vials were left on the surface of each treatment mound and replaced every other day for 14 days. Vials were placed directly on the mound surface to ensure that only the treated mound fed on the solution. Each treatment mound was fed a total of 160mg of ^{15}N-labeled glycine in 21mL of sugar solution over a 14-day period. Fire ant workers were observed feeding on the solution, and there was evidence of mound building over the vial opening, indicating worker foraging.

We collected workers from all treated and untreated mounds once before feeding the tracer to treated mounds and once after the 14-day treatment period. Workers were collected by disturbing a small section of each mound and aspirating 40-50 workers. Workers were frozen at -10˚C and never stored in EtOH to avoid possible effects of EtOH on isotopic signatures (Tillberg et al. 2006).

To verify that our isotope tracer methods could detect resource sharing over 30-m distances, we also fed a tracer solution (using the same amounts and methods as described above for fire ant colonies in the field) to a population of tawny crazy ants (*Nylanderia fulva*) near College Station, Texas (Appendix C S1). This species forms a single supercolony throughout its invaded range in North America, in which workers regularly share collected resources with each other and occupy transitory nests (Wang et al. 2016, Eyer et al. 2018). See Appendix C S3 for further details about this field validation with a unicolonial species.

5.3.2.2. Stable isotope analysis

Workers of both ant species were dried in an oven at 60˚C for 24-48 hours and then stored in airtight vials prior to processing. The abdomens of all ants were removed prior to weighing to avoid the effects of stomach contents on isotopic signatures (Tillberg et al. 2006). To achieve appropriate weights for each sample, 5-10 workers per sample were pooled and chopped in glass vials to fine homogeneous powders using small scissors. Approximately 0.400mg of each sample was weighed into tin capsules (Costech Analytical Technologies Inc., Valencia, CA, USA) using a microbalance (Mettler Toledo, Columbus, OH, USA). All samples were analyzed at the Texas A&M University Stable Isotopes for Biosphere Science Laboratory (https://sibs.tamu.edu/) using a Delta V Advantage Isotope Ratio Mass Spectrometer coupled with Costech Elemental Analyzer and Thermo ConFlo IV Universal Interface (Thermo Fisher Scientific, Waltham, MA, USA). All baseline samples (samples collected before mounds

were fed the tracer) were run before any post-feeding samples to ensure that natural abundance values were not influenced by memory effects from the high levels of ^{15}N in spiked samples, which can sometimes impact the measurements.

Stable isotope values are reported as "δX" (i.e., $\delta^{15}N$) and expressed in "per mil" or "‰" notation. Values were calculated by comparing the ratio of heavy to light stable isotopes in the sample to that in a standard (air for nitrogen, and Vienna Pee Dee Belemnite for carbon) using the following equation (Coplen 2011):

$$\delta X = [(R_{sample}/ R_{standard}) - 1] \times 1{,}000$$

5.3.2.3. Data analysis

We used logistic regression to determine the effects of spatial distance, genetic differentiation (using pairwise F_{ST} values), social form, within-colony relatedness coefficients between workers, and site on whether or not untreated mounds shared with the treated mound. To do this, we constructed generalized linear models with a binomial distribution using the *glm* function in base R statistical software v3.6.1 (R Core Team 2019). Distance from the treated mound, pairwise F_{ST} values (compared between the treated and untreated mounds), social form of both treated and untreated mounds (i.e., monogyne or polygyne), within-colony relatedness coefficients between workers in both treated and untreated mounds, and site were treated as independent variables. The sharing status of the untreated mounds (i.e., "shared with the treated mound" or "did not share with the treated mound") was the dependent, binary variable. Mounds that were

identified as having sharing with the treated mound had δ^{15}N values greater than 20‰ (but almost all had δ^{15}N values greater than 60‰), as these values were far higher than any natural abundance isotope values observed at our field sites (mean natural abundance δ^{15}N values before tracer treatment: 5.00‰ ± 0.15‰). Untreated mounds could only have attained δ^{15}N values greater than 20‰ by freely exchanging workers and/or resources with the treated mound. All other mounds were designated as "did not share with the treated mound." All plots were generated using *ggplot2* (Wickham 2016).

5.3.3. Within-colony nepotism

We conducted a laboratory experiment to determine if polygyne workers preferentially care for larvae from their natal colony over larvae from a different colony. To do this, we established six single-lineage colonies by collecting mated polygyne queens following mating flights near College Station (Brazos County) and Conroe (Montgomery County), Texas, USA. Fire ant queens are typically singly mated (Ross and Fletcher 1985, Ross 1993; but see Fritz et al. 2006, Lawson et al. 2012), so by maintaining colonies with an individual queen (rather than several queens), we were able to test worker discernment between brood that are close siblings (i.e., from the same mother) and brood that are not close siblings (i.e., from a different mother).

All colonies were kept in standardized laboratory conditions with a 14:10 light:dark cycle at approximately 25°C for at least two years to ensure they were large enough to be divided into smaller experimental colonies. Artificial nectar (Lanza 1988), crickets, and roaches were provided as food, with identical food items provided for each

colony to prevent the acquisition of distinct colony odors (Vander Meer and Morel 1998). Water was provided *ad libitum* in plastic tubes with cotton stoppers. Colonies were kept in plastic storage tubs lined with liquid Teflon (Insect-a-slip Insect Barrier, BioQuip Products, 2321 Gladwick St., Rancho Dominguez, CA) and provided nesting dishes consisting of 8cm petri dishes with a darkened lid and lined with plaster. Nesting dishes were kept moist by adding 10ml of water every other day to ensure high humidity.

Once incipient colonies were large enough, we used food dye to label the brood of each colony. At high enough concentrations, food dye is visible in the guts of larvae, which allowed us to easily distinguish between brood from different families. We dyed brood by giving workers two separate tubes of 15 mL of water and 15 mL of artificial nectar each containing 0.9 ml of food coloring (McCormick® Food Colors & Egg Dye, McCormick & Company, Inc., 18 Loveton Circle, Sparks, MD). We gave three colonies (colonies A, B, and C) yellow food coloring and three colonies (colonies D, E, and F) green food coloring (Table 5.1). Colors were randomly assigned. During this six-day period of brood dyeing, we did not give the ants any proteinaceous food so that dye would be highly visible in the guts of larvae. After six days of the food color treatment, the colony brood had taken up enough dye to be distinguishable with the naked eye.

Next, we created experimental colonies by combining workers and larvae from the same natal colony with larvae from a different colony (see Table 5.1 for complete family combinations). We separated workers and brood from each incipient colony by anesthetizing the workers with CO_2. Once separated from the brood, 0.1g of workers

(~120 workers) were placed in separate nesting containers and starved for 24 hours to empty their crop contents and allow acclimation before brood were introduced. After 24 hours, we added 50 larvae from the natal colony of the workers (i.e., close siblings) and 50 larvae from a different colony (i.e., not siblings). Not all permutations of families were logistically possible in this experiment, so only larval combinations of different colors were given to experimental colonies in such a way that all possible two-color combinations were created (Table 5.1).

Table 5.1 Single-lineage colony combinations to determine worker discrimination. Identity of the family (A, B, C, D, E, F), dyed color of the brood (yellow or green), and experimental colony combinations provided. Experimental colonies were constructed with 0.1g workers (~120) and 50 larvae (related brood) from the family indicated by the first letter in the experimental colony combinations. The second letter indicates the family of the other 50 larvae (unrelated brood).

	D (Green)	E (Green)	F (Green)
A (Yellow)	AD, DA	AE, EA	AF, FA
B (Yellow)	BD, DB	BE, EB	BF, FB
C (Yellow)	CD, DC	CE, EC	CF, FC

In all, there were 18 experimental colonies. Pupae and prepupae were omitted during brood sorting, so no experimental colonies contained any of these life stages; otherwise, experimental colonies contained a random sample of larval instars. Incipient colonies did not produce reproductive castes so only worker larvae were used in this experiment. Callow workers were not included in the sample of workers given to each experimental colony, thus only mature, adult workers were used.

In order to quantify the feeding of larvae by workers, experimental colonies were given 7.5ml artificial nectar containing 0.2g of non-toxic, fluorescent dye (DFDRY-C0 UV Dye from Risk Reactor, 2676 S. Grand Ave., Santa Ana, CA) for 18 hours. After 18 hours, we again anesthetized experimental colonies with CO_2, and separated workers and brood. We counted larvae of each color under normal lighting conditions in order to quantify larval survival. We then counted fluorescent larvae under a black light to quantify larval feeding by workers. To ensure accurate results for potentially variable behaviors, data for experimental colonies were averaged across the three iterations of this experiment. This allowed us to remove within colony temporal variation and estimate the general behaviors of each experimental colony instead of only looking at a single snapshot of their behavior.

Worker social form (i.e., monogyne or polygyne) was verified by screening for the presence of the *Gp-9*^{b} allele using the same methods as described in above. We extracted DNA from a pooled sample of eight mature, adult workers from each single-lineage colony using a Qiagen DNeasy Blood and Tissue kit (Qiagen Inc., Valencia, CA, USA). All colonies used in this experiment were confirmed as the polygyne form of red imported fire ants.

All data were analyzed using JMP® 9. Percentage data were arcsine-square-root transformed. All graphs were produced with untransformed data.

5.4. Results

5.4.1. Between-nest sharing and colony genetic structure

Results from our tawny crazy ant control site show that the isotope tracer was highly successful at detecting sharing in a unicolonial ant population at distances that were relevant for our study (i.e., up to 28.4m from the treated area; see Appendix C S3 and S5 for details).

We identified 38 monogyne and 35 polygyne nests across all six fire ant sites using the *Gp-9* method. We treated 11 monogyne and 8 polygyne nests with the isotope tracer, and all treated nests showed elevated δ^{15}N values, indicating that our methods were successful in treating individual mounds. Counter to our expectations, however, treated nests shared very little with neighboring nests, regardless of social form (Figure 5.1) and within-colony relatedness between workers (Appendix C S5). Only six of the 57 untreated mounds showed evidence of sharing with the treated mound (three monogyne and three polygyne), and sharing between mounds was independent of the social form of the treated mound ($\chi^2 = 0.0091$, df = 1, $P = 0.924$), the social form of the untreated mound ($\chi^2 = 0.0001$, df = 1, $P = 0.992$), and by the interaction between these variables ($\chi^2 = 0.0061$, df = 1, $P = 0.938$). Moreover, sharing was independent of within-colony relatedness between workers in the treated mound ($\chi^2 = 0.0681$, df = 1, $P = 0.794$), by relatedness between workers in the untreated mound ($\chi^2 = 0.7718$, df = 1, $P = 0.380$), and by the interaction between these variables ($\chi^2 = 0.000$, df = 1, $P = 1.000$).

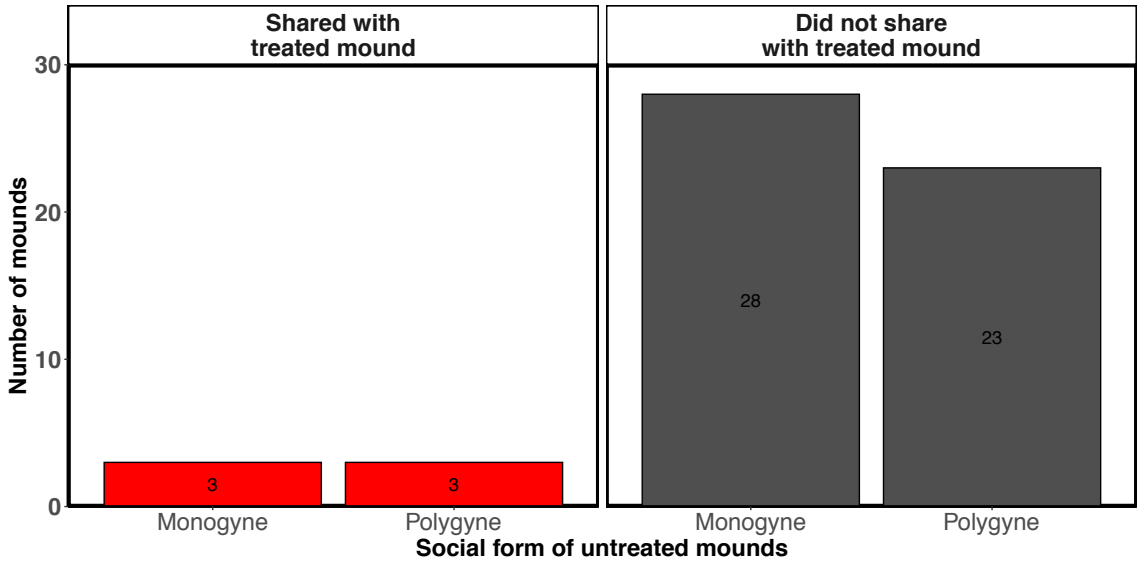


Figure 5.1 Very few untreated mounds shared with treated mounds, regardless of social form. Plot includes the status of an isotope tracer in untreated mounds by social form according to *Gp-9* results. Mounds with δ^{15}N values greater than 20‰ indicated that untreated mounds exchanged workers and/or resources with the treated mound ("Shared with treated mound"), and the values of all other mounds indicated that untreated mounds did not exchange workers or resources with the treated mound ("Did not share with treated mound").

There was a significant effect of distance on whether or not sharing was detected in untreated mounds ($\chi^2 = 10.0858$, df = 1, $P = 0.001$). All untreated mounds with elevated δ^{15}N values were within 5m of the treated mound (Figure 5.2a). However, not all nests within 5m of the treated mound shared (Appendix C S6), indicating that distance was not the only component influencing sharing between nests. There were 13 untreated nests within 5m from the treated mound that did not share with the treated mound, one of which was only 0.4m from the treated mound. Sharing between nests did not depend on site ($\chi^2 = 2.0408$, df = 5, $P = 0.843$).

Pairwise F_{ST} values between the treated and untreated mounds significantly affected whether or not untreated mounds shared with the treated mound ($\chi^2 = 14.5562$, df = 1, $P < 0.001$; Figure 5.2b), as all untreated mounds with elevated $\delta^{15}N$ values had pairwise F_{ST} values that were close to zero (mean and standard errors: 0.028 ± 0.015). Pairwise F_{ST} values were ~90% lower on average in untreated mounds that shared than in untreated mounds that did not share (Figure 5.2b). According to the log-likelihood (G)-based test of differentiation, all of the untreated mounds that shared with the treated mound could not be significantly genetically differentiated from the treated mound. There was no significant interaction between distance and pairwise F_{ST} values ($\chi^2 = 0.3395$, df = 1, $P = 0.560$). Pairwise F_{ST} between polygyne mounds within each site was lower than between monogyne mounds (mean and standard errors of 0.144 ± 0.008 and 0.356 ± 0.010, respectively), but there was no significant interaction between pairwise F_{ST} and social form ($\chi^2 = 0.0000$, df = 3, $P = 1.000$) on sharing between nests.

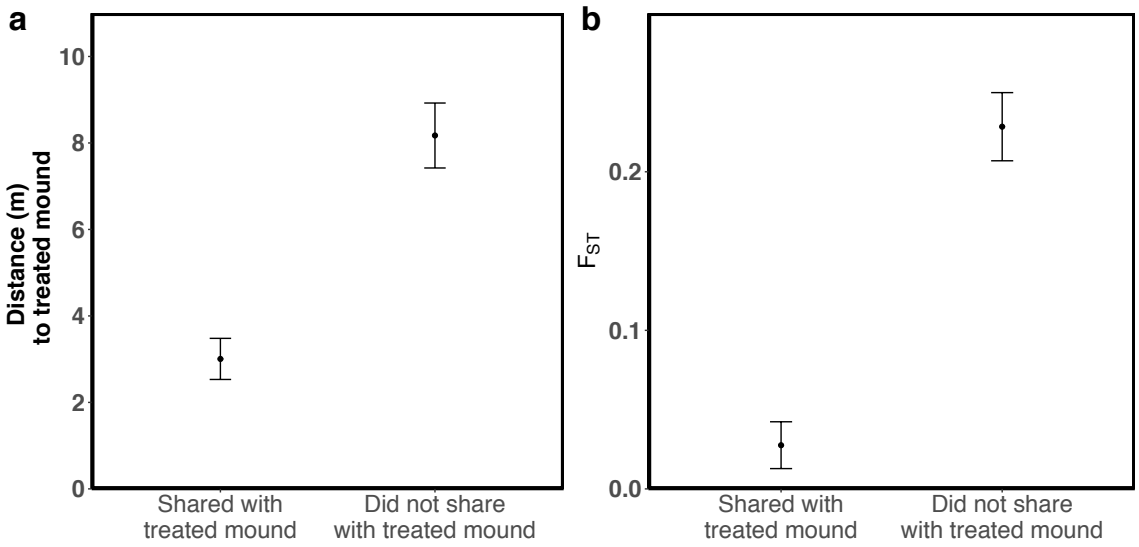


Figure 5.2 a) All untreated mounds that shared were within 5m of the treated mound. Plot includes status of an isotope tracer in untreated mounds by distance in meters to the treated mound. b) All untreated mounds that shared showed very low pairwise F_{ST} values with the treated mound. Plot includes status of an isotope tracer in untreated mounds by pairwise F_{ST} values compared to the treated mound. Results shown are from all six sites. Point and error bars represent mean ± SE. Mounds with δ^{15}N values greater than 20‰ indicated that untreated mounds exchanged workers and/or resources with the treated mound ("Shared with treated mound"), and the values of all other mounds indicated that untreated mounds did not exchange workers or resources with the treated mound ("Did not share with treated mound").

5.4.1.1. Within-colony relatedness and comparison with the literature

At our sites, within-colony relatedness was significantly higher in monogyne nests (mean and standard errors: 0.644 ± 0.024) than in polygyne nests (0.269 ± 0.037; $F_{1,60} = 75.832$, $P < 0.001$). Within-colony relatedness coefficients did not change by site ($F_{5,60} = 1.781$, $P = 0.130$), nor was there a significant interaction between site and *Gp-9* results on relatedness coefficients ($F_{3,60} = 0.382$, $P = 0.766$). As expected, relatedness in

monogyne nests was significantly greater than zero (t_{35} = 26.617, P < 0.001; 95% CI: 0.595-0.693). Counter to our expectations, however, relatedness in polygyne nests at our sites was also significantly greater than zero (t_{33} = 7.249, P < 0.001; 95% CI: 0.193-0.344). These results contrast with several previous studies of polygyne fire ant colonies in the USA (Figure 5.3). Pairwise F_{ST} between polygyne mounds within each site was lower than between monogyne mounds (mean and standard errors of 0.144 ± 0.008 and 0.356 ± 0.010, respectively).

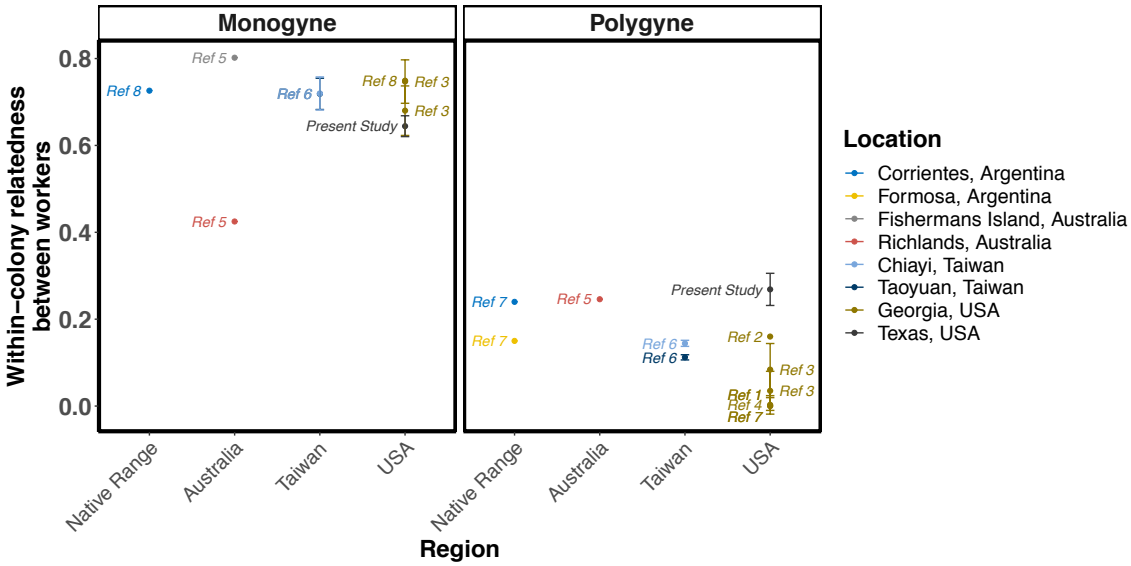


Figure 5.3 Within-colony relatedness coefficients between workers of fire ants (*Solenopsis invicta*) by social form from multiple populations. Data based on results extracted from the literature and results in the present study. Points and error bars (when available) represent mean ± SE. *Ref 1* = Goodisman et al. (2007); *Ref 2* = Ross (1993); *Ref 3* = Ross and Fletcher (1985); *Ref 4* = DeHeer and Ross (1997); *Ref 5* = Henshaw et al. (2005); *Ref 6* = Yang et al. (2008); *Ref 7* = Ross et al. (1996); *Ref 8* = Ross et al. (1993).

5.4.2. Within-colony nepotism

After 18 hours in experimental colonies with workers, significantly more brood from the same natal colony remained compared with brood from a different colony (Figure 5.4a; two-tailed *t* test: $t_{17} = 5.2284$, $P < 0.001$). The number of brood from different colonies diminished by 27% while the brood from the same natal colony diminished by only 14%. No dead larvae were found in the colonies.

Likewise, the feeding of larvae by workers differed depending on the relationship between workers and brood, with a significantly higher percentage of brood from the same natal colony being fed compared with brood from a different colony (Figure 5.4b; two-tailed *t* test: $t_{17} = 5.3881$, $P < 0.001$). There was a 10.5% difference between the percentage fed of brood from the same natal colony and the percentage fed of brood from a different colony.

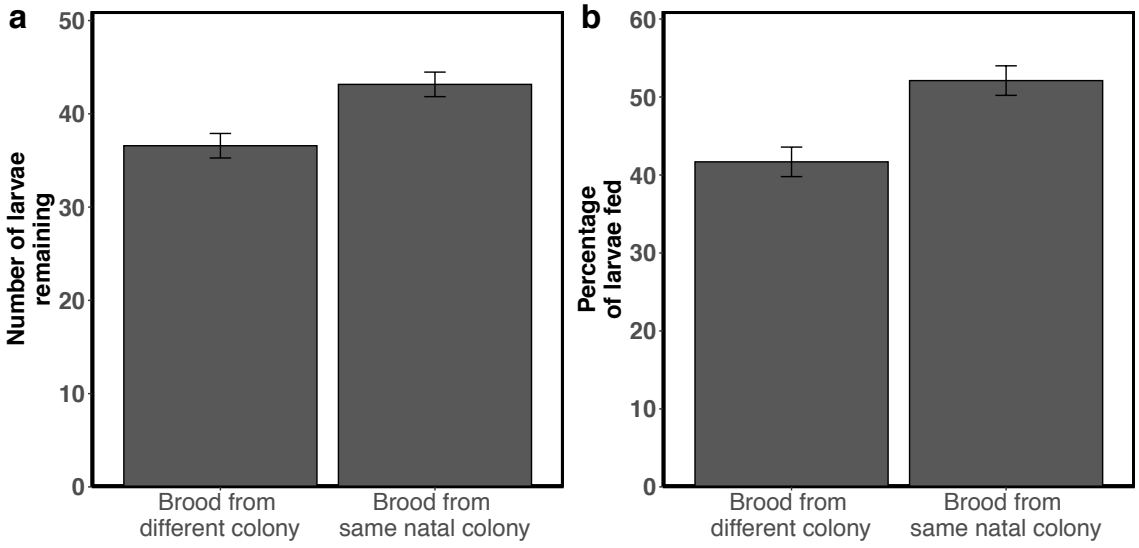


Figure 5.4 a) Number of brood remaining after 18 hours with workers. b) Percentage of brood fed after 18 hours with workers.

5.5. Discussion

Counter to our expectations, we detected distinct colony boundaries between almost all nests in the field regardless of social form (Figure 5.1) and within-colony relatedness between workers (Appendix C S5). The nests that did share with each other were likely part of the same polydomous colony based on genetic results and spatial distance between mounds (Figure 5.2), and sharing was not affected by colony social form (i.e., polygyne mounds were no more likely to share than monogyne mounds). Our results provide further support that polygyne nests are not completely interconnected (Weeks et al. 2004, Goodisman et al. 2007, Krushelnycky et al. 2010); in fact, polygyne colonies in our study were just as isolated as monogyne colonies, suggesting that polygyne fire ants in Texas are multicolonial rather than unicolonial. Moreover,

boundaries appear to be present at relatively small spatial scales, as many nests of both social forms did not exchange resources despite being within 5m, and sometimes even less than 1m, from each other (Appendix C S6). Weeks et al. (2004) found that most labeled polygyne fire ant workers remained within 4m of their colony, but it was surprising to find nests with distinct boundaries separated by less than 1m. These results imply that fire ants are able to distinguish nestmates from non-nestmates, even when environmental odor cues may be similar from living in close proximity. Heritable and environmental odor cues are thought to be additive in fire ants, but monogyne and polygyne fire ant workers have been shown to distinguish nestmate from non-nestmate despite similar environmental odor cues (Obin 1986, Obin et al. 1993).

Our laboratory results support the role of heritable odor cues in nestmate recognition, as workers in the laboratory preferentially fed nestmate over non-nestmate brood (Figure 5.4a). All colonies were kept in standardized laboratory conditions and fed standardized diets to minimize acquired, environmental identification cues (Obin et al. 1993), so worker recognition of nestmate over non-nestmate larvae is likely based on heritable as opposed to environmental odor cues. Additionally, we found evidence that workers may have preferentially cannibalized non-nestmate brood, as there was a significantly greater reduction in the number of non-nestmate brood remaining at the end of the experiment compared with nestmate brood (Figure 5.4b). One reason for the greater disappearance of non-nestmate brood could be due to differential brood viability between families, which can cause “sham nepotism” (Holzer et al. 2006). We believe this scenario is unlikely, however, given the short time frame and reciprocal nature of

our experiment. Furthermore, queens allowed to found in isolation do not express differential viability of offspring (Vargo and Ross 1989). Larvae were given to the colonies by placing them outside of the nest dishes and allowing the workers to bring them into the nest, so it is also possible that workers collected greater numbers of nestmate brood than non-nestmate brood. We found no desiccated larvae, however, in or around the experimental colonies. Instead, we hypothesize that polygyne fire ant workers preferentially cannibalize less related brood in times of stress. High levels of cannibalism are known from this species (Sorensen et al. 1983, Tschinkel 1993) and often occur when resources are in short supply. The lack of proteinaceous food for the six days preceding the creation of these experimental colonies may have driven the colonies to consume the brood, starting with brood that were less related to the workers. Any hereditary predisposition towards nepotism should be under positive selection in a eusocial system, as preferential care of related offspring should increase inclusive fitness (Keller 1997, Wilson and Hölldobler 2005, Wilson 2008); however, extremes in nepotism should be selected against in polygyne systems (Keller 1997), as they tend to create an environment of competition within the colony and diminish colony fitness. While strong nepotism should be selected against, slightly nepotistic ants may have an advantage over their less discriminating counterparts as nepotism toward sexual larvae, regardless of the strength of the interaction, should increase inclusive fitness (Nonacs 1988). This would suggest that eusociality should favor low levels of nepotism, such as those found in this study, which straddles the gap between selfless and selfish endeavors.

The behavior detected in polygyne colonies in the field and in the laboratory may be explained by the higher within-colony relatedness coefficients observed in the field. Although polygyne nests had lower within-colony relatedness values between workers than monogyne nests, relatedness coefficients in polygyne colonies were much higher on average (mean and standard errors: 0.269 ± 0.037) than those observed in previous studies of introduced polygyne fire ant populations in the USA (Figure 5.3; Ross and Fletcher 1985, Ross 1993, Ross et al. 1996, DeHeer and Ross 1997, Goodisman et al. 2007). Past studies have reported values that were not significantly different from zero (i.e., many unrelated queens producing workers within the same nest; but see Ross 1993), but our results suggest that workers within polygyne colonies in Texas may even be half-sisters (expected *r* for half-sisters = 0.25).

Much of the population genetics data of introduced polygyne fire ants in the USA has focused on one or a few geographic regions (Ross and Fletcher 1985, Ross 1993, Ross and Keller 1995, Ross et al. 1996, DeHeer and Ross 1997). Only a few studies have examined colony genetic structure in Texas (Ross et al. 1993, Ross et al. 1996, Chen et al. 2003), and none that we know of have reported within-colony relatedness between workers (Figure 5.3). Fire ant populations in Texas vary genetically from other parts of the country (Shoemaker et al. 2006), so geographic variation in colony genetic structure may be one reason why we found higher within-colony relatedness and pairwise F_{ST} values in polygyne nests compared with those in other states (Ross and Fletcher 1985, Ross 1993, Ross et al. 1996, DeHeer and Ross 1997, Goodisman et al. 2007). It is also possible that colony genetic structure has changed over time. For

example, relatedness was almost twice as high in older compared with younger populations (i.e., over 100 years old vs. 17 years old) in the polygynous ant *Formica fusca* (Hannonen et al. 2004). Past studies of polygyne fire ant queens in Texas reported a near zero relatedness between co-occurring queens (Ross et al. 1996, Chen et al. 2003), which should result in similarly low relatedness between workers, but it is possible that within-colony relatedness has increased over the past 20 years. The ecological impact of polygyne fire ants has significantly weakened over a ten-year period in parts of Texas (Morrison 2002), which may have corresponded with a change in genetic structure. Interestingly, our within-colony relatedness coefficients between workers were much more similar to those reported in native polygyne fire ant populations (Figure 5.3; Ross et al. 1996), where queens within the same colony are highly related (Ross et al. 1996), workers recognize nestmate from non-nestmate (Chirino et al. 2012), and colony densities are 4-7 times lower than those observed in the USA (Porter et al. 1997). Although we did not measure queen-queen relatedness, our behavioral results in the field and in the laboratory support the conclusion that polygyne fire ants in Texas likely function similarly to native conspecifics. Our within-colony relatedness coefficients between polygyne workers were also similar to those reported in Australia (Figure 5.3; Henshaw et al. 2005), so it would be interesting to determine if Australian polygyne fire ant colonies behave similarly to those in Texas and in the native range.

This does not explain, however, why some studies have identified distinct colony boundaries despite very low relatedness between polygyne fire ant nests (Goodisman et al. 2007). Our results suggest that relatedness alone does not predict sharing between

nests, as several neighboring nests had low pairwise F_{ST} and within-colony relatedness values but did not share with each other (Appendix C S6). In other ant species, kinship does not always correlate with cooperation between nests (Procter et al. 2016). For example, nests of the polygynous ant *Formica lugubris* did not share workers or resources with each other despite high genetic relatedness (Procter et al. 2016). Similarly, Argentine ants (*L. humile*) did not freely exchange workers between all nests within a single supercolony, even though there were no detectable genetic differences between nests (Heller et al. 2008). This highlights the importance of quantifying colony boundaries using several different methods (Ellis et al. 2017), as genetic relationships do not always imply a free exchange of workers or resources. Future research should examine the exchange of resources between polygyne fire ant nests in other parts of their invaded range where within-colony relatedness is much lower to ultimately determine the relationship between sharing and genetic relatedness.

Here we provide evidence that polygyne fire ant colonies in Texas are not unicolonial using a multidisciplinary and experimental approach (i.e., mapping physical boundaries and genetic structure). Polygyne colonies did not exchange resources or workers, indicating distinct colony boundaries. Polygyne workers also preferentially fed larval nestmates and may have even cannibalized non-nestmates during times of stress, suggesting that within-colony nepotism exists, at least in the case of worker-brood interactions. Polygyne colony behavior corresponded with higher levels of within-colony relatedness between workers in the field than those previously reported in North America, both of which closely resembled the behavior and relatedness found in

polygyne colonies in Argentina (Ross et al. 1996, Chirino et al. 2012). Our results suggest that polygyne fire ant colonies are actually multicolonial and may engage in high levels of intraspecific competition (Weeks et al. 2004). High levels of intraspecific competition likely reduce the ecological impacts of polygyne fire ant colonies on invaded communities and may explain why invaded habitats in parts of Texas have almost completely recovered since their initial introduction (Morrison 2002). Moreover, multicolonial polygyne fire ant colonies avoid the evolutionary consequences of unicoloniality (Helanterä et al. 2009). Many unicolonial species are associated with drastic population crashes (Cooling et al. 2012, Wetterer 2012, Abbott et al. 2014, Wetterer et al. 2014, Cooling and Hoffmann 2015, Lester and Gruber 2016), but we know of no such population collapse in polygyne fire ants. Monogyne fire ants maintain relatively consistent population dynamics due to high levels of intraspecific competition (Adams and Tschinkel 2001), and we suspect that polygyne fire ants may avoid population crashes through similar means. Our results, however, may not be universal across the entirety of their invaded range. The population genetics of fire ants can differ geographically (Shoemaker et al. 2006), which may correspond with differences in behavior. Future research should examine sharing between and within nests in other parts of their invaded range, including areas where within-colony relatedness coefficients are not significantly different from zero.

5.6. References

Abbott, K. L., P. T. Green, and D. J. O'Dowd. 2014. Seasonal shifts in macronutrient preferences in supercolonies of the invasive Yellow Crazy Ant *Anoplolepis gracilipes* (Smith, 1857)(Hymenoptera: Formicidae) on Christmas Island, Indian Ocean. Austral Entomology **53**:337-346.

Adams, E. S., and W. R. Tschinkel. 2001. Mechanisms of population regulation in the fire ant *Solenopsis invicta*: an experimental study. Journal of Animal Ecology **70**:355-369.

Bhatkar, A., and S. Vinson. 1987. Colony limits in *Solenopsis invicta* Buren. Pages 599-600 *in* J. Eder and H. Rembold, editors. Chemistry and biology of social insects. Munich: Verlag J. Paperny.

Björkman-Chiswell, B., E. Van Wilgenburg, M. Thomas, S. Swearer, and M. Elgar. 2008. Absence of aggression but not nestmate recognition in an Australian population of the Argentine ant *Linepithema humile*. Insectes Sociaux **55**:207-212.

Boomsma, J. J., and P. d'Ettorre. 2013. Nice to kin and nasty to non-kin: revisiting Hamilton's early insights on eusociality. Biology Letters **9**:20130444.

Boulay, R., X. Arnan, X. Cerdá, and J. Retana. 2014. The ecological benefits of larger colony size may promote polygyny in ants. Journal of Evolutionary Biology **27**:2856-2863.

Boutin-Ganache, I., M. Raposo, M. Raymond, and C. F. Deschepper. 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques **31**:25-28.

Buczkowski, G., and T. C. Wossler. 2019. Controlling invasive Argentine ants, *Linepithema humile*, in conservation areas using horizontal insecticide transfer. Scientific Reports **9**:1-7.

Chen, Y., L. Lu, L. Skow, and S. Vinson. 2003. Relatedness among co-existing queens within polygyne colonies of a Texas population of the fire ant, *Solenopsis invicta*. Southwestern Entomologist **28**:27-36.

Chirino, M. G., L. E. Gilbert, and P. J. Folgarait. 2012. Behavioral discrimination between monogyne and polygyne red fire ants (Hymenoptera: Formicidae) in their native range. Annals of the Entomological Society of America **105**:740-745.

Cooling, M., S. Hartley, D. A. Sim, and P. J. Lester. 2012. The widespread collapse of an invasive species: Argentine ants (*Linepithema humile*) in New Zealand. Biology Letters:rsbl20111014.

Cooling, M., and B. D. Hoffmann. 2015. Here today, gone tomorrow: declines and local extinctions of invasive ant populations in the absence of intervention. Biological Invasions **17**:3351-3357.

Coplen, T. B. 2011. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Communications in Mass Spectrometry **25**:2538-2560.

Crozier, R., and P. Pamilo. 1996. One into two will go. Nature **383**:574-575.

DeHeer, C. J., and K. G. Ross. 1997. Lack of detectable nepotism in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology **40**:27-33.

Earl, D. A. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources **4**:359-361.

Ellis, S., D. Procter, P. Buckham-Bonnett, and E. J. H. Robinson. 2017. Inferring polydomy: a review of functional, spatial and genetic methods for identifying colony boundaries. Insectes Sociaux **64**:19-37.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular ecology **14**:2611-2620.

Eyer, P.-A., B. McDowell, L. N. Johnson, L. A. Calcaterra, M. B. Fernandez, D. Shoemaker, R. T. Puckett, and E. L. Vargo. 2018. Supercolonial structure of invasive populations of the tawny crazy ant *Nylanderia fulva* in the US. BMC Evolutionary Biology **18**:209.

Eyer, P. A., E. M. Espinoza, A. J. Blumenfeld, and E. L. Vargo. 2020. The underdog invader: Breeding system and colony genetic structure of the dark rover ant (*Brachymyrmex patagonicus* Mayr). Ecology and Evolution **10**:493-505.

Fritz, G. N., R. K. Vander Meer, and C. A. Preston. 2006. Selective male mortality in the red imported fire ant, *Solenopsis invicta*. Genetics **173**:207-213.

Fry, B. 2006. Stable isotope ecology. Springer.

Giraud, T., J. S. Pedersen, and L. Keller. 2002. Evolution of supercolonies: the Argentine ants of southern Europe. Proceedings of the National Academy of Sciences **99**:6075-6079.

Goodisman, M. A., K. A. Sankovich, and J. L. Kovacs. 2007. Genetic and morphological variation over space and time in the invasive fire ant *Solenopsis invicta*. Biological Invasions **9**:571-584.

Gotzek, D., D. D. Shoemaker, and K. G. Ross. 2007. Molecular variation at a candidate gene implicated in the regulation of fire ant social behavior. PLoS One **2**:e1088.

Greenberg, L., S. Vinson, and S. Ellison. 1992. Nine-year study of a field containing both monogyne and polygyne red imported fire ants (Hymenoptera: Formicidae). Annals of the Entomological Society of America **85**:686-695.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. II. Journal of Theoretical Biology **7**:17-52.

Hannonen, M., H. Helanterä, and L. Sundström. 2004. Habitat age, breeding system and kinship in the ant *Formica fusca*. Molecular Ecology **13**:1579-1588.

Helanterä, H., J. E. Strassmann, J. Carrillo, and D. C. Queller. 2009. Unicolonial ants: where do they come from, what are they and where are they going? Trends in Ecology & Evolution **24**:341-349.

Heller, N., K. Ingram, and D. Gordon. 2008. Nest connectivity and colony structure in unicolonial Argentine ants. Insectes Sociaux **55**:397-403.

Henshaw, M. T., N. Kunzmann, C. Vanderwoude, M. Sanetra, and R. H. Crozier. 2005. Population genetics and history of the introduced fire ant, *Solenopsis invicta*

Buren (Hymenoptera: Formicidae), in Australia. Australian Journal of Entomology **44**:37-44.

Hölldobler, B., and E. O. Wilson. 1977. The number of queens: an important trait in ant evolution. Naturwissenschaften **64**:8-15.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Harvard University Press.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, and T. J. Case. 2002. The Causes and Consequences of Ant Invasions. Annual Review of Ecology and Systematics **33**:181-233.

Holway, D. A., and A. V. Suarez. 2004. Colony-structure variation and interspecific competitive ability in the invasive Argentine ant. Oecologia **138**:216-222.

Holzer, B., R. Kümmerli, L. Keller, and M. Chapuisat. 2006. Sham nepotism as a result of intrinsic differences in brood viability in ants. Proceedings of the Royal Society B: Biological Sciences **273**:2049-2052.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics **24**:1403-1405.

Kearse, M., R. Moir, A. Wilson, S. Stones Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28**:1647-1649.

Keller, L. 1995. Social life: the paradox of multiple-queen colonies. Trends in Ecology & Evolution **10**:355-360.

Keller, L. 1997. Indiscriminate altruism: unduly nice parents and siblings. Trends in Ecology & Evolution **12**:99-103.

Krieger, M., and L. Keller. 1997. Polymorphism at dinucleotide microsatellite loci in fire ant Solenopsis invicta populations. Molecular Ecology **6**:997-999.

Krieger, M. J., and K. G. Ross. 2002. Identification of a major gene regulating complex social behavior. Science **295**:328-332.

Krushelnycky, P. D., D. A. Holway, and E. G. LeBrun. 2010. Invasion processes and causes of success. Pages 245-260 *in* L. Lach, C. L. Parr, and K. L. Abbott, editors. Ant ecology. Oxford University Press.

Lanza, J. 1988. Ant preferences for *Passiflora* nectar mimics that contain amino acids. Biotropica **20**:341-344.

Lawson, L. P., R. K. Vander Meer, and D. Shoemaker. 2012. Male reproductive fitness and queen polyandry are linked to variation in the supergene Gp-9 in the fire ant *Solenopsis invicta*. Proceedings of the Royal Society B: Biological Sciences **279**:3217-3222.

LeBrun, E. G., J. Abbott, and L. E. Gilbert. 2013. Imported crazy ant displaces imported fire ant, reduces and homogenizes grassland ant and arthropod assemblages. Biological Invasions **15**:2429-2442.

Lester, P. J., and M. A. Gruber. 2016. Booms, busts and population collapses in invasive ants. Biological Invasions **18**:1-11.

Morel, L., R. K. Vander Meer, and C. S. Lofgren. 1990. Comparison of nestmate recognition between monogyne and polygyne populations of *Solenopsis invicta*

(Hymenoptera: Formicidae). Annals of the Entomological Society of America **83**:642-647.

Morrison, L. W. 2002. Long-term impacts of an arthropod-community invasion by the imported fire ant, *Solenopsis invicta*. Ecology **83**:2337-2345.

Nonacs, P. 1988. Queen number in colonies of social Hymenoptera as a kin-selected adaptation. Evolution **42**:566-580.

Obin, M., L. Morel, R. Vander Meer, and R. K. Vandermeer. 1993. Unexpected, well-developed nestmate recognition in laboratory colonies of polygyne imported fire ants (Hymenoptera: Formicidae). Journal of Insect Behavior **6**:579-589.

Obin, M. S. 1986. Nestmate recognition cues in laboratory and field colonies of *Solenopsis invicta* buren (Hymenoptera: Formicidae). Journal of Chemical Ecology **12**:1965-1975.

Passera, L. 1994. Characteristics of tramp species. Pages 23-43 *in* D. F. Williams, editor. Exotic ants. Biology, impact, and control of introduced species. Westview Press.

Plowes, R. M., J. G. Dunn, and L. E. Gilbert. 2007. The urban fire ant paradox: native fire ants persist in an urban refuge while invasive fire ants dominate natural habitats. Biological Invasions **9**:825-836.

Porter, S., and D. Savignano. 1990. Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. Ecology **71**:2095-2106.

Porter, S. D., H. G. Fowler, and W. P. Mackay. 1992. Fire ant mound densities in the United States and Brazil (Hymenoptera: Formicidae). Journal of Economic Entomology **85**:1154-1161.

Porter, S. D., D. F. Williams, R. S. Patterson, and H. G. Fowler. 1997. Intercontinental differences in the abundance of *Solenopsis* fire ants (Hymenoptera: Formicidae): escape from natural enemies? Environmental Entomology **26**:373-384.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics **155**:945-959.

Procter, D. S., J. E. Cottrell, K. Watts, S. W. A'Hara, M. Hofreiter, and E. J. Robinson. 2016. Does cooperation mean kinship between spatially discrete ant nests? Ecology and Evolution **6**:8846-8856.

Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. Evolution **43**:258-275.

Queller, D. C., and J. E. Strassmann. 1998. Kin selection and social insects. Bioscience **48**:165-175.

Ross, K., E. Vargo, L. Keller, and J. Trager. 1993. Effect of a founder event on variation in the genetic sex-determining system of the fire ant *Solenopsis invicta*. Genetics **135**:843-854.

Ross, K. G. 1993. The breeding system of the fire ant *Solenopsis invicta*: effects on colony genetic structure. The American Naturalist **141**:554-576.

Ross, K. G., and J. M. Carpenter. 1991. Phylogenetic analysis and the evolution of queen number in eusocial Hymenoptera. Journal of Evolutionary Biology **4**:117-130.

Ross, K. G., and D. J. Fletcher. 1985. Comparative study of genetic and social structure in two forms of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology **17**:349-356.

Ross, K. G., and L. Keller. 1995. Ecology and evolution of social organization: insights from fire ants and other highly eusocial insects. Annual Review of Ecology and Systematics **26**:631-656.

Ross, K. G., and L. Keller. 1998. Genetic control of social organization in an ant. Proceedings of the National Academy of Sciences **95**:14232-14237.

Ross, K. G., E. L. Vargo, and L. Keller. 1996. Social evolution in a new environment: the case of introduced fire ants. Proceedings of the National Academy of Sciences **93**:3021-3025.

Rousset, F. 2008. genepop '007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources **8**:103-106.

Schmid-Hempel, P. 1997. Infection and colony variability in social insects. Pages 43-51 Infection, Polymorphism and Evolution. Springer.

Schmid-Hempel, P., and R. H. Crozier. 1999. Ployandry versus polygyny versus parasites. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **354**:507-515.

Shoemaker, D. D., C. J. Deheer, M. J. Krieger, and K. G. Ross. 2006. Population genetics of the invasive fire ant *Solenopsis invicta* (Hymenoptera: Formicidae) in the United States. Annals of the Entomological Society of America **99**:1213-1233.

Snyder, L. E. 1993. Non-random behavioural interactions among genetic subgroups in a polygynous ant. Animal Behaviour **46**:431-439.

Sorensen, A. A., T. M. Busch, and S. B. Vinson. 1983. Factors affecting brood cannibalism in laboratory colonies of the imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Journal of the Kansas Entomological Society:140-150.

R Core Team 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Tillberg, C. V., D. P. McCarthy, A. G. Dolezal, and A. V. Suarez. 2006. Measuring the trophic ecology of ants using stable isotopes. Insectes Sociaux **53**:65-69.

Tschinkel, W. R. 1993. Resource allocation, brood production and cannibalism during colony founding in the fire ant, *Solenopsis invicta*. Behavioral Ecology and Sociobiology **33**:209-223.

Tschinkel, W. R. 2006. The fire ants. Harvard University Press.

Tsutsui, N. D., A. V. Suarez, D. A. Holway, and T. J. Case. 2000. Reduced genetic variation and the success of an invasive species. Proceedings of the National Academy of Sciences of the United States of America **97**:5948-5953.

Valles, S. M., and S. D. Porter. 2003. Identification of polygyne and monogyne fire ant colonies (*Solenopsis invicta*) by multiplex PCR of Gp-9 alleles. Insectes Sociaux **50**:199-200.

Vander Meer, R., M. Obin, and L. Morel. 1990. Nestmate recognition in fire ants: monogyne and polygyne populations. Pages 322-328. Applied myrmecology: a world perspective. Westview Press.

Vander Meer, R. K., and L. Morel. 1998. Nestmate recognition in ants. Pages 79-103 *in* M. Breed, editor. Pheromone communication in social insects. Westview Press.

Vargo, E. L., and K. G. Ross. 1989. Differential viability of eggs laid by queens in polygyne colonies of the fire ant, *Solenopsis invicta*. Journal of Insect Physiology **35**:587-593.

Wang, J. 2011. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Molecular Ecology Resources **11**:141-145.

Wang, Z., L. Moshman, E. Kraus, B. Wilson, N. Acharya, and R. Diaz. 2016. A review of the tawny crazy ant, *Nylanderia fulva*, an emergent ant invader in the southern United States: is biological control a feasible management option? Insects **7**:77.

Weeks, R. D., L. Wilson, and S. Vinson. 2004. Resource partitioning among colonies of polygyne red imported fire ants (Hymenoptera: Formicidae). Environmental Entomology **33**:1602-1608.

Wetterer, J., O. Davis, and J. Williamson. 2014. Boom and bust of the tawny crazy ant, *Nylanderia fulva* (Hymenoptera: Formicidae), on St. Croix, US Virgin Islands. The Florida Entomologist **97**:1099-1103.

Wetterer, J. K. 2012. Worldwide spread of the African big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae). Myrmecological News **17**:51-62.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer.

Wilson, E. O. 2008. One giant leap: how insects achieved altruism and colonial life. Bioscience **58**:17-25.

Wilson, E. O., and B. Hölldobler. 2005. Eusociality: origin and consequences. Proceedings of the National Academy of Sciences **102**:13367-13371.

Yang, C., D. D. Shoemaker, W.-J. Wu, and C.-J. Shih. 2008. Population genetic structure of the red imported fire ant, *Solenopsis invicta*, in Taiwan. Insectes Sociaux **55**:54-65.

6. CONCLUSIONS

This study identifies the causes and consequences of variation in the baselines and methodologies used to estimate trophic position from stable isotope data, correlates trophic interactions with the abundance and foraging activity of an ecologically important invasive ant, develops methods for use of stable isotope tracers with large, ground-nesting ant species, and explores fundamental assumptions about inter- and intracolonial cooperation in invasive ants with widespread distributions. The relationship between trophic interactions, species abundance, and competition can determine the distribution of a species (Davis et al. 1998, Cunningham et al. 2009), which can in turn influence ecosystem processes and the evolution of a species. In this study, I used stable isotope analysis to show that a highly omnivorous diet likely drives the abundance of the tawny crazy ant, and that multiple-queen (polygyne) colonies of the red imported fire ant are not cooperating indiscriminately with each other. In addition, I helped develop best-practices for important ecological tools by 1) demonstrating the consequences of appropriate baselines, methodologies, and sample sizes in accurately estimating trophic position from stable isotope data, and 2) applying stable isotope tracers to invasive ant species in the laboratory and in the field.

I first conducted a literature review to quantify the variation in baselines and models/ methodologies used to estimate trophic position from stable isotope data (Section 2). I assessed the potential consequences of this variation on the estimates of individual species, as well as on conclusions about the overall food web structure by

extracting hundreds of published trophic positions and applying various baselines and methodologies to existing stable isotope datasets. I identified 10 different baselines and eight models/ methodologies commonly used in the literature, and the baseline and methodology that authors used depended on their study system (i.e., aquatic or terrestrial). Moreover, baseline and methodology significantly impacted the trophic position estimates of individual species, as well as conclusions about food web structure. Increased sample size may mitigate some of the variation caused by different baselines and methodologies, but an alarmingly large proportion of studies collected only one sample in at least one trophic group (41% of all studies) or did not report how many they collected at all (26%). Authors should collect a minimum of five samples per trophic group (but ten for best-practices) from as many trophic groups as possible to increase statistical power and redundancy in comparisons. I also recommend moving away from biological interpretations of absolute trophic positions (as these are prone to a number of biases), and instead focus on comparisons of these values relative to other organisms collected from the same habitat. Reviews that compile and average trophic positions of organisms from various studies (Pauly et al. 1998, Das et al. 2003, Potapov et al. 2019) often do not account for possible effects of methodology or baseline (but see Potapov et al. 2019), so it will be important for future studies to incorporate these effects in their models to avoid introducing additional biases in the literature.

It may also be beneficial to complement trophic position estimates with more traditional approaches such as gut content analysis or direct observation (Seminoff et al. 2012, Kloskowski and Trembaczowski 2015). By pairing both techniques, specific food

items can be identified using gut content analysis or direct observation (a snap-shot of the diet), and then the relative importance of each food item can be assessed using stable isotope analysis (a more time-integrated evaluation of diet). I applied this combined approach to determine the relationship between tawny crazy ant diet and abundance in invaded habitats (Section 3). Classical food web theory predicts that individuals at the base of the food web will be more abundant than those at the top (i.e., herbivores are more abundant than predators; Elton 1927, Lindeman 1942). Many invasive ant species reflect this prediction, as colony densities are often highest when feeding as herbivores (i.e., on high amounts of carbohydrate resources; Wetterer et al. 2006, Tillberg et al. 2007, Wilder et al. 2011a, Wilder et al. 2011b, Wittman et al. 2018). Counter to my expectations, however, tawny crazy ant densities at baits were highest when ants were more predaceous. Moreover, when compared with known predators and herbivores in the community, tawny crazy ants were much more predaceous than traditional food web theory would predict. Rather than feeding as herbivores, tawny crazy ants were actually highly omnivorous. My direct observations provide further support that tawny crazy ants take advantage of a broad range of food items, as I observed workers foraging on plant nectaries, tending aphids on a variety of plants, and consuming arthropod prey (dead and alive). My results suggest that tawny crazy ants reach high densities not by feeding as herbivores, as classical food web theory would predict, but by feeding flexibly on many different resources. So-called 'top-heavy' food webs, in which predators achieve a greater biomass than herbivores, are not uncommon in nature (Snyder and Evans 2006, Hall 2011), especially in the case of omnivores (McCauley et al. 2018, Woodson et al.

2018). I propose that extreme omnivory, rather than strict herbivory, may drive invasive ant abundance, especially in the case of tawny crazy ants. Past research has suggested controlling tawny crazy ant numbers by excluding workers from insect mutualists (Sharma et al. 2019), but my results imply that this may be an ineffective strategy due to the incredible dietary flexibility of tawny crazy ants in the field.

Another factor that may promote the spread and establishment of many invasive ant species is their proclivity towards intraspecific cooperation (Holway et al. 2002, Krushelnycky et al. 2010). Many invasive ant species are unicolonial (Passera 1994, Holway et al. 2002), a state that is characterized by reduced intraspecific aggression and an absence of boundaries between nests (Helanterä et al. 2009). Unfortunately, it can be difficult to determine the extent of boundaries between ant colonies in the field. Labeling food using stable isotope tracers is an effective way to quantify resource sharing and identify boundaries between nests, but it has rarely been applied to ants (Fischer et al. 2003, Defossez et al. 2010, Wagner and Fleur Nicklen 2010), and never to large colonies of ground-nesting species. To develop the methods for this approach, I applied this technique to laboratory and field colonies of the red imported fire ant, as well as field colonies of the tawny crazy ant (Section 4). Both ^{15}N-labeled glycine and ^{13}C-labeled glucose tracers were sufficient to detect highly enriched isotope values in laboratory colonies of fire ants after 24 hours, and these signals remained detectable for up to 48 hours for carbon and 72 hours for nitrogen. My methods were also successful in the field for both ant species, especially after 14 days of feeding.

Due to the success in feeding field colonies the isotope tracer, I applied this technique to quantify resource sharing between fire ant colonies to test fundamental assumptions about inter- and intracolonial cooperation in fire ants (Section 5). Fire ants occur as two social forms; monogyne (i.e., colonies with a single egg-laying queen) and polygyne (i.e., colonies with multiple egg-laying queens), the latter of which is commonly referred to as unicolonial throughout its invaded range. The ecological benefits of unicoloniality may be one reason why polygyne fire ant colonies are often more abundant and ecologically dominant in invaded habitats than monogyne colonies (Porter and Savignano 1990). However, interconnectedness between polygyne colonies in the field is poorly documented (e.g., Bhatkar and Vinson 1987). My results suggest that polygyne fire ant colonies are actually multicolonial and may engage in high levels of intraspecific competition with neighboring colonies and may exhibit low levels of nepotism within the colony. Polygyne colonies from my study were similar in some ways to colonies in the native range, where queens within the same colony are highly related (Ross et al. 1996), workers recognize nestmate from non-nestmate (Chirino et al. 2012), and colony densities are four to seven times lower than those observed in the USA (Porter et al. 1997). Within-colony relatedness in polygyne nests from my study was much higher than those reported in previous studies in the USA (Ross and Fletcher 1985, Ross 1993, Ross et al. 1996, DeHeer and Ross 1997, Goodisman et al. 2007), so future research should examine polygyne colony behavior in other parts of their invaded range where within-colony relatedness is lower.

This study examines the relationships between trophic interactions, species abundance, and intraspecific cooperation (or lack thereof) by applying stable isotope analysis to two invasive ant species. My dissertation is a first step in developing best practices for estimating trophic position from stable isotope analysis. Information based on stable isotope data is being used to answer a variety of important basic and applied questions, including disease vector management (Rasgon 2008, Stapp and Salkeld 2009, Gilbreath et al. 2013, Heylen et al. 2019), conservation (Darimont and Reimchen 2002, Alves-Stanley et al. 2010, Merkle et al. 2011, Evans et al. 2012), and climate change assessment (Pomerleau et al. 2017), so it is critical to establish more standardized practices to reduce potential biases in the literature. My dissertation also tests fundamental hypotheses about the association between biomass and food web structure (Elton 1927, Lindeman 1942, France 2014, McCauley et al. 2018), suggesting that omnivory may result in top-heavy food webs. Finally, my dissertation investigates central assumptions in kin selection theory (Hamilton 1964, Keller 1995, 1997, Boomsma and d'Ettorre 2013), showing a high degree of kin recognition and intraspecific competition in a species that was thought to be cooperative.

6.1. References

Alves-Stanley, C. D., G. A. Worthy, and R. K. Bonde. 2010. Feeding preferences of West Indian manatees in Florida, Belize, and Puerto Rico as indicated by stable isotope analysis. Marine Ecology Progress Series **402**:255-267.

Bhatkar, A., and S. Vinson. 1987. Colony limits in *Solenopsis invicta* Buren. Pages 599-600 *in* J. Eder and H. Rembold, editors. Chemistry and biology of social insects. Munich: Verlag J. Paperny.

Boomsma, J. J., and P. d'Ettorre. 2013. Nice to kin and nasty to non-kin: revisiting Hamilton's early insights on eusociality. Biology Letters **9**:20130444.

Chirino, M. G., L. E. Gilbert, and P. J. Folgarait. 2012. Behavioral discrimination between monogyne and polygyne red fire ants (Hymenoptera: Formicidae) in their native range. Annals of the Entomological Society of America **105**:740-745.

Cunningham, H. R., L. J. Rissler, and J. J. Apodaca. 2009. Competition at the range boundary in the slimy salamander: using reciprocal transplants for studies on the role of biotic interactions in spatial distributions. Journal of Animal Ecology **78**:52-62.

Darimont, C. T., and T. E. Reimchen. 2002. Intra-hair stable isotope analysis implies seasonal shift to salmon in gray wolf diet. Canadian Journal of Zoology **80**:1638-1642.

Das, K., G. Lepoint, Y. Leroy, and J.-M. Bouquegneau. 2003. Marine mammals from the southern North Sea: feeding ecology data from $\delta^{13}C$ and $\delta^{15}N$ measurements. Marine Ecology Progress Series **263**:287-298.

Davis, A. J., L. S. Jenkinson, J. H. Lawton, B. Shorrocks, and S. Wood. 1998. Making mistakes when predicting shifts in species range in response to global warming. Nature **391**:783-786.

Defossez, E., C. Djiéto-Lordon, D. McKey, M.-A. Selosse, and R. Blatrix. 2010. Plant-ants feed their host plant, but above all a fungal symbiont to recycle nitrogen. Proceedings of the Royal Society B: Biological Sciences **278**:1419-1426.

DeHeer, C. J., and K. G. Ross. 1997. Lack of detectable nepotism in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology **40**:27-33.

Elton, C. 1927. Animal ecology. The Macmillan Company, New York.

Evans, K. L., J. Newton, J. W. Mallord, and S. Markman. 2012. Stable isotope analysis provides new information on winter habitat use of declining avian migrants that is relevant to their conservation. PLoS One **7**:e34542.

Fischer, R. C., W. Wanek, A. Richter, and V. Mayer. 2003. Do ants feed plants? A ^{15}N labelling study of nitrogen fluxes from ants to plants in the mutualism of Pheidole and Piper. Journal of Ecology **91**:126-134.

France, R. 2014. Rebuilding and comparing pyramids of numbers (Elton) and energy (Lindeman) with selected global $\delta^{15}N$ data. Hydrobiologia **722**:1-7.

Gilbreath, T. M., E. J. Kweka, Y. A. Afrane, A. K. Githeko, and G. Yan. 2013. Evaluating larval mosquito resource partitioning in western Kenya using stable isotopes of carbon and nitrogen. Parasites & Vectors **6**:353.

Goodisman, M. A., K. A. Sankovich, and J. L. Kovacs. 2007. Genetic and morphological variation over space and time in the invasive fire ant *Solenopsis invicta*. Biological Invasions **9**:571-584.

Hall, R. J. 2011. Eating the competition speeds up invasions. Biology Letters:rsbl20100766.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. II. Journal of Theoretical Biology **7**:17-52.

Helanterä, H., J. E. Strassmann, J. Carrillo, and D. C. Queller. 2009. Unicolonial ants: where do they come from, what are they and where are they going? Trends in Ecology & Evolution **24**:341-349.

Heylen, D., O. Schmidt, H. Dautel, L. Gern, H. Kampen, J. Newton, and J. Gray. 2019. Host identification in unfed ticks from stable isotope compositions ($\delta^{13}C$ and $\delta^{15}N$). Medical and Veterinary Entomology **33**:360-366.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, and T. J. Case. 2002. The causes and consequences of ant invasions. Annual Review of Ecology and Systematics **33**:181-233.

Keller, L. 1995. Social life: the paradox of multiple-queen colonies. Trends in Ecology & Evolution **10**:355-360.

Keller, L. 1997. Indiscriminate altruism: unduly nice parents and siblings. Trends in Ecology & Evolution **12**:99-103.

Kloskowski, J., and A. Trembaczowski. 2015. Fish reduce habitat coupling by a waterbird: evidence from combined stable isotope and conventional dietary approaches. Aquatic Ecology **49**:21-31.

Krushelnycky, P. D., D. A. Holway, and E. G. LeBrun. 2010. Invasion processes and causes of success. Pages 245-260 *in* L. Lach, C. L. Parr, and K. L. Abbott, editors. Ant ecology. Oxford University Press.

Lindeman, R. 1942. The trophic-dynamic aspect of ecology. Ecology **23**:399-417.

McCauley, D. J., G. Gellner, N. D. Martinez, R. J. Williams, S. A. Sandin, F. Micheli, P. J. Mumby, and K. S. McCann. 2018. On the prevalence and dynamics of inverted trophic pyramids and otherwise top-heavy communities. Ecology Letters **21**:439-454.

Merkle, J. A., J. J. Derbridge, and P. R. Krausman. 2011. Using stable isotope analysis to quantify anthropogenic foraging in black bears. Human-Wildlife Interactions **5**:159-167.

Passera, L. 1994. Characteristics of tramp species. Pages 23-43 *in* D. F. Williams, editor. Exotic ants. Biology, impact, and control of introduced species. Westview Press.

Pauly, D., A. Trites, E. Capuli, and V. Christensen. 1998. Diet composition and trophic levels of marine mammals. ICES Journal of Marine Science **55**:467-481.

Pomerleau, C., M. Peter Heide-Jørgensen, S. H. Ferguson, H. L. Stern, J. L. Høyer, and G. A. Stern. 2017. Reconstructing variability in West Greenland ocean biogeochemistry and bowhead whale (*Balaena mysticetus*) food web structure using amino acid isotope ratios. Polar Biology **40**:2225-2238.

Porter, S., and D. Savignano. 1990. Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. Ecology **71**:2095-2106.

Porter, S. D., D. F. Williams, R. S. Patterson, and H. G. Fowler. 1997. Intercontinental differences in the abundance of *Solenopsis* fire ants (Hymenoptera: Formicidae): escape from natural enemies? Environmental Entomology **26**:373-384.

Potapov, A. M., U. Brose, S. Scheu, and A. V. Tiunov. 2019. Trophic position of consumers and size structure of food webs across aquatic and terrestrial ecosystems. The American Naturalist **194**:823-839.

Rasgon, J. L. 2008. Stable isotope analysis can potentially identify completely-digested bloodmeals in mosquitoes. PLoS One **3**:e2198.

Ross, K. G. 1993. The breeding system of the fire ant *Solenopsis invicta*: effects on colony genetic structure. The American Naturalist **141**:554-576.

Ross, K. G., and D. J. Fletcher. 1985. Comparative study of genetic and social structure in two forms of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology **17**:349-356.

Ross, K. G., E. L. Vargo, and L. Keller. 1996. Social evolution in a new environment: the case of introduced fire ants. Proceedings of the National Academy of Sciences **93**:3021-3025.

Seminoff, J. A., S. R. Benson, K. E. Arthur, T. Eguchi, P. H. Dutton, R. F. Tapilatu, and B. N. Popp. 2012. Stable isotope tracking of endangered sea turtles: validation with satellite telemetry and $\delta^{15}N$ analysis of amino acids. PLoS One **7**:e37403.

Sharma, S., E. A. Buss, G. S. Hodges, and D. H. Oi. 2019. Effect of soil treatments for cottony cushion scale (Hemiptera: Monophlebidae) control on *Nylanderia fulva*

(Hymenoptera: Formidicae) survival and trailing activity. Florida Entomologist **102**:202-206.

Snyder, W., and E. Evans. 2006. Ecological effects of invasive arthropod generalist predators. Annual Review of Ecology, Evolution, and Systematics **37**:95-122.

Stapp, P., and D. J. Salkeld. 2009. Inferring host–parasite relationships using stable isotopes: implications for disease transmission and host specificity. Ecology **90**:3268-3273.

Tillberg, C., D. Holway, E. LeBrun, and A. Suarez. 2007. Trophic ecology of invasive Argentine ants in their native and introduced ranges. Proceedings of the National Academy of Sciences of the United States of America **104**:20856-20861.

Wagner, D., and E. Fleur Nicklen. 2010. Ant nest location, soil nutrients and nutrient uptake by ant-associated plants: does extrafloral nectar attract ant nests and thereby enhance plant nutrition? Journal of Ecology **98**:614-624.

Wetterer, J. K., X. Espadaler, A. L. Wetterer, D. Aguin-Pombo, and A. M. Franquinho-Aguiar. 2006. Long-term impact of exotic ants on the native ants of Madeira. Ecological Entomology **31**:358-368.

Wilder, S., D. Holway, A. Suarez, and M. Eubanks. 2011a. Macronutrient content of plant-based food affects growth of a carnivorous arthropod. Ecology **92**:325-332.

Wilder, S., D. Holway, A. Suarez, E. LeBrun, and M. Eubanks. 2011b. Intercontinental differences in resource use reveal the importance of mutualisms in fire ant invasions. Proceedings of the National Academy of Sciences of the United States of America **108**:20639-20644.

Wittman, S. E., D. J. O'Dowd, and P. T. Green. 2018. Carbohydrate supply drives colony size, aggression, and impacts of an invasive ant. Ecosphere **9**:e02403.

Woodson, C. B., J. R. Schramski, and S. B. Joye. 2018. A unifying theory for top-heavy ecosystem structure in the ocean. Nature Communications **9**:23.

APPENDIX A

SECTION 2

Appendix A S1. Statistics used by reviewed studies (out of 200 publications total).

Ecosystem	Statistics		Number of studies:
Terrestrial	**Linear models**		**28**
		ANOVA	15
		Linear mixed model	6
		Generalized linear model	2
		Multiple regression	2
		t tests	2
		General linear model	1
		Linear regression	1
	Nonparametric		**7**
		Kruskal Wallis	4
		Mann Whitney U	1
		PERMANOVA	1
		ANOSIM	1
	Other		**4**
		Correlation	2
		Circular statistics	1
		Discriminant function analysis	1
	Layman's Community Metrics		**2**
	Bayesian		**2**
	Total:		**54**
Aquatic	**Linear models**		**7**
		Regression	2
		t tests	2
		ANOVA	1
		General linear model	1
		Hierarchical model	1
	Nonparametric		**2**
		Mann Whitney U	1
		Spearmans Rank Sum	1
	Other		**3**
		Correlation	1
		PCA	1
		Procrustes	1
	Bayesian		**2**
	NS		**1**
	Total:		**20**

Appendix A S2. Number of trophic positions extracted from the literature (out of 834 total) by methodology, taxonomic class, and trophic enrichment factor (TEF).

Methodology	Class	TEF	Number of estimates
CSIA	Actinopterygii	7.6	22
		5.7	6
		7	1
	Arachnida	7.6	3
	Cephalopoda	7.6	4
	Insecta	7.6	39
	Magnoliopsida	7.6	16
	Mammalia	7.6	7
		4.3	2
	Maxillopoda	7	3
Single source	Actinopterygii	3.4	127
		2.4	20
		3.8	6
		2.1	5
		2.3	1
		3	1
	Arachnida	3.4	3
	Aves	3.8	22
		2.4	9
		3.4	3
	Cephalopoda	3.3	4
	Chondrichthyes	3.8	11
		3.4	2
		2.3	1
	Insecta	3.4	48
	Magnoliopsida	3.4	13
	Mammalia	2.4	67
		3.8	30
		3.4	22
		2.5	7
	Maxillopoda	3.4	8
		2	3
		3.8	3
Two source	Actinopterygii	3.4	117
		3.46	9

Appendix A S2 Continued

Methodology	Class	TEF	Number of estimates
Two source	Actinopterygii	3.15	4
	Insecta	NS	13
	Mammalia	3.15	1
Three source	Actinopterygii	3.4	3
Bayesian	Actinopterygii	Multiple	4
		3.4	1
Stomach content	Actinopterygii	None	153
	Chondrichthyes	None	1
	Mammalia	None	7
	Maxillopoda	None	2

Appendix A S3. Tests of random-effect terms in each model used to analyze extracted trophic position estimates (*ranova* in the package *LmerTest*). Each model is specified above tables.

Complete model: TP ~ Method.simple + Class + (1 | Starting.Year) + (1 | Species) + (1 | Region.Country) + Method.simple:Class

	Number of parameters	Log-likelihood	AIC	Likelihood ratio test	Df	*P*
No random effects	29	-674.05232	1406.10463	NA	NA	NA
(1 | Starting.Year)	28	-687.33089	1430.66178	26.5571429	1	**2.56E-07**
(1 | Species)	28	-771.81941	1599.63883	195.534191	1	**1.97E-44**
(1 | Region.Country)	28	-681.10197	1418.20394	14.0993048	1	**0.00017341**

Bulk model: TP ~ Method.simple + Baseline.simple + Class + (1 | Starting.Year) + (1 | Species) + (1 | Region.Country) + Method.simple:Baseline.simple + Method.simple:Class

	Number of parameters	Log-likelihood	AIC	Likelihood ratio test	Df	*P*
No random effects	24	-466.61408	981.228158	NA	NA	NA
(1 | Starting.Year)	23	-475.48261	996.965226	17.7370678	1	**2.54E-05**
(1 | Species)	23	-508.35872	1062.71744	83.4892777	1	**6.41E-20**
(1 | Region.Country)	23	-470.19314	986.386289	7.15813148	1	**0.00746249**

Appendix A S3 Continued

Actinopterygii model: TP ~ Method.simple * Species + (1|Starting.Year) + (1|Region.Country)

	Number of parameters	Log-likelihood	AIC	Likelihood ratio test	Df	*P*
No random effects	41	-329.78908	741.578168	NA	NA	NA
(1 | Starting.Year)	40	-337.44584	754.891671	15.3135028	1	**9.11E-05**
(1 | Region.Country)	40	-335.72842	751.456848	11.8786798	1	**0.0005678**

Insecta model: TP ~ Method.simple + Species + (1|Region.Country)

	Number of parameters	Log-likelihood	AIC	Likelihood ratio test	Df	*P*
No random effects	23	-76.550842	199.101684	NA	NA	NA
(1 | Region.Country)	22	-78.915258	201.830517	4.72883228	1	**0.02966102**

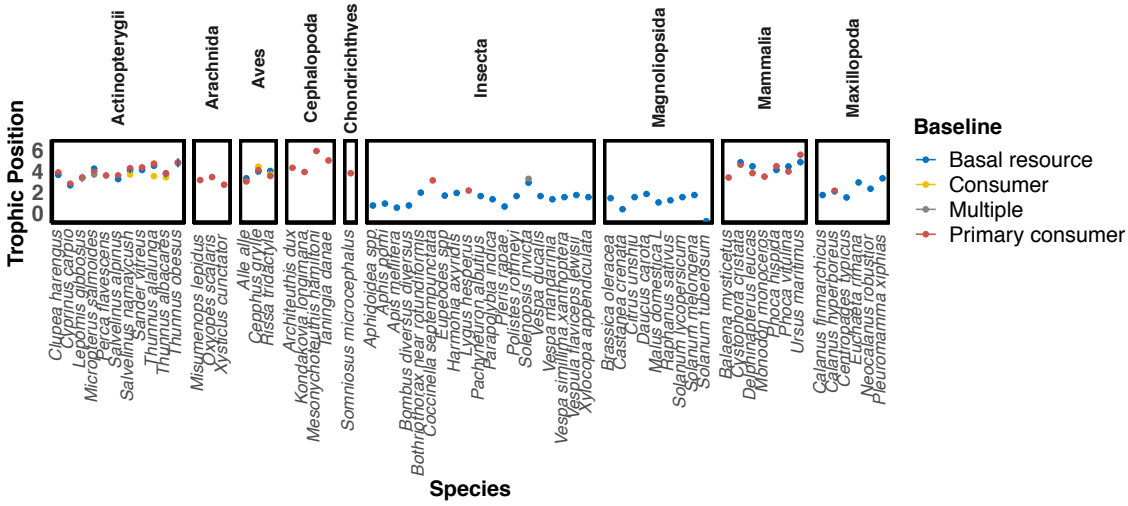
Mammalia model: TP ~ Method.simple * Species + (1|Starting.Year) + (1|Region.Country)

	Number of parameters	Log-likelihood	AIC	Likelihood ratio test	Df	*P*
No random effects	20	-119.86113	279.722252	NA	NA	NA
(1 | Starting.Year)	19	-124.99435	287.988693	10.2664405	1	**0.00135472**
(1 | Region.Country)	19	-119.86113	277.722252	-1.95E-07	1	1

Aves model: TP ~ Baseline.simple * Species + (1|Region.Country)

	Number of parameters	Log-likelihood	AIC	Likelihood ratio test	Df	*P*
No random effects	11	3.92139784	14.1572043	NA	NA	NA
(1 | Region.Country)	10	-12.349348	44.6986958	32.5414915	1	**1.17E-08**

Appendix A S4. Baseline used for each published trophic position estimate by species. Results show values from bulk isotope analysis only (no CSIA or stomach content analysis). Points represent means, and error bars represent standard error.



Appendix A S5. Summaries of ten stable isotope datasets. All trophic positions were calculated using the formulas included in Table 2.1. $\Delta n = 3.4‰$ for all single source and two source calculations. For all compound-specific isotope analysis (CSIA) calculations $\Delta n = 7.6‰$, $\beta = -3.4‰$ for aquatic organisms, and $\beta = +8.4‰$ for terrestrial organisms.

Ecosystem studied	Publication	Short name	Trophic groups analyzed	Sites	Summary	Sample size per trophic group	Trophic position estimates
Terrestrial	Chikaraishi et al. 2014	ChikT	1.Plant, 2.Herbivore, Consumer 1 (Katydid), Consumer 2 (Paper wasp), Consumer 3 (Lady beetle), Consumer 4 (Hornet)	NA	Plants and arthropods collected from a fruit & vegetable farm in Japan	Range: 2-12, Median: 4, Mean: 5.2	Raw values ($\partial^{15}N$), Single source - Var1 (plant baseline), Single source - Var2 (herbivore baseline), Baseline subtraction, CSIA
	Korobushkin et al. 2014	Koro	1.Litter (Leaf litter), Consumer 1 (Saprophages), Consumer 2 (Predators)	3 mixed forests	Soil macrofauna collected in temperate forests in Russia	Range: 2-27, Median: 18, Mean: 16.9	Raw values ($\partial^{15}N$), Single source - Var1 (plant baseline), Baseline subtraction
	Pringle & Fox-Dobbs 2008	Pringle	1.Plant1 (*Acacia drepanolobium*), 1.Plant2 (*Pennisetum sp.*), 2.Prey1 (Arboreal prey arthropod), 2.Prey2 (Terrestrial prey arthropod), Consumer 1 (Arboreal predatory arthropod), Consumer 2 (Predatory gecko), Consumer 3 (Terrestrial predatory arthropod)	NA	Arthropods and geckos collected from wooded grassland in Kenya	Range:20-44 , Median:37 , Mean: 33	Raw values ($\partial^{15}N$), Single source - Var1 (plant base), Single source - Var2 (herbivore base), Two source, Baseline subtraction
	Sanders et al. 2007	Sanders	1.Plant, 2.Herbivore, Consumer 1 (Detrivore), Consumer 2 (Ant), Consumer 3 (Wandering spider), Consumer 4 (Web-building spider)	NA	Arthropods collected in a grassland food web in Germany	Range: 3-13, Median: 6.5, Mean: 7.2	Raw values ($\partial^{15}N$), Single source - Var1 (plant baseline), Single source - Var2 (herbivore baseline), Baseline subtraction
	Steffan et al. 2013	Steffan	1.Plant, 2.Herbivore, Consumer 1 (Primary predator), Consumer 2 (Secondary predator)	Feeding trial 1, Feeding trial 2, Field collection	Two controlled feeding trials and one apple orchard field collection of plants and arthropods in USA	Range: 3-4, Median: 3.5, Mean: 3.5	Raw values ($\partial^{15}N$), Single source - Var1 (plant baseline), Single source - Var2 (herbivore baseline), Baseline subtraction, CSIA

Appendix A S5 Continued

Ecosystem studied	Publication	Short name	Trophic groups analyzed	Sites	Summary	Sample size per trophic group	Trophic position estimates
Aquatic	Quezada-Romegialli et al. 2018	Bilagay	1.Benthic baseline (sea snails), 2.Pelagic baseline (mussels), Consumer 1 (Bilagay fish: *Cheilodactylus variegatus*)	10 coastal kelp forests	All samples collected from around Northern Chile	Range: 10-74, Median: 26, Mean: 28	Raw values (∂^{15}N), Single source - Var2 (herbivore baseline), Two source, Baseline subtraction
	Chikaraishi et al. 2014	ChikA	1.Algae1 (Brown algae), 1.Algae2 (Red algae), 2.Gastropod,2. Crustacean, Consumer1 (Fish)	NA	All samples collected from a stony shore in Japan	Range: 3-24, Median: 4, Mean: 8	Raw values (∂^{15}N), Single source - Var1 (plant baseline), Single source - Var2 (herbivore baseline), Baseline subtraction, CSIA
	Fox et al. 2019	Fox	1.Plankton (Heterotrophic source: plankton), 2.Endosymbiont (Autotrophic source: Coral endosymbiont), Coral	16 sites around an atoll	Coral and potential food sources collected around Palmyra Atoll National Wildlife Refuge in Northern Line Islands	Range: 1-6, Median: 5, Mean: 3.7	Raw values (∂^{15}N), Single source - Var1 (plant baseline), Two source, Baseline subtraction
	Ishikawa et al. 2014	Ishi	1.Litter, 1.Periphyton, Consumer 1 (Invertebrate filter feeder), Consumer 2 (Invertebrate grazer), Consumer 3 (Invertebrate shredder), Consumer 4 (Other invertebrates), Consumer 5 (Other fishes), Consumer 6 (Invertebrate predator), Consumer 7 (Goby fish)	Upper and lower sites of Ado and Yasu rivers (4 sites total)	Aquatic invertebrates, fishes, & potential food sources collected from two rivers in central Japan	Range: 1-8, Median: 2.5, Mean: 2.9	Raw values (∂^{15}N), Single source - Var1 (plant baseline), Two source, Baseline subtraction, CSIA
	Price et al. 2019	Price	1.Allochthonous source (riparian vegetation, FPOM, CPOM), 1.Autochthonous source (periphyton), Consumer 1 (detrivore), Consumer 2 (filter feeder), Consumer 3 (grazer), Consumer 4 (shredder), Consumer 5 (predator)	3 locations differing in land use within 5 streams (15 sites total)	Macroinvertebrates and potential food sources collected from sites varying in land use (woodland, agricultural, & urban) in Croatia	Range: 2-27, Median: 6.5, Mean: 8.7	Raw values (∂^{15}N), Single source - Var1 (plant baseline), Two source, Baseline subtraction

Appendix A S6. Species names and corresponding number in Figure 2.6 from eight stable isotope datasets.

Dataset	Species	Species Number
Pringle	*Acacia drepanolobium*	1
ChikA	*Acanthopagrus schlegeli*	2
Sanders	*Adarrus multinotatus*	3
Sanders	*Alopecosa trabalis*	4
Sanders	*Anoscopus albifrons*	5
Sanders	*Aphidinae a*	6
Sanders	*Aphidinae b*	7
ChikT	*Aphidoidea sp*	8
Sanders	*Aphrophora alni*	9
ChikT	*Apis mellifera (#1)*	10
ChikA	*Apogon semilineatus*	11
Steffan	*Apple leaf*	12
Sanders	*Arboridia parvula*	13
Sanders	*Arctosa lutetiana*	14
Sanders	*Argiope bruennichi*	15
Sanders	*Atypus piceus*	16
Sanders	*Auchenorrhyncha group a*	17
Sanders	*Auchenorrhyncha group b*	18
Sanders	*Aulonia albimana*	19
Ishi	*Baetis spp*	20
ChikA	*Batillus cornutus*	21
Steffan	*Bean plant*	22
ChikA	*Binghamia californica*	23
ChikT	*Bombus diversus diversus (#1)*	24
ChikT	*Brassica oleracea (#1)*	25
ChikA	*Canthigaster rivulata*	26
ChikT	*Castanea crenata*	27
Bilagay	*Cheilodactylus variegatus*	28
Ishi	*Chloroperlidae spp*	29
Sanders	*Chrysomelidae Alticinae*	30
ChikT	*Citrus unshiu*	31
Sanders	*Clubiona juv*	32
ChikT	*Coccinella septempunctata (#1)*	33
ChikT	*Cucurbita moschata*	34
ChikT	*Daucus carota*	35
ChikT	*Diospyros kaki Thunberg*	36

Appendix A S6 Continued

Dataset	Species	Species Number
ChikA	*Ditrema temmincki temmincki*	37
Bilagay	*Echinolittorina peruviana*	38
ChikA	*Ecklonia cava*	39
Steffan	*Fall armyworm*	40
Sanders	*Formica cunicularia*	41
ChikT	*Gampsocleis mikado*	42
ChikA	*Gelidium japonicum*	43
ChikA	*Girella punctata*	44
Ishi	*Goerodes spp*	45
Ishi	*Gomphidae spp*	46
ChikA	*Goniistius zonatus*	47
ChikA	*Gymnothorax kidako*	48
ChikA	*Halichoeres poecilopterus*	49
ChikA	*Haliotis discus*	50
ChikT	*Harmonia axyridis (#3)*	51
Ishi	*Heptageniidae spp*	52
ChikT	*Holochlora japonica*	53
Steffan	*Hover fly*	54
Ishi	*Hydropsychidae spp*	55
Ishi	*Kamimuria tibialis*	56
Steffan	*Lacewing*	57
Bilagay	*Lapa*	58
Sanders	*Lasius alienus*	59
Sanders	*Lasius flavus*	60
ChikA	*Lutjanus stellatus*	61
Pringle	*Lygodactylus keniensis*	62
Sanders	*Mangora acalypha*	63
Sanders	*Meioneta rurestris*	64
ChikA	*Microcanthus strigatus*	65
Sanders	*Mocydiopsis attenuata*	66
Sanders	*Myrmica sabuleti*	67
Bilagay	*Mytilus chilensis*	68
Ishi	*Niponiella limbatella*	69
Ishi	*Nipponocypris temminckii*	70
ChikA	*Omphalius pfeifferi*	71
Ishi	*Oncorhynchus masou ishikawae*	72
ChikA	*Oplegnathus fasciatus*	73

Appendix A S6 Continued

Dataset	Species	Species Number
ChikA	*Oplegnathus punctatus*	74
ChikA	*Pachygrapsus crassipes*	75
ChikA	*Panulirus japonicus*	76
ChikT	*Papilio machaon 1*	77
ChikT	*Papilio protenor*	78
ChikT	*Parapolybia indica 9*	79
ChikA	*Parapristipoma trilineatum*	80
Sanders	*Pardosa juv*	81
Sanders	*Pardosa lugubris*	82
Steffan	*Pea aphids*	83
Pringle	*Pennisetum sp*	84
ChikA	*Percnon planissimum*	85
Bilagay	*Perumytilus purpuratus*	86
ChikT	*Pieris rapae (#5)*	87
Sanders	*Pisaura juv*	88
Sanders	*Pisaura mirabilis*	89
ChikA	*Plagusia dentipes*	90
Fox	*Pocillopora meandrina*	91
ChikT	*Polistes jokahamae jokahamae (#3)*	92
ChikT	*Polistes mandarinus*	93
ChikT	*Polistes rothneyi iwatai (#12)*	94
Sanders	*Ponera coarctata*	95
Bilagay	*Prisogaster niger*	96
Ishi	*Protohermes grandis*	97
ChikT	*Prunus avium*	98
ChikA	*Pseudoblennius percoides*	99
ChikA	*Pseudolabrus siebold*	100
ChikA	*Pteragogus flagellifer*	101
ChikT	*Raphanus sativus*	102
Ishi	*Rhinogobius flumineus*	103
Ishi	*Rhinogobius kurodai*	104
Ishi	*Rhynchocypris oxycephalus jouyi*	105
Ishi	*Rhynchocypris sp*	106
ChikA	*Sargassum filicinum*	107
Bilagay	*Scurria viridula*	108
ChikA	*Sebastes inermis*	109
ChikA	*Sebastiscus marmoratus*	110

Appendix A S6 Continued

Dataset	Species	Species Number
ChikT	*Solanum lycopersicum*	111
ChikT	*Solanum melongena*	112
ChikT	*Solanum tuberosum*	113
Ishi	*Stenopsyche marmorata*	114
ChikA	*Takifugu niphobles*	115
Bilagay	*Tegula*	116
Bilagay	*Tegula atra*	117
Sanders	*Tenuiphantes tenuis*	118
Sanders	*Tibellus juv*	119
Sanders	*Tibellus oblongus*	120
Ishi	*Tipulidae spp*	121
Sanders	*Trochosa terricola*	122
Bilagay	*Turritella*	123
ChikA	*Undaria pinnatifida*	124
ChikT	*Vespa ducalis pulchra (#1)*	125
ChikT	*Vespa mandarinia japonica (#1)*	126
ChikT	*Vespa simillima xanthoptera*	127
ChikT	*Vespula flaviceps lewisii*	128
Sanders	*Walckenaeria acuminata*	129
Steffan	*Wasp 1*	130
ChikT	*Xylocopa appendiculata (#1)*	131
Sanders	*Zora sylvestris*	132

Appendix A S7. Literature cited from review of 200 studies.

Akin, S., and K. O. Winemiller. 2008. Body size and trophic position in a temperate estuarine food web. Acta Oecologica **33**:144-153.

Albers, D., M. Schaefer, and S. Scheu. 2006. Incorporation of plant carbon into the soil animal food web of an arable system. Ecology **87**:235-245.

Anderson, C., and G. Cabana. 2007. Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. Journal of the North American Benthological Society **26**:273-285.

Anderson, C., and G. Cabana. 2009. Anthropogenic alterations of lotic food web structure: evidence from the use of nitrogen isotopes. Oikos **118**:1929-1939.

Aresco, M. J., J. Travis, and P. S. MacRae. 2015. Trophic interactions of turtles in a north Florida lake food web: prevalence of omnivory. Copeia **103**:343-356.

Azevedo-Silva, C. E., R. Almeida, D. P. Carvalho, J. P. Ometto, P. B. de Camargo, P. R. Dorneles, A. Azeredo, W. R. Bastos, O. Malm, and J. P. Torres. 2016. Mercury biomagnification and the trophic structure of the ichthyofauna from a remote lake in the Brazilian Amazon. Environmental Research **151**:286-296.

Beaudoin, C. P., E. E. Prepas, W. M. Tonn, L. I. Wassenaar, and B. G. Kotak. 2001. A stable carbon and nitrogen isotope study of lake food webs in Canada's Boreal Plain. Freshwater Biology **46**:465-477.

Berezina, N. A., A. Razinkovas-Baziukas, and A. V. Tiunov. 2017. Non-indigenous amphipods and mysids in coastal food webs of eastern Baltic Sea estuaries. Journal of the Marine Biological Association of the United Kingdom **97**:581-590.

Blanke, C., Y. Chikaraishi, and M. J. Vander Zanden. 2018. Historical niche partitioning and long-term trophic shifts in Laurentian Great Lakes deepwater coregonines. Ecosphere **9**:e02080.

Bode, A., M. T. Alvarez-Ossorio, M. E. Cunha, S. Garrido, J. B. Peleteiro, C. Porteiro, L. Valdés, and M. Varela. 2007. Stable nitrogen isotope studies of the pelagic food web on the Atlantic shelf of the Iberian Peninsula. Progress in Oceanography **74**:115-131.

Bode, A., P. Carrera, G. González-Nuevo, E. Nogueira, I. Riveiro, and M. B. Santos. 2018. A trophic index for sardine (*Sardina pilchardus*) and its relationship to population abundance in the southern Bay of Biscay and adjacent waters of the NE Atlantic. Progress in Oceanography **166**:139-147.

Bodin, N., F. Le Loc'h, X. Caisey, A.-M. Le Guellec, A. Abarnou, V. Loizeau, and D. Latrouite. 2008. Congener-specific accumulation and trophic transfer of polychlorinated biphenyls in spider crab food webs revealed by stable isotope analysis. Environmental Pollution **151**:252-261.

Bowes, R. E., and J. H. Thorp. 2015. Consequences of employing amino acid vs. bulk-tissue, stable isotope analysis: a laboratory trophic position experiment. Ecosphere **6**:1-12.

Carscallen, W. M. A., K. Vandenberg, J. M. Lawson, N. D. Martinez, and T. N. Romanuk. 2012. Estimating trophic position in marine and estuarine food webs. Ecosphere **3**:1-20.

Caut, S., M. J. Jowers, X. Arnan, J. Pearce-Duvet, A. Rodrigo, X. Cerda, and R. R. Boulay. 2014. The effects of fire on ant trophic assemblage and sex allocation. Ecology and Evolution **4**:35-49.

Chang, C.-W., T. Miki, F.-K. Shiah, S.-j. Kao, J.-T. Wu, A. R. Sastri, and C.-h. Hsieh. 2014. Linking secondary structure of individual size distribution with nonlinear size-trophic level relationship in food webs. Ecology **95**:897-909.

Codron, D., J. Codron, J. A. Lee-Thorp, M. Sponheimer, D. de Ruiter, and J. S. Brink. 2007. Stable isotope characterization of mammalian predator–prey relationships in a South African savanna. European Journal of Wildlife Research **53**:161-170.

Coelho, J., C. Mieiro, E. Pereira, A. Duarte, and M. Pardal. 2013. Mercury biomagnification in a contaminated estuary food web: effects of age and trophic position using stable isotope analyses. Marine Pollution Bulletin **69**:110-115.

Colaço, A., E. Giacomello, F. Porteiro, and G. Menezes. 2013. Trophodynamic studies on the Condor seamount (Azores, Portugal, North Atlantic). Deep Sea Research Part II: Topical Studies in Oceanography **98**:178-189.

Corral-Hernández, E., M. Maraun, and J. C. Iturrondobeitia. 2015. Trophic structure of oribatid mite communities from six different oak forests (*Quercus robur*). Soil Biology and Biochemistry **83**:93-99.

Croteau, M. N., S. N. Luoma, and A. R. Stewart. 2005. Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnology and Oceanography **50**:1511-1519.

Crotty, F. V., R. P. Blackshaw, S. M. Adl, R. Inger, and P. J. Murray. 2014. Divergence of feeding channels within the soil food web determined by ecosystem type. Ecology and Evolution **4**:1-13.

Da Silva, D. S., M. Lucotte, M. Roulet, H. Poirier, D. Mergler, E. O. Santos, and M. Crossa. 2005. Trophic structure and bioaccumulation of mercury in fish of three natural lakes of the Brazilian Amazon. Water, Air, and Soil Pollution **165**:77-94.

Dalponti, G., R. D. Guariento, and A. Caliman. 2018. Hunting high or low: body size drives trophic position among and within marine predators. Marine Ecology Progress Series **597**:39-46.

Dalu, T., R. J. Wasserman, P. W. Froneman, and O. L. Weyl. 2017. Trophic isotopic carbon variation increases with pond's hydroperiod: evidence from an Austral ephemeral ecosystem. Scientific Reports **7**:7572.

De Visser, S. N., B. P. Freymann, and H. Schnyder. 2008. Trophic interactions among invertebrates in termitaria in the African savanna: a stable isotope approach. Ecological Entomology **33**:758-764.

deHart, P. A., and S. E. Strand. 2012. Effects of garlic mustard invasion on arthropod diets as revealed through stable-isotope analyses. Southeastern Naturalist **11**:575-589.

Des Roches, S., L. J. Harmon, and E. B. Rosenblum. 2016. Colonization of a novel depauperate habitat leads to trophic niche shifts in three desert lizard species. Oikos **125**:343-353.

Dharampal, P. S., and R. H. Findlay. 2017. Mercury levels in largemouth bass (*Micropterus salmoides*) from regulated and unregulated rivers. Chemosphere **170**:134-140.

Di Beneditto, A. P. M., V. T. Bittar, P. B. Camargo, C. E. Rezende, and H. A. Kehrig. 2012. Mercury and nitrogen isotope in a marine species from a tropical coastal food web. Archives of Environmental Contamination and Toxicology **62**:264-271.

Dominik, J., D. Tagliapietra, A. Bravo, M. Sigovini, J. Spangenberg, D. Amouroux, and R. Zonta. 2014. Mercury in the food chain of the Lagoon of Venice, Italy. Marine Pollution Bulletin **88**:194-206.

Dörner, H., C. Skov, S. Berg, T. Schulze, D. Beare, and G. Van der Velde. 2009. Piscivory and trophic position of *Anguilla anguilla* in two lakes: importance of macrozoobenthos density. Journal of Fish Biology **74**:2115-2131.

Doubek, J. P., and J. T. Lehman. 2014. Historical trophic position of *Limnocalanus macrurus* in Lake Michigan. Journal of Great Lakes Research **40**:1027-1032.

Duyck, P.-F., A. Lavigne, F. Vinatier, R. Achard, J. Okolle, and P. Tixier. 2011. Addition of a new resource in agroecosystems: do cover crops alter the trophic positions of generalist predators? Basic and Applied Ecology **12**:47-55.

East, J. L., C. Wilcut, and A. A. Pease. 2017. Aquatic food-web structure along a salinized dryland river. Freshwater Biology **62**:681-694.

Edwards, D. P., P. Woodcock, R. J. Newton, F. A. Edwards, D. J. Andrews, T. D. Docherty, S. L. Mitchell, T. Ota, S. Benedick, S. H. Bottrell, and K. C. Hamer. 2013. Trophic flexibility and the persistence of understory birds in intensively logged rainforest. Conservation Biology **27**:1079-1086.

Elliott, K. H., and J. E. Elliott. 2016. Origin of sulfur in diet drives spatial and temporal mercury trends in seabird eggs from Pacific Canada 1968–2015. Environmental Science & Technology **50**:13380-13386.

Erdmann, G., V. Otte, R. Langel, S. Scheu, and M. Maraun. 2007. The trophic structure of bark-living oribatid mite communities analysed with stable isotopes (^{15}N, ^{13}C) indicates strong niche differentiation. Experimental and Applied Acarology **41**:1-10.

Fanelli, E., E. Azzurro, M. Bariche, J. E. Cartes, and F. Maynou. 2015. Depicting the novel Eastern Mediterranean food web: a stable isotopes study following Lessepsian fish invasion. Biological Invasions **17**:2163-2178.

Faye, D., L. Tito de Morais, J. Raffray, O. Sadio, O. Thiaw, F. Le-Loch, and L. de Morais. 2011. Structure and seasonal variability of fish food webs in an estuarine tropical marine protected area (Senegal): Evidence from stable isotope analysis. Estuarine, Coastal and Shelf Science **92**:607-617.

Ferlian, O., and S. Scheu. 2014. Shifts in trophic interactions with forest type in soil generalist predators as indicated by complementary analyses of fatty acids and stable isotopes. Oikos **123**:1182-1191.

Fetcher, N., S. J. Agosta, J. C. Moore, J. A. Stratford, and M. A. Steele. 2015. The food web of a severely contaminated site following reclamation with warm season grasses. Restoration Ecology **23**:421-429.

Fiedler, K., F. Kuhlmann, B. Schlick-Steiner, F. Steiner, and G. Gebauer. 2007. Stable N-isotope signatures of central European ants–assessing positions in a trophic gradient. Insectes Sociaux **54**:393-402.

Fiera, C. 2014. Application of stable isotopes and lipid analysis to understand trophic interactions in springtails. North-Western Journal of Zoology **10**: 227-235.

Fisk, A. T., K. A. Hobson, and R. J. Norstrom. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. Environmental Science & Technology **35**:732-738.

Franca, S., S. França, R. Vasconcelos, S. Tanner, C. Máguas, M. Costa, H. Cabral, and C. Maguas. 2011. Assessing food web dynamics and relative importance of organic matter sources for fish species in two Portuguese estuaries: A stable isotope approach. Marine Environmental Research **72**:204-215.

France, R. 1996. Absence or masking of metabolic fractionations of ^{13}C in a freshwater benthic food web. Freshwater Biology **36**:1-6.

France, R. 2014. Rebuilding and comparing pyramids of numbers (Elton) and energy (Lindeman) with selected global $\delta^{15}N$ data. Hydrobiologia **722**:1-7.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnology and Oceanography **33**:1182-1190.

Gaines, K. F., C. S. Romanek, C. S. Boring, C. G. Lord, M. Gochfeld, and J. Burger. 2002. Using raccoons as an indicator species for metal accumulation across trophic levels: a stable isotope approach. The Journal of Wildlife Management **66**:811-821.

Gan, H., D. R. Zak, and M. D. Hunter. 2014. Trophic stability of soil oribatid mites in the face of environmental change. Soil Biology and Biochemistry **68**:71-77.

Gantner, N., M. Power, D. Iqaluk, M. Meili, H. Borg, M. Sundbom, K. R. Solomon, G. Lawson, and D. C. Muir. 2010. Mercury concentrations in landlocked Arctic char (*Salvelinus alpinus*) from the Canadian Arctic. Part I: insights from trophic relationships in 18 lakes. Environmental Toxicology and Chemistry: An International Journal **29**:621-632.

Gewurtz, S. B., N. Gandhi, G. A. Stern, W. G. Franzin, B. Rosenberg, and M. L. Diamond. 2006. Dynamics of PCBs in the food web of Lake Winnipeg. Journal of Great Lakes Research **32**:712-727.

Gibb, H., and S. A. Cunningham. 2011. Habitat contrasts reveal a shift in the trophic position of ant assemblages. Journal of Animal Ecology **80**:119-127.

Gibb, H., J. Stoklosa, D. I. Warton, A. M. Brown, N. R. Andrew, and S. A. Cunningham. 2015. Does morphology predict trophic position and habitat use of ant species and assemblages? Oecologia **177**:519-531.

Gómez-Díaz, E., and J. González-Solís. 2010. Trophic structure in a seabird host-parasite food web: insights from stable isotope analyses. PLoS One **5**:e10454.

Goncharov, A., E. Y. Khramova, and A. Tiunov. 2014. Spatial variations in the trophic structure of soil animal communities in boreal forests of Pechora-Ilych Nature Reserve. Eurasian Soil Science **47**:441-448.

Goncharov, A. A., M. Zalewski, D. Dudek-Godeau, J.-F. Godeau, P. Sienkiewicz, W. Ulrich, and A. V. Tiunov. 2015. Sex-related variation in δ15N values of ground beetles (Coleoptera, Carabidae): a case study. Pedobiologia **58**:147-151.

González-Bergonzoni, I., F. Landkildehus, M. Meerhoff, T. L. Lauridsen, K. Özkan, T. A. Davidson, N. Mazzeo, and E. Jeppesen. 2014. Fish determine macroinvertebrate food webs and assemblage structure in Greenland subarctic streams. Freshwater Biology **59**:1830-1842.

Gorokhova, E., S. Hansson, H. Höglander, and C. M. Andersen. 2005. Stable isotopes show food web changes after invasion by the predatory cladoceran *Cercopagis pengoi* in a Baltic Sea bay. Oecologia **143**:251-259.

Guzzo, M. M., G. D. Haffner, S. Sorge, S. A. Rush, and A. T. Fisk. 2011. Spatial and temporal variabilities of $\delta^{13}C$ and $\delta^{15}N$ within lower trophic levels of a large lake: implications for estimating trophic relationships of consumers. Hydrobiologia **675**:41-53.

Hallanger, I. G., N. A. Warner, A. Ruus, A. Evenset, G. Christensen, D. Herzke, G. W. Gabrielsen, and K. Borgå. 2011. Seasonality in contaminant accumulation in Arctic marine pelagic food webs using trophic magnification factor as a measure of bioaccumulation. Environmental Toxicology and Chemistry **30**:1026-1035.

Hamer, K. C., R. J. Newton, F. A. Edwards, S. Benedick, S. H. Bottrell, and D. P. Edwards. 2015. Impacts of selective logging on insectivorous birds in Borneo: the importance of trophic position, body size and foraging height. Biological Conservation **188**:82-88.

Haynert, K., M. Kiggen, B. Klarner, M. Maraun, and S. Scheu. 2017. The structure of salt marsh soil mesofauna food webs–The prevalence of disturbance. PLoS One **12**:e0189645.

Hebert, C. E., D. C. Weseloh, L. T. Gauthier, M. T. Arts, and R. J. Letcher. 2009. Biochemical tracers reveal intra-specific differences in the food webs utilized by individual seabirds. Oecologia **160**:15-23.

Hellmann, C., B. Wissel, and C. Winkelmann. 2013. Omnivores as seasonally important predators in a stream food web. Freshwater Science **32**:548-562.

Hertz, E., J. P. Robinson, M. Trudel, A. Mazumder, and J. K. Baum. 2014. Estimation of predator-prey mass ratios using stable isotopes: sources of errors. Marine Ecology Progress Series **516**:1-6.

Hette-Tronquart, N., T. Oberdorff, E. Tales, A. Zahm, and J. Belliard. 2018. Biological impacts of local vs. regional land use on a small tributary of the Seine River (France): insights from a food web approach based on stable isotopes. Environmental Science and Pollution Research **25**:23583-23594.

Hobson, K., A. Fisk, N. Karnovsky, M. Holst, J.-M. Gagnon, and M. Fortier. 2002. A stable isotope ($\delta^{13}C$, $\delta^{15}N$) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. Deep Sea Research Part II: Topical Studies in Oceanography **49**:5131-5150.

Hobson, K. A., and H. E. Welch. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series **84**:9-18.

Hoeinghaus, D. J., K. O. Winemiller, and A. A. Agostinho. 2008. Hydrogeomorphology and river impoundment affect food-chain length of diverse Neotropical food webs. Oikos **117**:984-995.

Hoekstra, P., T. O'hara, A. Fisk, K. Borgå, K. R. Solomon, and D. Muir. 2003. Trophic transfer of persistent organochlorine contaminants (OCs) within an Arctic marine food web from the southern Beaufort–Chukchi Seas. Environmental Pollution **124**:509-522.

Hurd, L. E., P. A. Dehart, J. M. Taylor, M. C. Campbell, and M. M. Shearer. 2015. The ontogenetically variable trophic niche of a praying mantid revealed by stable isotope analysis. Environmental Entomology **44**:239-245.

Hyodo, F., T. Matsumoto, Y. Takematsu, T. Kamoi, D. Fukuda, M. Nakagawa, and T. Itioka. 2010. The structure of a food web in a tropical rain forest in Malaysia based on carbon and nitrogen stable isotope ratios. Journal of Tropical Ecology **26**:205-214.

Iglesias, C., M. Meerhoff, L. S. Johansson, I. González-Bergonzoni, N. Mazzeo, J. P. Pacheco, F. Teixeira-de Mello, G. Goyenola, T. L. Lauridsen, M. Søndergaard, T.A. Davidson, and E. Jeppesen. 2017. Stable isotope analysis confirms substantial differences between subtropical and temperate shallow lake food webs. Hydrobiologia **784**:111-123.

Iken, K., B. Bluhm, and K. Dunton. 2010. Benthic food-web structure under differing water mass properties in the southern Chukchi Sea. Deep Sea Research Part II: Topical Studies in Oceanography **57**:71-85.

Illig, J., R. Langel, R. A. Norton, S. Scheu, and M. Maraun. 2005. Where are the decomposers? Uncovering the soil food web of a tropical montane rain forest in southern Ecuador using stable isotopes (^{15}N). Journal of Tropical Ecology **21**:589-593.

Ingimarsdóttir, M., A. Michelsen, J. Ripa, and K. Hedlund. 2014. Food sources of early colonising arthropods: the importance of allochthonous input. Pedobiologia **57**:21-26.

Ishikawa, N., Y. Kato, H. Togashi, M. Yoshimura, C. Yoshimizu, N. Okuda, and I. Tayasu. 2014. Stable nitrogen isotopic composition of amino acids reveals food web structure in stream ecosystems. Oecologia **175**:911-922.

Jassey, V. E., C. Meyer, C. Dupuy, N. Bernard, E. A. Mitchell, M.-L. Toussaint, M. Metian, A. P. Chatelain, and D. Gilbert. 2013. To what extent do food preferences explain the trophic position of heterotrophic and mixotrophic microbial consumers in a *Sphagnum* peatland? Microbial Ecology **66**:571-580.

Jepsen, D. B., and K. O. Winemiller. 2002. Structure of tropical river food webs revealed by stable isotope ratios. Oikos **96**:46-55.

Jones, H., K. Swadling, E. Butler, L. Barry, and C. Macleod. 2014. Application of stable isotope mixing models for defining trophic biomagnification pathways of mercury and selenium. Limnology and Oceanography **59**:1181-1192.

Kato, Y., M. Kondoh, N. F. Ishikawa, H. Togashi, Y. Kohmatsu, M. Yoshimura, C. Yoshimizu, T. F. Haraguchi, Y. Osada, and N. Ohte. 2018. Using food network unfolding to evaluate food–web complexity in terms of biodiversity: theory and applications. Ecology Letters **21**:1065-1074.

Kennedy, S. R., T. E. Dawson, and R. G. Gillespie. 2018. Stable isotopes of Hawaiian spiders reflect substrate properties along a chronosequence. PeerJ **6**:e4527.

Kidd, K. A., D. Schindler, R. H. Hesslein, and D. Muir. 1995. Correlation between stable nitrogen isotope ratios and concentrations of organochlorines in biota from a freshwater food web. Science of The Total Environment **160**:381-390.

Kim, T., S. Bartel, and C. Gratton. 2019. Grassland harvesting alters ant community trophic structure: an isotopic study in tallgrass prairies. Ecology and Evolution **9**:9815-9826.

Klarner, B., R. B. Ehnes, G. Erdmann, B. Eitzinger, M. M. Pollierer, M. Maraun, and S. Scheu. 2014. Trophic shift of soil animal species with forest type as indicated by stable isotope analysis. Oikos **123**:1173-1181.

Kohzu, A., T. Iwata, M. Kato, J. Nishikawa, E. Wada, N. Amartuvshin, B. Namkhaidorj, and N. Fujita. 2009. Food webs in Mongolian grasslands: the analysis of ^{13}C and ^{15}N natural abundances. Isotopes in Environmental and Health Studies **45**:208-219.

König, T., R. Kaufmann, and S. Scheu. 2011. The formation of terrestrial food webs in glacier foreland: evidence for the pivotal role of decomposer prey and intraguild predation. Pedobiologia **54**:147-152.

Kopp, D., S. Lefebvre, M. Cachera, M. C. Villanueva, and B. Ernande. 2015. Reorganization of a marine trophic network along an inshore–offshore gradient due to stronger pelagic–benthic coupling in coastal areas. Progress in Oceanography **130**:157-171.

Korobushkin, D. I., K. B. Gongalsky, and A. V. Tiunov. 2014. Isotopic niche ($\delta^{13}C$ and $\delta^{15}N$ values) of soil macrofauna in temperate forests. Rapid Communications in Mass Spectrometry **28**:1303-1311.

Korotkevich, A. Y., A. M. Potapov, A. V. Tiunov, and N. A. Kuznetsova. 2018. Collapse of trophic-niche structure in belowground communities under anthropogenic disturbance. Ecosphere **9**:e02528.

Kovalenko, K. E., and E. D. Dibble. 2011. Effects of invasive macrophyte on trophic diversity and position of secondary consumers. Hydrobiologia **663**:167-173.

Kruse, S., E. A. Pakhomov, B. P. Hunt, Y. Chikaraishi, N. O. Ogawa, and U. Bathmann. 2015. Uncovering the trophic relationship between *Themisto gaudichaudii* and *Salpa thompsoni* in the Antarctic Polar Frontal Zone. Marine Ecology Progress Series **529**:63-74.

Kudrin, A. A., S. M. Tsurikov, and A. V. Tiunov. 2015. Trophic position of microbivorous and predatory soil nematodes in a boreal forest as indicated by stable isotope analysis. Soil Biology and Biochemistry **86**:193-200.

Lach, L., C. V. Tillberg, and A. V. Suarez. 2010. Contrasting effects of an invasive ant on a native and an invasive plant. Biological Invasions **12**:3123-3133.

Ladygina, N., T. Caruso, and K. Hedlund. 2008. Dietary switching of collembola in grassland soil food webs. Soil Biology & Biochemistry **40**:2898-2903.

Lagerlöf, J., C. Maribie, and J. M. John. 2017. Trophic interactions among soil arthropods in contrasting land-use systems in Kenya, studied with stable isotopes. European Journal of Soil Biology **79**:31-39.

Lavoie, R. A., C. E. Hebert, J.-F. Rail, B. M. Braune, E. Yumvihoze, L. G. Hill, and D. R. Lean. 2010. Trophic structure and mercury distribution in a Gulf of St. Lawrence (Canada) food web using stable isotope analysis. Science of the Total Environment **408**:5529-5539.

Layman, C. A., D. A. Arrington, C. G. Montaña, and D. M. Post. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology **88**:42-48.

Layman, C. A., K. O. Winemiller, D. A. Arrington, and D. B. Jepsen. 2005. Body size and trophic position in a diverse tropical food web. Ecology **86**:2530-2535.

Le Bourg, B., Y. Letourneur, D. Bănaru, J. Blanchot, C. Chevalier, G. Mou-Tham, B. Lebreton, and M. Pagano. 2018. The same but different: stable isotopes reveal two distinguishable, yet similar, neighbouring food chains in a coral reef. Journal of the Marine Biological Association of the United Kingdom **98**:1589-1597.

Lorrain, A., B. S. Graham, B. N. Popp, V. Allain, R. J. Olson, B. P. Hunt, M. Potier, B. Fry, F. Galván-Magaña, and C. E. Menkes. 2015. Nitrogen isotopic baselines and implications for estimating foraging habitat and trophic position of yellowfin tuna in the Indian and Pacific Oceans. Deep Sea Research Part II: Topical Studies in Oceanography **113**:188-198.

Lucey, J. M., N. Tawatao, M. J. Senior, V. K. Chey, S. Benedick, K. C. Hamer, P. Woodcock, R. J. Newton, S. H. Bottrell, and J. K. Hill. 2014. Tropical forest fragments contribute to species richness in adjacent oil palm plantations. Biological Conservation **169**:268-276.

Marty, J., K. Smokorowski, and M. Power. 2009. The influence of fluctuating ramping rates on the food web of boreal rivers. River Research and Applications **25**:962-974.

Matich, P., M. R. Heithaus, and C. A. Layman. 2011. Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. Journal of Animal Ecology **80**:294-305.

McClelland, J. W., and J. P. Montoya. 2002. Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. Ecology **83**:2173-2180.

McHugh, P. A., A. R. McIntosh, and P. G. Jellyman. 2010. Dual influences of ecosystem size and disturbance on food chain length in streams. Ecology Letters **13**:881-890.

McKinney, M. A., B. C. McMeans, G. T. Tomy, B. Rosenberg, S. H. Ferguson, A. Morris, D. C. Muir, and A. T. Fisk. 2012. Trophic transfer of contaminants in a changing arctic marine food web: Cumberland Sound, Nunavut, Canada. Environmental Science & Technology **46**:9914-9922.

McMeans, B. C., M. T. Arts, and A. T. Fisk. 2015. Impacts of food web structure and feeding behavior on mercury exposure in Greenland Sharks (*Somniosus microcephalus*). Science of the Total Environment **509**:216-225.

McMullin, R. M., S. R. Wing, L. C. Wing, and O. A. Shatova. 2017. Trophic position of Antarctic ice fishes reflects food web structure along a gradient in sea ice persistence. Marine Ecology Progress Series **564**:87-98.

McNabb, D. M., J. Halaj, and D. H. Wise. 2001. Inferring trophic positions of generalist predators and their linkage to the detrital food web in agroecosystems: a stable isotope analysis. Pedobiologia **45**:289-297.

McParland, C., C. Paszkowski, and J. Newbrey. 2010. Trophic relationships of breeding Red-necked Grebes (*Podiceps grisegena*) on wetlands with and without fish in the Aspen Parkland. Canadian Journal of Zoology **88**:186-194.

Menke, S. B., A. V. Suarez, C. V. Tillberg, C. T. Chou, and D. A. Holway. 2010. Trophic ecology of the invasive argentine ant: spatio-temporal variation in resource assimilation and isotopic enrichment. Oecologia **164**:763-771.

Mestre, L., J. Piñol, J. A. Barrientos, X. Espadaler, K. Brewitt, C. Werner, and C. Platner. 2013. Trophic structure of the spider community of a Mediterranean citrus grove: a stable isotope analysis. Basic and Applied Ecology **14**:413-422.

Milessi, A. C., C. Danilo, R.-G. Laura, C. Daniel, S. Javier, and L. Rodríguez-Gallego. 2010. Trophic mass-balance model of a subtropical coastal lagoon, including a comparison with a stable isotope analysis of the food-web. Ecological Modelling **221**:2859-2869.

Miyachi, S., T. Mayahara, K. Tsushima, K. Sasada, E. Kohno, N. O. Ogawa, Y. Chikaraishi, and N. Ohkouchi. 2015. Approach to determine individual trophic level and the difference in food sources of Japanese anchovy *Engraulis japonicus* in Sagami Bay, based on compound-specific nitrogen stable isotope analysis of amino acids. Fisheries Science **81**:1053-1062.

Molina, C. I., F.-M. Gibon, T. Oberdorff, E. Dominguez, J. Pinto, R. Marín, and M. Roulet. 2011. Macroinvertebrate food web structure in a floodplain lake of the Bolivian Amazon. Hydrobiologia **663**:135-153.

Molina-Burgos, B. E., A. Valenzuela-Sánchez, M. Alvarado-Rybak, S. Klarian, and C. Soto-Azat. 2018. Trophic ecology of the Endangered Darwin's frog inferred by stable isotopes. Endangered Species Research **36**:269-278.

Mooney, K. A., and C. V. Tillberg. 2005. Temporal and spatial variation to ant omnivory in pine forests. Ecology **86**:1225-1235.

Mothapo, N. P., and T. C. Wossler. 2016. Patterns of floral resource use by two dominant ant species in a biodiversity hotspot. Biological Invasions:1-15.

Ng, E. L., J. P. Fredericks, and M. C. Quist. 2017. Stable isotope evaluation of population- and individual-level diet variability in a large, oligotrophic lake with non-native lake trout. Ecology of Freshwater Fish **26**:271-279.

Nordström, M., K. Aarnio, and E. Bonsdorff. 2009. Temporal variability of a benthic food web: patterns and processes in a low-diversity system. Marine Ecology Progress Series **378**:13-26.

Oelbermann, K., and S. Scheu. 2010. Trophic guilds of generalist feeders in soil animal communities as indicated by stable isotope analysis ($^{15}N/^{14}N$). Bulletin of Entomological Research **100**:511-520.

Ohshimo, S., H. Tanaka, K. Nishiuchi, and T. Yasuda. 2016. Trophic positions and predator–prey mass ratio of the pelagic food web in the East China Sea and Sea of Japan. Marine and Freshwater Research **67**:1692-1699.

Okuzaki, Y., I. Tayasu, N. Okuda, and T. Sota. 2009. Vertical heterogeneity of a forest floor invertebrate food web as indicated by stable-isotope analysis. Ecological Research **24**:1351-1359.

Okuzaki, Y., I. Tayasu, N. Okuda, and T. Sota. 2010. Stable isotope analysis indicates trophic differences among forest floor carabids in Japan. Entomologia Experimentalis et Applicata **135**:263-270.

Ou, C., C. G. Montaña, and K. O. Winemiller. 2017. Body size–trophic position relationships among fishes of the lower Mekong basin. Royal Society Open Science **4**:160645.

Page, H. M., A. J. Brooks, M. Kulbicki, R. Galzin, R. J. Miller, D. C. Reed, R. J. Schmitt, S. J. Holbrook, and C. Koenigs. 2013. Stable isotopes reveal trophic relationships and diet of consumers in temperate kelp forest and coral reef ecosystems. Oceanography **26**:180-189.

Pasquaud, S., M. Pillet, V. David, B. Sautour, and P. Elie. 2010. Determination of fish trophic levels in an estuarine system. Estuarine, Coastal and Shelf Science **86**:237-246.

Perdomo, G., A. Evans, M. Maraun, P. Sunnucks, and R. Thompson. 2012. Mouthpart morphology and trophic position of microarthropods from soils and mosses are strongly correlated. Soil Biology and Biochemistry **53**:56-63.

Pereira, A., E. Benedito, and C. Sakuragui. 2007. Spatial variation in the stable isotopes of ^{13}C and ^{15}N and trophic position of *Leporinus friderici* (Characiformes, Anostomidae) in Corumbá Reservoir, Brazil. Anais da Academia Brasileira de Ciências **79**:41-49.

Perkins, M., R. Inger, S. Bearhop, and D. Sanders. 2018. Multichannel feeding by spider functional groups is driven by feeding strategies and resource availability. Oikos **127**:23-33.

Persic, A., H. Roche, and F. Ramade. 2004. Stable carbon and nitrogen isotope quantitative structural assessment of dominant species from the Vaccarès Lagoon trophic web (Camargue Biosphere Reserve, France). Estuarine, Coastal and Shelf Science **60**:261-272.

Pethybridge, H., E. Butler, D. Cossa, R. Daley, and A. Boudou. 2012. Trophic structure and biomagnification of mercury in an assemblage of deepwater chondrichthyans from southeastern Australia. Marine Ecology Progress Series **451**:163-174.

Pfeiffer, M., D. Mezger, and J. Dyckmans. 2014. Trophic ecology of tropical leaf litter ants (Hymenoptera: Formicidae)–a stable isotope study in four types of Bornean rain forest. Myrmecological News **19**:31-41.

Platner, C., J. Pinol, D. Sanders, and X. Espadaler. 2012. Trophic diversity in a Mediterranean food web—stable isotope analysis of an ant community of an organic citrus grove. Basic and Applied Ecology **13**:587-596.

Pollierer, M., R. Langel, S. Scheu, and M. Maraun. 2009. Compartmentalization of the soil animal food web as indicated by dual analysis of stable isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$). Soil Biology & Biochemistry **41**:1221-1226.

Ponsard, S., and R. Arditi. 2000. What can stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) tell about the food web of soil micro-invertebrates? Ecology **81**:852-864.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**:703-718.

Poste, A. E., D. C. Muir, D. Mbabazi, and R. E. Hecky. 2012. Food web structure and mercury trophodynamics in two contrasting embayments in northern Lake Victoria. Journal of Great Lakes Research **38**:699-707.

Potapov, A. M., A. V. Tiunov, S. Scheu, T. Larsen, and M. M. Pollierer. 2019. Combining bulk and amino acid stable isotope analyses to quantify trophic level and basal resources of detritivores: a case study on earthworms. Oecologia **189**:447-460.

Protopapa, M., R. Koppelmann, S. Zervoudaki, C. Wunsch, J. Peters, C. Parinos, F. Paraschos, A. Gogou, and C. Möllmann. 2019. Trophic positioning of prominent copepods in the epi-and mesopelagic zone of the ultra-oligotrophic eastern Mediterranean Sea. Deep Sea Research Part II: Topical Studies in Oceanography **164**: 144-155.

Qiu, G., K. S. Abeysinghe, X.-D. Yang, Z. Xu, X. Xu, K. Luo, and E. Goodale. 2019. Effects of selenium on mercury bioaccumulation in a terrestrial food chain from an abandoned mercury mining region. Bulletin of Environmental Contamination and Toxicology **102**:329-334.

Quevedo, M., R. Svanbäck, and P. Eklöv. 2009. Intrapopulation niche partitioning in a generalist predator limits food web connectivity. Ecology **90**:2263-2274.

Quinn, M. R., X. Feng, C. L. Folt, and C. P. Chamberlain. 2003. Analyzing trophic transfer of metals in stream food webs using nitrogen isotopes. Science of the Total Environment **317**:73-89.

Quiroga, E., D. Gerdes, A. Montiel, R. Knust, and U. Jacob. 2014. Normalized biomass size spectra in high Antarctic macrobenthic communities: linking trophic position and body size. Marine Ecology Progress Series **506**:99-113.

Reid, M., M. Delong, and M. Thoms. 2012. The influence of hydrological connectivity on food web structure in floodplain lakes. River Research and Applications **28**:827-844.

Reis, P. C., L. A. Martinelli, and F. A. Barbosa. 2016. Basal carbon sources and planktonic food web in a tropical lake: an isotopic approach. Marine and Freshwater Research **68**:429-441.

Renaud, P. E., M. Tessmann, A. Evenset, and G. N. Christensen. 2011. Benthic food-web structure of an Arctic fjord (Kongsfjorden, Svalbard). Marine Biology Research **7**:13-26.

Resasco, J., D. J. Levey, and E. I. Damschen. 2012. Habitat corridors alter relative trophic position of fire ants. Ecosphere **3**:art100.

Resasco, J., K. Tuff, S. Cunningham, B. Melbourne, A. Hicks, S. Newsome, and K. Davies. 2018. Generalist predator's niche shifts reveal ecosystem changes in an experimentally fragmented landscape. Ecography **41**:1209-1219.

Reum, J. C., and K. N. Marshall. 2013. Evaluating $\delta^{15}N$–body size relationships across taxonomic levels using hierarchical models. Oecologia **173**:1159-1168.

Roach, K. A., J. H. Thorp, and M. D. Delong. 2009. Influence of lateral gradients of hydrologic connectivity on trophic positions of fishes in the Upper Mississippi River. Freshwater Biology **54**:607-620.

Robinson, J. P. W., and J. Baum. 2016. Trophic roles determine coral reef fish community size structure. Canadian Journal of Fisheries and Aquatic Sciences **73**:496-505.

Rowell, K., D. L. Dettman, and R. Dietz. 2010. Nitrogen isotopes in otoliths reconstruct ancient trophic position. Environmental Biology of Fishes **89**:415-425.

Ruus, A., K. I. Ugland, and J. U. Skaare. 2002. Influence of trophic position on organochlorine concentrations and compositional patterns in a marine food web. Environmental Toxicology and Chemistry: An International Journal **21**:2356-2364.

Sabo, J. L., M. Caron, R. Doucett, K. L. Dibble, A. Ruhi, J. C. Marks, B. A. Hungate, and T. A. Kennedy. 2018. Pulsed flows, tributary inputs and food-web structure in a highly regulated river. Journal of Applied Ecology **55**:1884-1895.

Saigo, M., L. Ruffener, P. Scarabotti, and M. Marchese. 2017. Food chain length in a large floodplain river: planktonic or benthic reliance as a limiting factor. Marine and Freshwater Research **68**:1336-1341.

Saigo, M., F. Zilli, M. Marchese, and D. Demonte. 2015. Trophic level, food chain length and omnivory in the Paraná River: a food web model approach in a floodplain river system. Ecological Research **30**:843-852.

Sampedro, L., and J. Domínguez. 2008. Stable isotope natural abundances ($\delta^{13}C$ and $\delta^{15}N$) of the earthworm *Eisenia fetida* and other soil fauna living in two different vermicomposting environments. Applied Soil Ecology **38**:91-99.

Sanders, D., and C. Platner. 2007. Intraguild interactions between spiders and ants and top-down control in a grassland food web. Oecologia **150**:611-624.

Sardenne, F., S. Hollanda, S. Lawrence, R. Albert-Arrisol, M. Degroote, and N. Bodin. 2017. Trophic structures in tropical marine ecosystems: a comparative

investigation using three different ecological tracers. Ecological Indicators **81**:315-324.

Scheunemann, N., C. Digel, S. Scheu, and O. Butenschoen. 2015. Roots rather than shoot residues drive soil arthropod communities of arable fields. Oecologia **179**:1135-1145.

Schmidt, O., J. P. Curry, J. Dyckmans, E. Rota, and C. M. Scrimgeour. 2004. Dual stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of soil invertebrates and their food sources. Pedobiologia **48**:171-180.

Schneider, K., S. Migge, R. A. Norton, S. Scheu, R. Langel, A. Reineking, and M. Maraun. 2004. Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios ($^{15}N/^{14}N$). Soil Biology and Biochemistry **36**:1769-1774.

Schneider, L., W. A. Maher, J. Potts, A. M. Taylor, G. E. Batley, F. Krikowa, A. A. Chariton, and B. Gruber. 2015. Modeling food web structure and selenium biomagnification in lake macquarie, New South Wales, Australia, using stable carbon and nitrogen isotopes. Environmental Toxicology and Chemistry **34**:608-617.

Smith, C. R., and A. V. Suarez. 2010. The trophic ecology of castes in harvester ant colonies. Functional Ecology **24**:122-130.

Sokołowski, A., M. Wołowicz, H. Asmus, R. Asmus, A. Carlier, Z. Gasiunaite, A. Grémare, H. Hummel, J. Lesutiené, and A. Razinkovas. 2012. Is benthic food web structure related to diversity of marine macrobenthic communities? Estuarine, Coastal and Shelf Science **108**:76-86.

Starrfelt, J., K. Borgå, A. Ruus, and E. Fjeld. 2013. Estimating trophic levels and trophic magnification factors using Bayesian inference. Environmental Science & Technology **47**:11599-11606.

Steffan, S. A., Y. Chikaraishi, D. R. Horton, N. Ohkouchi, M. E. Singleton, E. Miliczky, D. B. Hogg, and V. P. Jones. 2013. Trophic hierarchies illuminated via amino acid isotopic analysis. PLoS One **8**:e76152.

Stenroth, P., N. Holmqvist, P. Nyström, O. Berglund, P. Larsson, and W. Granéli. 2008. The influence of productivity and width of littoral zone on the trophic position of a large-bodied omnivore. Oecologia **156**:681-690.

Stowasser, G., A. Atkinson, R. McGill, R. Phillips, M. A. Collins, and D. Pond. 2012. Food web dynamics in the Scotia Sea in summer: a stable isotope study. Deep Sea Research Part II: Topical Studies in Oceanography **59**:208-221.

Svanbäck, R., M. Quevedo, J. Olsson, and P. Eklöv. 2015. Individuals in food webs: the relationships between trophic position, omnivory and among-individual diet variation. Oecologia **178**:103-114.

Syväranta, J., P. Högmander, T. Keskinen, J. Karjalainen, and R. I. Jones. 2011. Altered energy flow pathways in a lake ecosystem following manipulation of fish community structure. Aquatic Sciences **73**:79-89.

Takamura, K. 2009. Population structuring by weirs and the effect on trophic position of a freshwater fish *Zacco platypus* in the middle reaches of Japanese rivers. Fundamental and applied limnology **174**:307-315.

Takimoto, G., D. A. Spiller, and D. M. Post. 2008. Ecosystem size, but not disturbance, determines food-chain length on islands of the Bahamas. Ecology **89**:3001-3007.

Tillberg, C., D. Holway, E. LeBrun, and A. Suarez. 2007. Trophic ecology of invasive Argentine ants in their native and introduced ranges. Proceedings of the National Academy of Sciences of the United States of America **104**:20856-20861.

Tillberg, C. V., and M. D. Breed. 2004. Placing an omnivore in a complex food web: dietary contributions to adult biomass of an ant. Biotropica **36**:266-271.

Tillberg, C. V., B. Edmonds, A. Freauff, P. E. Hanisch, C. Paris, C. R. Smith, N. D. Tsutsui, B. D. Wills, S. E. Wittman, and A. V. Suarez. 2014. Foraging ecology of the tropical giant hunting ant *Dinoponera australis* (Hymenoptera: Formicidae)—evaluating mechanisms for high abundance. Biotropica **46**:229-237.

Traugott, M., N. Schallhart, R. Kaufmann, and A. Juen. 2008. The feeding ecology of elaterid larvae in central European arable land: new perspectives based on naturally occurring stable isotopes. Soil biology & biochemistry **40**:342-349.

Tsurikov, S. M., S. G. Ermilov, and A. V. Tiunov. 2019. Trophic structure of a tropical soil-and litter-dwelling oribatid mite community and consistency of trophic niches across biomes. Experimental and Applied Acarology **78**:29-48.

Urton, E. J., and K. A. Hobson. 2005. Intrapopulation variation in gray wolf isotope ($\delta^{15}N$ and $\delta^{13}C$) profiles: implications for the ecology of individuals. Oecologia **145**:316-325.

van der Merwe, J., and E. C. Hellgren. 2016. Spatial variation in trophic ecology of small mammals in wetlands: support for hydrological drivers. Ecosphere **7**:e01567.

Vander Zanden, J. M., and W. W. Fetzer. 2007. Global patterns of aquatic food chain length. Oikos **116**:1378-1388.

Vander Zanden, J. M., and J. B. Rasmussen. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology **80**:1395-1404.

Vander Zanden, M. J., and J. B. Rasmussen. 1996. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. Ecological Monographs **66**:451-477.

Vander Zanden, M. J., and J. B. Rasmussen. 2002. Food web perspectives on studies of bass populations in north-temperate lakes. Pages 173-184 *in* American Fisheries Society Symposium.

Vander Zanden, M. J., B. J. Shuter, N. Lester, and J. B. Rasmussen. 1999. Patterns of food chain length in lakes: a stable isotope study. The American Naturalist **154**:406-416.

Vinagre, C., C. Máguas, H. Cabral, and M. Costa. 2012. Food web structure of the coastal area adjacent to the Tagus estuary revealed by stable isotope analysis. Journal of Sea Research **67**:21-26.

Vinagre, C., V. Mendonça, L. Narciso, and C. Madeira. 2015. Food web of the intertidal rocky shore of the west Portuguese coast–determined by stable isotope analysis. Marine Environmental Research **110**:53-60.

Vizzini, S., G. Signa, and A. Mazzola. 2016. Guano-derived nutrient subsidies drive food web structure in coastal ponds. PLoS One **11**:e0151018.

Wang, Y., X. Xiao, X. Yu, J. Xu, Y. Cai, and G. Lei. 2016. Resource availability determines food chain length in Chinese subtropical rivers. Aquatic Ecology **50**:187-195.

Wilder, S., D. Holway, A. Suarez, E. LeBrun, and M. Eubanks. 2011. Intercontinental differences in resource use reveal the importance of mutualisms in fire ant invasions. Proceedings of the National Academy of Sciences of the United States of America **108**:20639-20644.

Winder, V. L., A. K. Michaelis, and S. D. Emslie. 2012. Understanding associations between nitrogen and carbon isotopes and mercury in three Ammodramus sparrows. Science of the Total Environment **419**:54-59.

Winemiller, K. O., S. Akin, and S. C. Zeug. 2007. Production sources and food web structure of a temperate tidal estuary: integration of dietary and stable isotope data. Marine Ecology Progress Series **343**:63-76.

Woodcock, P., D. P. Edwards, R. J. Newton, C. V. Khen, S. H. Bottrell, and K. C. Hamer. 2013. Impacts of intensive logging on the trophic organisation of ant communities in a biodiversity hotspot. PLoS One **8**:e60756.

Woodland, R. J., F. Y. Warry, V. Evrard, R. H. Clarke, P. Reich, and P. L. Cook. 2015. Niche-dependent trophic position distributions among primary, secondary and tertiary consumers. Oikos **125**:556-565.

Xu, J., Z. Wen, Z. Ke, M. Zhang, M. Zhang, N. Guo, L.-A. Hansson, and P. Xie. 2014. Contrasting energy pathways at the community level as a consequence of regime shifts. Oecologia **175**:231-241.

Zheng, Y., J. Niu, Q. Zhou, C. Xie, Z. Ke, D. Li, and Y. Gao. 2018. Effects of resource availability and hydrological regime on autochthonous and allochthonous carbon in the food web of a large cross-border river (China). Science of the Total Environment **612**:501-512.

Appendix A S8. Literature cited for published trophic positions.

Aresco, M. J., J. Travis, and P. S. MacRae. 2015. Trophic interactions of turtles in a north Florida lake food web: prevalence of omnivory. Copeia **103**:343-356.

Blévin, P., J. Aars, M. Andersen, M.-A. Blanchet, L. Hanssen, D. Herzke, R. M. Jeffreys, E. S. Nordøy, M. Pinzone, and C. de la Vega. 2019. Pelagic vs Coastal—Key Drivers of Pollutant Levels in Barents Sea Polar Bears with Contrasted Space-Use Strategies. Environmental Science & Technology **54**: 985-995.

Brown, T. M., A. T. Fisk, X. Wang, S. H. Ferguson, B. G. Young, K. J. Reimer, and D. C. Muir. 2016. Mercury and cadmium in ringed seals in the Canadian Arctic: influence of location and diet. Science of The Total Environment **545**:503-511.

Buckman, A. H., R. J. Norstrom, K. A. Hobson, N. J. Karnovsky, J. Duffe, and A. T. Fisk. 2004. Organochlorine contaminants in seven species of Arctic seabirds from northern Baffin Bay. Environmental Pollution **128**:327-338.

Cherel, Y., P. Bustamante, and P. Richard. 2019. Amino acid $\delta^{13}C$ and $\delta^{15}N$ from sclerotized beaks: a new tool to investigate the foraging ecology of cephalopods, including giant and colossal squids. Marine Ecology Progress Series **624**:89-102.

Chikaraishi, Y., N. O. Ogawa, H. Doi, and N. Ohkouchi. 2011. $^{15}N/^{14}N$ ratios of amino acids as a tool for studying terrestrial food webs: a case study of terrestrial insects (bees, wasps, and hornets). Ecological Research **26**:835-844.

Chumchal, M., R. Drenner, B. Fry, K. D. Hambright, and L. Newland. 2008. Habitat-specific differences in mercury concentration in a top predator from a shallow lake. Transactions of the American Fisheries Society **137**:195-208.

Costantini, M., P. Carlino, E. Calizza, G. Careddu, D. Cicala, S. Sporta Caputi, F. Fiorentino, and L. Rossi. 2018. The role of alien fish (the centrarchid *Micropterus salmoides*) in lake food webs highlighted by stable isotope analysis. Freshwater Biology **63**:1130-1142.

Cox, S. P., T. E. Essington, J. F. Kitchell, S. J. Martell, C. J. Walters, C. Boggs, and I. Kaplan. 2002. Reconstructing ecosystem dynamics in the central Pacific Ocean, 1952-1998. II. A preliminary assessment of the trophic impacts of fishing and effects on tuna dynamics. Canadian Journal of Fisheries and Aquatic Sciences **59**:1736-1747.

Cullon, D. L., M. B. Yunker, J. R. Christensen, R. W. Macdonald, M. J. Whiticar, N. J. Dangerfield, and P. S. Ross. 2012. Biomagnification of polychlorinated biphenyls in a harbor seal (*Phoca vitulina*) food web from the strait of Georgia, British Columbia, Canada. Environmental Toxicology and Chemistry **31**:2445-2455.

Das, K., G. Lepoint, Y. Leroy, and J.-M. Bouquegneau. 2003. Marine mammals from the southern North Sea: feeding ecology data from $\delta^{13}C$ and $\delta^{15}N$ measurements. Marine Ecology Progress Series **263**:287-298.

Dharampal, P. S., and R. H. Findlay. 2017. Mercury levels in largemouth bass (*Micropterus salmoides*) from regulated and unregulated rivers. Chemosphere **170**:134-140.

Dolson, R., K. McCann, N. Rooney, and M. Ridgway. 2009. Lake morphometry predicts the degree of habitat coupling by a mobile predator. Oikos **118**:1230-1238.

Eloranta, A. P., K. K. Kahilainen, P. A. Amundsen, R. Knudsen, C. Harrod, and R. I. Jones. 2015. Lake size and fish diversity determine resource use and trophic position of a top predator in high-latitude lakes. Ecology and Evolution **5**:1664-1675.

Feiner, Z., J. Rice, and D. D. Aday. 2013. Trophic niche of invasive white perch and potential interactions with representative reservoir species. Transactions of the American Fisheries Society **142**:628-641.

Franco, M., G. Guevara, L. Correa, and M. Soto-Gamboa. 2013. Trophic interactions of the endangered Southern river otter (*Lontra provocax*) in a Chilean Ramsar wetland inferred from prey sampling, fecal analysis, and stable isotopes. Naturwissenschaften **100**:299-310.

Franssen, N., and K. Gido. 2006. Use of stable isotopes to test literature-based trophic classifications of small-bodied stream fishes. The American Midland Naturalist **156**:1-10.

Fujibayashi, M., K. Okano, Y. Takada, H. Mizutani, N. Uchida, O. Nishimura, and N. Miyata. 2018. Transfer of cyanobacterial carbon to a higher trophic-level fish community in a eutrophic lake food web: fatty acid and stable isotope analyses. Oecologia **188**:901-912.

Gantner, N., M. Power, D. Iqaluk, M. Meili, H. Borg, M. Sundbom, K. R. Solomon, G. Lawson, and D. C. Muir. 2010. Mercury concentrations in landlocked Arctic char (*Salvelinus alpinus*) from the Canadian Arctic. Part I: insights from trophic relationships in 18 lakes. Environmental Toxicology and Chemistry: An International Journal **29**:621-632.

Germain, L. R., P. L. Koch, J. Harvey, and M. D. McCarthy. 2013. Nitrogen isotope fractionation in amino acids from harbor seals: implications for compound-specific trophic position calculations. Marine Ecology Progress Series **482**:265-277.

Germain, L. R., M. D. McCarthy, P. L. Koch, and J. T. Harvey. 2012. Stable carbon and nitrogen isotopes in multiple tissues of wild and captive harbor seals (*Phoca vitulina*) off the California coast. Marine Mammal Science **28**:542-560.

Gewurtz, S. B., N. Gandhi, G. A. Stern, W. G. Franzin, B. Rosenberg, and M. L. Diamond. 2006. Dynamics of PCBs in the food web of Lake Winnipeg. Journal of Great Lakes Research **32**:712-727.

Guzzo, M. M., G. D. Haffner, S. Sorge, S. A. Rush, and A. T. Fisk. 2011. Spatial and temporal variabilities of $\delta^{13}C$ and $\delta^{15}N$ within lower trophic levels of a large lake:

implications for estimating trophic relationships of consumers. Hydrobiologia **675**:41-53.

Hannides, C. C. S., B. Popp, M. Landry, and B. Graham. 2009. Quantification of zooplankton trophic position in the North Pacific Subtropical Gyre using stable nitrogen isotopes. Limnology and Oceanography **54**:50-61.

Haubrock, P. J., P. Balzani, M. Azzini, A. F. Inghilesi, L. Veselý, W. Guo, and E. Tricarico. 2019. Shared histories of co-evolution may affect trophic interactions in a freshwater community dominated by alien species. Frontiers in Ecology and Evolution **7**:355.

Hetherington, E. D., R. J. Olson, J. C. Drazen, C. E. Lennert-Cody, L. T. Ballance, R. S. Kaufmann, and B. N. Popp. 2017. Spatial foodweb structure in the eastern tropical Pacific Ocean based on compound-specific nitrogen isotope analysis of amino acids. Limnology and Oceanography **62**:541-560.

Hobson, K., A. Fisk, N. Karnovsky, M. Holst, J.-M. Gagnon, and M. Fortier. 2002a. A stable isotope ($\delta^{13}C$, $\delta^{15}N$) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. Deep Sea Research Part II: Topical Studies in Oceanography **49**:5131-5150.

Hobson, K., G. Gilchrist, and K. Falk. 2002b. Isotopic investigations of seabirds of the north water polynya: contrasting trophic relationships between the eastern and western sectors. The Condor **104**:1-11.

Hobson, K. A. 1993. Trophic relationships among high Arctic seabirds: insights from tissue-dependent stable-isotope models. Marine Ecology Progress Series **95**:7-18.

Hobson, K. A., W. G. Ambrose, and P. E. Renaud. 1995. Sources of primary production, benthic-pelagic coupling, and trophic relationships within the Northeast Water Polynya: insights from $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series **128**:1-10.

Hobson, K. A., and H. E. Welch. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series **84**:9-18.

Hoekstra, P., T. O'hara, A. Fisk, K. Borgå, K. R. Solomon, and D. Muir. 2003. Trophic transfer of persistent organochlorine contaminants (OCs) within an Arctic marine food web from the southern Beaufort–Chukchi Seas. Environmental Pollution **124**:509-522.

Houde, M., X. Wang, S. Ferguson, P. Gagnon, T. Brown, S. Tanabe, T. Kunito, M. Kwan, and D. Muir. 2017. Spatial and temporal trends of alternative flame retardants and polybrominated diphenyl ethers in ringed seals (*Phoca hispida*) across the Canadian Arctic. Environmental Pollution **223**:266-276.

Houssard, P., A. Lorrain, L. Tremblay-Boyer, V. Allain, B. S. Graham, C. E. Menkes, H. Pethybridge, L. I. Couturier, D. Point, and B. Leroy. 2017. Trophic position increases with thermocline depth in yellowfin and bigeye tuna across the Western and Central Pacific Ocean. Progress in Oceanography **154**:49-63.

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

Kainz, M., H. Hager, S. Rasconi, K. Kahilainen, P. A. Amundsen, and B. Hayden. 2017. Polyunsaturated fatty acids in fishes increase with total lipids irrespective of feeding sources and trophic position. Ecosphere **8**:e01753.

Karlsson, J., and P. Byström. 2005. Littoral energy mobilization dominates energy supply for top consumers in subarctic lakes. Limnology and Oceanography **50**:538-543.

Kelleway, J., D. Mazumder, G. G. Wilson, N. Saintilan, L. Knowles, J. Iles, and T. Kobayashi. 2010. Trophic structure of benthic resources and consumers varies across a regulated floodplain wetland. Marine and Freshwater Research **61**:430-440.

Kennedy, P. J., P. J. Blanchfield, K. A. Kidd, M. J. Paterson, C. L. Podemski, and M. D. Rennie. 2019. Changes in the condition, early growth, and trophic position of lake trout (*Salvelinus namaycush*) in response to an experimental aquaculture operation. Canadian Journal of Fisheries and Aquatic Sciences **76**:1376-1387.

Kiljunen, M., H. Peltonen, M. Lehtiniemi, L. Uusitalo, T. Sinisalo, J. Norkko, M. Kunnasranta, J. Torniainen, A. J. Rissanen, and J. Karjalainen. 2020. Benthic-pelagic coupling and trophic relationships in northern Baltic Sea food webs. Limnology and Oceanography.

Kovalenko, K. E., and E. D. Dibble. 2011. Effects of invasive macrophyte on trophic diversity and position of secondary consumers. Hydrobiologia **663**:167-173.

Kruger, B. R., J. P. Werne, D. K. Branstrator, T. R. Hrabik, Y. Chikaraishi, N. Ohkouchi, and E. C. Minor. 2016. Organic matter transfer in Lake Superior's food web: insights from bulk and molecular stable isotope and radiocarbon analyses. Limnology and Oceanography **61**:149-164.

Kürten, B., S. J. Painting, U. Struck, N. V. Polunin, and J. J. Middelburg. 2013. Tracking seasonal changes in North Sea zooplankton trophic dynamics using stable isotopes. Biogeochemistry **113**:167-187.

Lake, J., J. Serbst, A. Kuhn, N. Smucker, P. Edwards, A. Libby, M. Charpentier, and K. Miller. 2019. Use of stable isotopes in benthic organic material as a baseline for estimating fish trophic positions in lakes. Canadian Journal of Fisheries and Aquatic Sciences **76**:1227-1237.

Lepak, J., C. Kraft, B. Weidel, and B. C. Weldel. 2006. Rapid food web recovery in response to removal of an introduced apex predator. Canadian Journal of Fisheries and Aquatic Sciences **63**:569-575.

Lesage, V., M. O. Hammill, and K. M. Kovacs. 2001. Marine mammals and the community structure of the Estuary and Gulf of St Lawrence, Canada: evidence from stable isotope analysis. Marine Ecology Progress Series **210**:203-221.

Linnebjerg, J., K. Hobson, J. Fort, T. Nielsen, P. Møller, K. Wieland, E. Born, F. Rigét, A. Mosbech, and P. Moller. 2016. Deciphering the structure of the West Greenland marine food web using stable isotopes ($\delta^{13}C$, $\delta^{15}N$). Marine Biology **163**:230.

Logan, J. M., and M. E. Lutcavage. 2013. Assessment of trophic dynamics of cephalopods and large pelagic fishes in the central North Atlantic Ocean using stable isotope analysis. Deep Sea Research Part II: Topical Studies in Oceanography **95**:63-73.

Lorrain, A., B. S. Graham, B. N. Popp, V. Allain, R. J. Olson, B. P. Hunt, M. Potier, B. Fry, F. Galván-Magaña, and C. E. Menkes. 2015. Nitrogen isotopic baselines and implications for estimating foraging habitat and trophic position of yellowfin tuna in the Indian and Pacific Oceans. Deep Sea Research Part II: Topical Studies in Oceanography **113**:188-198.

Mazumder, D., M. Johansen, N. Saintilan, J. Iles, T. Kobayashi, L. Knowles, and L. Wen. 2012. trophic shifts involving native and exotic fish during hydrologic recession in floodplain wetlands. Wetlands **32**:267-275.

McKinney, M. A., B. C. McMeans, G. T. Tomy, B. Rosenberg, S. H. Ferguson, A. Morris, D. C. Muir, and A. T. Fisk. 2012. Trophic transfer of contaminants in a changing arctic marine food web: Cumberland Sound, Nunavut, Canada. Environmental Science & Technology **46**:9914-9922.

McMeans, B., M. Arts, C. Lydersen, K. Kovacs, H. Hop, S. Falk Petersen, and A. Fisk. 2013. The role of Greenland sharks (*Somniosus microcephalus*) in an Arctic

ecosystem: assessed via stable isotopes and fatty acids. Marine Biology **160**:1223-1238.

McMeans, B. C., M. T. Arts, and A. T. Fisk. 2015. Impacts of food web structure and feeding behavior on mercury exposure in Greenland Sharks (*Somniosus microcephalus*). Science of The Total Environment **509**:216-225.

Mercado Silva, N., M. Helmus, M. J. V. Zanden, and M. J. Vander Zanden. 2009. The effects of impoundment and non-native species on a river food web in Mexico's central plateau. River Research and Applications **25**:1090-1108.

Ng, E. L., J. P. Fredericks, and M. C. Quist. 2017. Stable isotope evaluation of population- and individual-level diet variability in a large, oligotrophic lake with non-native lake trout. Ecology of Freshwater Fish **26**:271-279.

Olson, R. J., and G. M. Watters. 2003. A model of the pelagic ecossystem in the eastern tropical Pacific Ocean. Inter-American Tropical Tuna Commission Bulletin **22**:135-218.

Øverjordet, I. B., G. W. Gabrielsen, T. Berg, A. Ruus, A. Evenset, K. Borgå, G. Christensen, S. Lierhagen, and B. M. Jenssen. 2015. Effect of diet, location and sampling year on bioaccumulation of mercury, selenium and cadmium in pelagic feeding seabirds in Svalbard. Chemosphere **122**:14-22.

Pauly, D., A. Trites, E. Capuli, and V. Christensen. 1998. Diet composition and trophic levels of marine mammals. ICES Journal of Marine Science **55**:467-481.

Pethybridge, H., C. A. Choy, J. Logan, V. Allain, A. Lorrain, N. Bodin, C. Somes, J. Young, F. Ménard, C. Langlais, L. Duffy, A. Hobday, P. Kuhnert, B. Fry, C. Menkes, and R. Olson. 2018. A global meta-analysis of marine predator nitrogen stable isotopes: Relationships between trophic structure and environmental conditions. Global Ecology and Biogeography **27**:1043-1055.

Pomerleau, C., M. Peter Heide-Jørgensen, S. H. Ferguson, H. L. Stern, J. L. Høyer, and G. A. Stern. 2017. Reconstructing variability in West Greenland ocean biogeochemistry and bowhead whale (*Balaena mysticetus*) food web structure using amino acid isotope ratios. Polar Biology **40**:2225-2238.

Popp, B. N., B. S. Graham, R. J. Olson, C. C. Hannides, M. J. Lott, G. A. López-Ibarra, F. Galván-Magaña, and B. Fry. 2007. Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids. Terrestrial Ecology **1**:173-190.

Ramírez-Herrejón, J., R. Moncayo-Estrada, E. Balart, L. García Camacho, B. Vital Rodríguez, R. Alvarado Villanueva, R. Ortega Murillo, and J. Caraveo Patiño. 2014. Trophic interrelations between introduced common carp, *Cyprinus carpio* (Actinopterygii: Cypriniformes: Cyprinidae), and fish community in a eutrophic shallow lake. Acta Ichthyologica et Piscatoria **44**:45-58.

Roach, K. A., J. H. Thorp, and M. D. Delong. 2009. Influence of lateral gradients of hydrologic connectivity on trophic positions of fishes in the Upper Mississippi River. Freshwater Biology **54**:607-620.

Roeder, K., and M. Kaspari. 2017. From cryptic herbivore to predator: stable isotopes reveal consistent variability in trophic levels in an ant population. Ecology **98**:297-303.

Ruus, A., K. I. Ugland, and J. U. Skaare. 2002. Influence of trophic position on organochlorine concentrations and compositional patterns in a marine food web. Environmental Toxicology and Chemistry: An International Journal **21**:2356-2364.

Steffan, S. A., Y. Chikaraishi, D. R. Horton, E. Miliczky, J. E. Zalapa, V. P. Jones, and N. Ohkouchi. 2015. Beneficial or not? Decoding carnivore roles in plant protection. Biological Control **91**:34-41.

Steffan, S. A., Y. Chikaraishi, D. R. Horton, N. Ohkouchi, M. E. Singleton, E. Miliczky, D. B. Hogg, and V. P. Jones. 2013. Trophic hierarchies illuminated via amino acid isotopic analysis. PLoS One **8**:e76152.

Teffer, A. K., M. D. Staudinger, and F. Juanes. 2015. Trophic niche overlap among dolphinfish and co-occurring tunas near the northern edge of their range in the western North Atlantic. Marine Biology **162**:1823-1840.

Vander Zanden, J. M., G. Cabana, and J. B. Rasmussen. 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios ($\delta^{15}N$) and literature dietary data. Canadian Journal of Fisheries and Aquatic Sciences **54**:1142-1158.

Vander Zanden, M. J., J. M. Casselman, and J. B. Rasmussen. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature **401**:464.

Vander Zanden, M. J., S. Chandra, B. Allen, J. Reuter, and C. Goldman. 2003. Historical food web structure and restoration of native aquatic communities in the Lake Tahoe (California–Nevada) Basin. Ecosystems **6**:274-288.

Vander Zanden, M. J., and J. B. Rasmussen. 1996. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. Ecological monographs **66**:451-477.

Vander Zanden, M. J., B. J. Shuter, N. P. Lester, and J. B. Rasmussen. 2000. Within- and among-population variation in the trophic position of a pelagic predator, lake trout (*Salvelinus namaycush*). Canadian Journal of Fisheries and Aquatic Sciences **57**:725-731.

Wilder, S., D. Holway, A. Suarez, E. LeBrun, and M. Eubanks. 2011. Intercontinental differences in resource use reveal the importance of mutualisms in fire ant invasions. Proceedings of the National Academy of Sciences of the United States of America **108**:20639-20644.

Yurkowski, D. J., S. H. Ferguson, C. A. Semeniuk, T. M. Brown, D. C. Muir, and A. T. Fisk. 2016. Spatial and temporal variation of an ice-adapted predator's feeding ecology in a changing Arctic marine ecosystem. Oecologia **180**:631-644.

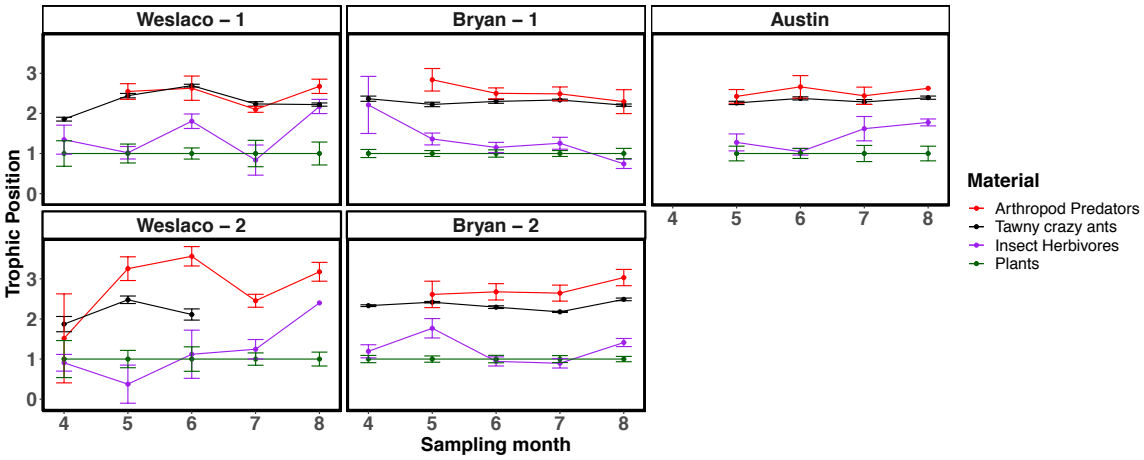
Zambrano, L., E. Valiente, and M. J. Vander Zanden. 2010. Food web overlap among native axolotl (*Ambystoma mexicanum*) and two exotic fishes: carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*) in Xochimilco, Mexico City. Biological Invasions **12**:3061-3069.

Ziegler, J., C. Solomon, B. Finney, and I. Gregory Eaves. 2015. Macrophyte biomass predicts food chain length in shallow lakes. Ecosphere **6**:art5.

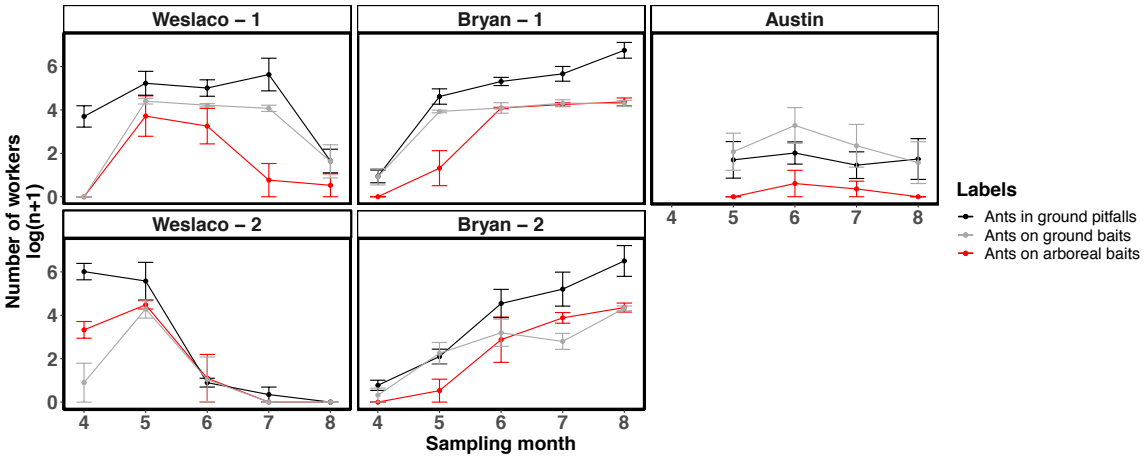
APPENDIX B

SECTION 3

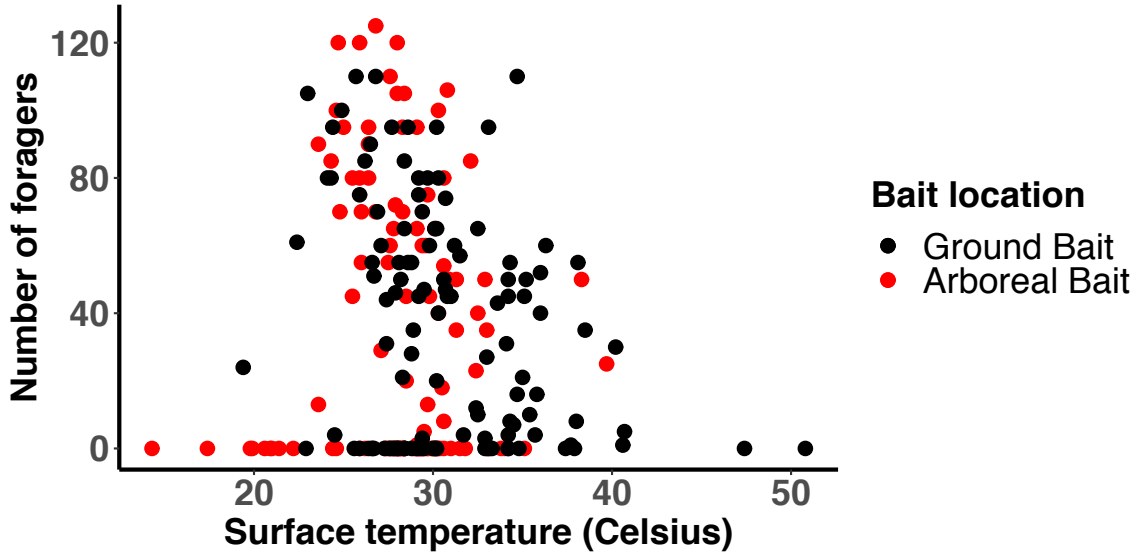
Appendix B S1. Tawny crazy ant trophic position compared with plants, known insect herbivores (i.e., planthoppers and caterpillars), and known arthropod predators (i.e., spiders) by site and month. Points and error bars represent means ± SE. Sampling month ranges from April (4) to August (8). Sampling began in May (5) at the Austin site, so there are no data for April at this site. Arthropod predators were not collected at several sites in April. Tawny crazy ants almost completely disappeared at Weslaco – 2 by July (Appendix S2), so there was not enough material for stable isotope analysis at this site for July and August.



Appendix B S2. Number of workers (log-transformed) in pitfall traps and at baits by site and month. Points and error bars represent means ± SE. Sampling month ranges from April (4) to August (8). Sampling began in May (5) at the Austin site, so there is no data for April at this site.



Appendix B S3. Number of ant foragers at baits on the ground and in nearby trees by the surface temperature measured next to each bait.



The following video files accompany this dissertation:

Appendix B Supplementary Video 1. Tawny crazy ants foraging on the extrafloral nectaries of partridge pea (*Chamaecrista fasciculata*). Video was taken by M. Kjeldgaard on June 5, 2018 at the Bryan – 1 site (30.72˚N, 96.32˚W).

Appendix B Supplementary Video 2. Tawny crazy ants tending aphids on post oak (*Quercus stellata*). Video was taken by M. Kjeldgaard on July 10, 2018 at the Bryan – 1 site (30.72˚N, 96.32˚W).

Appendix B Supplementary Video 3. Tawny crazy ants tending aphids on post oak (*Quercus stellata*). Video was taken by M. Kjeldgaard on August 2, 2018 at the Bryan – 2 site (30.72˚N, 96.32˚W).

Appendix B Supplementary Video 4. Tawny crazy ants tending aphids on wild petunia (*Ruellia spp.*). Video was taken by M. Kjeldgaard on July 11, 2018 at the Bryan – 2 site (30.72˚N, 96.32˚W).

Appendix B Supplementary Video 5. Tawny crazy ants foraging on dead grasshopper caught in a sticky trap. Video was taken by M. Kjeldgaard on July 11, 2018 at the Bryan – 2 site (30.72˚N, 96.32˚W).

Appendix B Supplementary Video 6. Tawny crazy ants biting an injured cockroach. Video was taken by M. Kjeldgaard on June 21, 2018 at the Austin site (30.46˚N, 97.83˚W).

Appendix B Supplementary Video 7. Moments after Supplementary Video 6, tawny crazy ants subdue the cockroach. Video was taken by M. Kjeldgaard on June 21, 2018 at the Austin site (30.46˚N, 97.83˚W).

Appendix B Supplementary Video 8. Tawny crazy ant workers walking up and down the leaves of ashe juniper (*Juniperus ashei*). Video was taken by M. Kjeldgaard on June 6, 2018 at the Bryan – 1 site (30.72˚N, 96.32˚W).

APPENDIX C

SECTION 5

Appendix C S1. Geographic coordinates of sampling locations in Texas for fire ants (*Solenopsis invicta)* and the tawny crazy ant control site (*Nylanderia fulva*).

Ant species sampled	County	Site	Shortened site name	Geographic coordinates
Solenopsis invicta	Brazos	Texas A&M Ecology and Natural Resource Teaching Area, Site 1	O	30˚34'23.1" N, 96˚22'01.5" W
	Brazos	Texas A&M Ecology and Natural Resource Teaching Area, Site 2	A	30˚34'33.3" N, 96˚21'50.2" W
	Brazos	Coulter Airfield	C	30˚43'13.0" N, 96˚20'11.2" W
	Brazos	Hearne, Site 1	T	30˚56'21.9" N, 96˚14'21.9" W
	Brazos	Hearne, Site 2	T2	30˚56'13.6" N, 96˚14'24.4" W
	Robertson	Bremond	B	31˚12'14.6" N, 96˚35'47.1" W
Nylanderia fulva	Brazos	Silver Horse Ranch	TCA	30˚43'40.5"N, 96˚19'27.6"W

Appendix C S2. Explanation of results of colony clustering for each site.

In all but one monogyne nest, the presence of a single reproducing queen was confirmed using microsatellite markers, as all workers could be assigned to a unique queen. The reproduction of multiple queens was confirmed in all but three polygyne nests. In the sites including only monogyne mounds (Sites O & C), all mounds were genetically distinct from each other, likely representing separate colonies. A similar result was obtained in the sites including a mixture of both monogyne and polygyne mounds (B & T). In sites A and T2, some neighboring monogyne mounds were not genetically distinct from each other, likely belonging to the same colonies. In site T2, two monogyne mounds were not significantly distinct from polygyne mounds, which likely stems from the overlapping genetic diversity with highly diverse polygyne mounds. The absence of clear separation between most polygyne mounds may be similarly explained by the high genetic diversity included in most of them or by an absence of colony boundaries between polygyne mounds.

Appendix C S3. Validation of tracer isotope technique methods and results with a unicolonial species (*Nylanderia fulva*)

Methods

To verify that our isotope tracer methods could detect resource sharing over 30-m distances, we also fed a tracer solution (using the same amounts and methods as described above for fire ant colonies in the field) to a population of tawny crazy ants (*Nylanderia fulva*) near College Station, Texas (Appendix S1). This site is primarily grassy habitat that is located near fire ant Site C (~1.6km away). Tawny crazy ants have displaced fire ants at this location due to high population densities, which can sometimes reach more than one million ants per hectare (Kjeldgaard et al., *unpubl. data*). We chose to use this species because tawny crazy ants form a single supercolony throughout their invaded range, in which workers regularly share collected resources with each other and occupy transitory nests. We wanted to determine: 1) if sharing can be detected in a species that is known to share food resources with each other, and 2) if there was any effect of distance on detection of the isotope tracer. Results from this field validation were used to inform our analysis of the fire ant data.

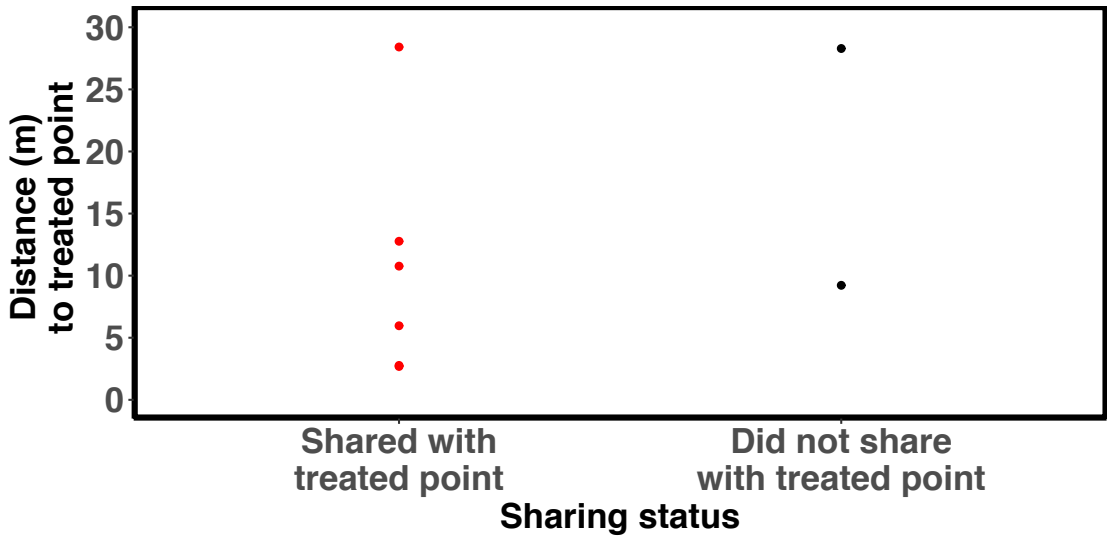
We marked three clusters of four sampling points within the tawny crazy ant location. We treated a single point within each cluster and then sampled at 3m, 10m, and 30m from the treated area to determine the effect of distance on tracer detection. Each cluster was separated by at least 50m to avoid contamination from neighboring clusters. Because tawny crazy ants do not form mounds, we sampled ants at each point using baits that consisted of a single hotdog piece (~4g wet weight) and honey on a laminated card. Baits were left out for five minutes and then approximately 40 workers were aspirated from each bait card. We left three 1-mL vials of the tracer mixed with sugar water (as described in *Treatment with the tracer* section in the main text) at each treated point and sampled ants before treatment and after 14 days of treatment. All ants were frozen at -10˚C prior to processing for stable isotope analysis.

For analysis, we constructed a generalized linear model with a binomial distribution. Distance from the treated point was the independent variable, and the feeding status of untreated points (i.e., "fed on the tracer" or "did not feed on the tracer") was the dependent variable.

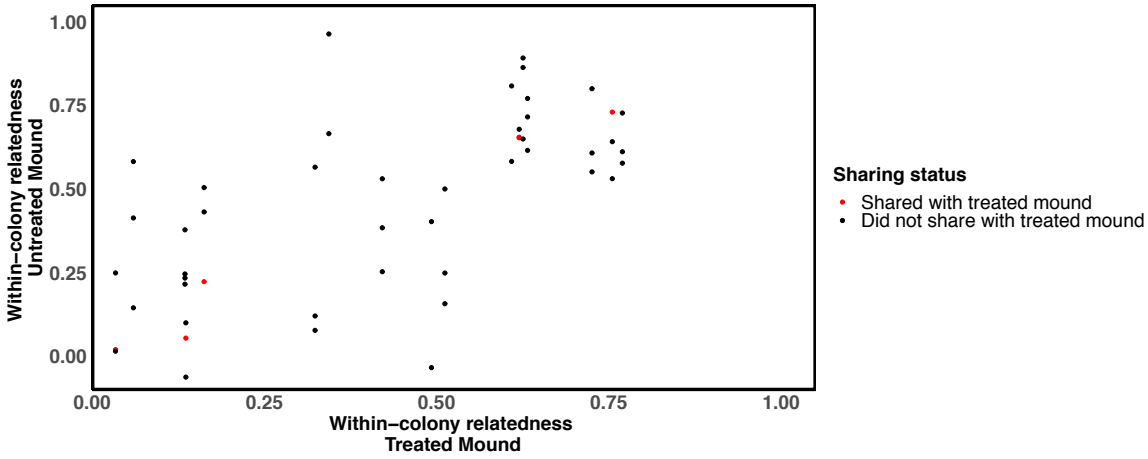
Results

Our methods were successful in elevating the δ^{15}N values of all treated sampling points in the tawny crazy ant control site. In addition, almost all of the untreated sampling points fed on the tracer (Appendix S5), indicating that resources and workers were shared and dispersed throughout the site. There was no significant effect of distance on tracer detection ($\chi^2 = 1.03$, df = 1, $P = 0.310$), and we detected elevated δ^{15}N values in sampling points up to 28.4m from the treated area (Appendix S5).

Appendix C S4. Status of an isotope tracer in the unicolonial tawny crazy ant control site. Results shown are untreated points by distance in meters to the treated point. Each point represents the results from a single point. Sampling points with δ^{15}N values greater than 20‰ indicated that workers and/or resources were exchanged with the treated point ("Shared with treated point"), and the values of all other points indicated that workers did not share ("Did not share with treated point").



Appendix C S5. Status of an isotope tracer in all six fire ant sites. Results shown are from untreated mounds only, with within-colony relatedness between workers of the untreated mound by within-colony relatedness between workers of the treated mound. Each point represents the results from a single mound. Mounds with δ^{15}N values greater than 20‰ indicated that workers and/or resources were exchanged with the treated mound ("Shared with treated mound"), and the values of all other mounds indicated that there was no sharing with the treated mound ("Did not share with treated mound").



Appendix C S6. Status of an isotope tracer in all six fire ant sites. Results shown are untreated mounds by pairwise F_{ST} values and distance in meters to the treated mound. Each point represents the results from a single mound. Mounds with δ^{15}N values greater than 20‰ indicated that workers and/or resources were exchanged with the treated mound ("Shared with treated mound"), and the values of all other mounds indicated that there was no sharing with the treated mound ("Did not share with treated mound").