Food Web Analysis of Hawai‘i Island’s *Blackburnia hawaiiensis* (Coleoptera: Carabidae) Using Next-generation Sequencing and Stable Isotope Techniques

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Abstract

Tropical montane forests are valuable ecosystems in Hawai‘i, providing fresh water to the people of the islands as well as acting as reservoirs of biodiversity. These forests are experiencing rapid alterations due to anthropogenic effects such as climate change, habitat degradation, invasive species, and industrialization. Some of the detrimental effects caused by these ecosystem alterations can be mitigated through understanding the genetics and ecology of the organisms within it. Despite the importance of these arthropod-dominated ecosystems, knowledge of food webs and predator-prey interactions is sparse. In order to supplement the understanding of Hawai‘i’s montane forest ecosystems, we have implemented two different methods of diet analysis on the endemic Hawaiian carabid beetle, *Blackburnia hawaiiensis*. This understudied carabid may provide important ecosystem functions, being a numerically dominant predatory insect and widely distributed throughout Hawai‘i Island. *B. hawaiiensis* populations and potential prey in similar, highly isolated geographic locations were used to employ two different yet complimentary laboratory techniques: natural abundance stable isotope analysis (SIA) and metagenomics of gut contents using next-generation sequencing (NGS). Both NGS and SIA have revealed *B. hawaiiensis* to be a high trophic consumer with evidence of intraguild predation in three study sites: Ka‘iholena, Thurston, and Pu‘u Maka‘ala. In a broader context, the combined SIA and NGS techniques have great potential to further our understanding of the arthropod food webs of the montane forests of Hawai‘i Island, ultimately improving conservation efforts for the entire arthropod community. These two methods in combination could potentially be implemented in any ecosystem globally to better determine the diets of species within complex food webs, enhancing ecosystem management strategies.

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Ecological Importance of Insects

Some of the most significant questions about how life works have first been asked of insects, with the answers later found to apply to all eukaryotes (Harrison et al. 2012). Outweighing humans 200 million to 1, insects are the most successful eukaryotic organisms on Earth (Pedigo 1999). Many insect species have extensive breeding capacity and rapid turnover rates, offering considerable scope for speciation in a relatively short period (Myers & Knoll 2001).

More than 900,000 species of insects have been described, and it is estimated that 2 to 50 million species actually exist (Stork 1993). Their massive species count can be attributed to their ability to adapt to their environment, developing diverse mechanisms to survive and reproduce. Insects are uniquely suited for the study of physiological response to environmental variation because one can often ask both proximate and ultimate questions of behavioral feedback (Harrison et al. 2012). Because of their small size and high reproductive rate, multiple individuals of some species are easily manipulated and reared in the lab, making for superlative experimental design (Harrison et al. 2012). For example, the genetic marvels of sympatric speciation have been well studied in the Hawaiian picture-winged *Drosophila heteroneura* and *D. silvestris* species (Kaneshiro & Val 1977; Pierce 2012; Price et al. 2013).

Insects have an immense value as indicator species, considering their quick response to environmental perturbations and their easy manipulation in the lab (Holling 1978; Murphy & Noon 1991). For example, insect-plant associations are important for ecosystem function, thus gaining an understanding of these associations could improve management strategies. Insects

span a variety of ecological niches and display diverse distributional, population, and dispersal traits, potentially providing a wide array of ecological and biogeographical tools for use in almost all conservation management (Kremen et al. 1993).

Carabid Life History

Coleoptera, or beetles, are the largest and most diverse order of insects (Lövei & Sunderland 1996). One of Coleoptera's most distinctive features is the structure of their wings. Their front wings or elytra act as protective sheaths, while their hind wings are usually used for flight (Ball & Bousquet 2001).

The family Carabidae is the third largest family in the order Coleoptera, comprised of 40,000 described species (Lövei & Sunderland 1996). The Carabidae generally have a large prognathous head and mandibles, margined protnotum, and striated elytra (Ball & Bousquet 2001). They are most often nocturnal, and some such as the bombardier beetles, emit a glandular fluid from the anus both offensively and defensively (Ball & Bousquet 2001; Triplehorn & Johnson 2005).

Carabid habitat ranges from arboreal to cavernicolous, with a moisture preference ranging from geophiles to xerophiles (Ball & Bousquet 2001). Particularly in the tropics, many carabids are arboreal; inhabiting vegetation and hunting for prey on tree trunks, branches, leaves, moss mats, and/or other epiphytes (Ball & Bousquet 2001).

Like all beetles, carabids are holometabolous insects, undergoing complete metamorphosis with four life stages (egg, larva, pupa, and adult) (Ball & Bousquet 2001). Many carabids are subject to diapause during some portion of the year, for example the species *Calathus micropterus* breed in the summer months and overwinter as adults (Niemela et al.

1989). Carabids were once categorized into either autumn breeders with larval hibernation or spring breeders with imaginal hibernation (Larsson 1939), but current research has revealed several species reproduce multiple times per year, even during the winter (Jaskula & Sosztnska-Maj 2011). However, the activity of most ground beetles begins to decrease in late autumn and increases again in early spring (Jaskula & Soszynska-Maj 2011). This activity is due to environmental changes as well as availability of prey, depending on a species' diet.

Carabidae as Environmental Indicator Species and Top-Predators

Carabid beetles are a good model taxon for elucidating hierarchical interactions and structures at various spatial scales because they have been extensively studied both taxonomically and ecologically (Niemela et al. 1993; Lövei & Sunderland 1996; Dufrene & Legendre 1997). This group is often used as indicator species, being high-level consumers as well as sensitive to the state of the environment (Thiele 1977; Luff et al. 1992; Dufrene & Legendre 1997). Indicator species can be defined as being sensitive to disturbances in the environment and reflecting the responses of other species, typically the overall biodiversity of the ecosystem (Raino & Niemela 2002). With the increasing rate of invasive species in biodiversity hot spots, it is important to monitor those systems with potential bioindicators (Mittermeirer et al. 2011). Because carabids are dependent on a balance of abiotic and biotic factors such as temperature, humidity, food conditions, competitors, and life history/season (Lövei & Sunderland 1996), they are well qualified as indicator species (Raino & Niemela 2002). Several studies have shown carabids respond quickly to disturbances such as habitat fragmentation, grazing, forest cutting, and fertilization (Eyre & Luff 1990; Niemela et al. 1993; Blake et al. 1996; Davies & Margules 1998). In Hawai‘i, endemic carabid populations have been

observed to recover quickly after mowing in comparison to other groups of beetles such as Staphylinidae, Nitidulidae, and Curculionidae (Ewing pers. com. 2015).

Pearson & Cassola (1992) suggested tiger beetles, a derived and charismatic clade of carabids, would be good indicator species in the tropics because they have specialized morphology adapted to certain habitat types and reflect the diversity of birds and butterflies. Raino & Niemela (2002) caution using carabids as indicator species in the tropics because of the lack of surveys identifying beetles to species. Additionally, pitfall trapping, the best technique currently available to assess arthropod species assemblages at any particular site (Thiele 1977), may fail because more than 30% of carabid species are arboreal (Stork 1987). However, in tropical areas where carabids and the ecosystems in which they live have been extensively studied such as Hawai‘i (Liebherr & Zimmerman 2000), carabids may make excellent indicator species by implementing other collection techniques such as beating and litter sifting.

Using predators such as carabids as indicator species can reveal change in the entire community because predation is one of the primary organizing forces in community ecology (Withgott & Brennan 2008). Anthropogenic effects on carabid beetles may have "cascading" effects on the distribution and abundance of their prey species, potentially altering the competition among prey species. Predator success is often directly correlated to prey population densities, therefore, a predator's diet in any given environment is dependent on what is quantitatively and qualitatively available (Piñol et al. 2014a).

Carabidae are often generalist predators in arthropod communities, being beneficial as biological control in many situations (McNabb et al. 2001; Wise et al. 2006; Dinter 2009; Hatteland 2010; Lee & Edwards 2011). Top-down control can be an important determinant of ecosystem structure and function, the abundant top-predators having superior influence on the

entire community (Baum & Worm 2009). By studying the diet of a top-predator, such as carabids, food web dynamics may be revealed, furthering the understanding of how feeding habits of different species can affect the community as a whole (Valentini et al. 2008).

Carabid Diet and the Use of Molecular and Ecological Tools

Diet analysis of a species in a given environment can improve knowledge of community ecology and ecosystem function (Valentini et al. 2008; Pompanon et al. 2012). Allen (1979) documented the diet of 150 carabid species in North America, finding most species to be opportunists, feeding on available prey. According to Ball & Bousquet (2001), most ground beetles are facultative predators and may also scavenge on dying and dead arthropods. Larval stages of Carabidae are often predacious as well. Diets of carabids can range from seeds to Lepidoptera (Hagley et al. 1982; Fawki & Toft 2005). Many carabids are beneficial to the environment, feeding on some of the worst pests such as gypsy moth larvae, cankerworms, and cutworms (Borror & White 1970). Numerous studies have investigated the diets of this diverse group, and several food-preference experiments have revealed their generalist behavior (Hagley et al. 1982; Allen 1988; Bilde & Toft 1997). However, food preference studies are limited to the prey offered and therefore are not as comprehensive as studying predation in a natural environment.

With the advances in modern technology, the study of invertebrate diet has become increasingly accessible (Hoogendoorn & Heimpel 2001; Shokralla et al. 2012; Piñol et al. 2014a). Direct observation of invertebrate eating habits can be fruitless, for many are cryptic in habit and small in size (Heimpel et al. 1997; Hoogendoorn & Heimpel 2001). Microscopic observation of gut contents is still practiced (Powell et al. 1996; Triltsch 1997), but is only useful

for analyzing the guts of species that ingest relatively large and indigestible substances (Hoogendoorn & Heimpel 2001). Polymerase chain reaction (PCR) has been used to detect the presence or absence of specific species within gut content; for example, Hoogendoorn & Heimpel (2001) used this technique on field-caught coccinellid beetles. Initial feeding trials and Sanger sequencing has revealed the capabilities to design species-specific primers for prey detection in field-caught carabids. For example, this method has been employed on *Pterostichus melanarius*, a carabid that eats many pests in agricultural systems such as aphids and slugs (King et al. 2010). Although PCR techniques are reliable, they are often costly and timely and require previous knowledge of diet, therefore using a different approach is beneficial.

Metagenomic Analyses

The sequencing of DNA from environmental samples containing complex mixtures of hundreds or even thousands of DNA sequences has surpassed the capabilities of traditional Sanger sequencing (Hajibabaei et al. 2011; Shokralla et al. 2012). When analyzing the diets of generalist predators, prey-specific primers are not ideal for sequencing a large number of prey species, particularly when the full range of prey is unknown (Piñol et al. 2014a). Next-generation sequencing (NGS) metagenomics using general primers to amplify entire gut contents allows for the analysis of potential and unknown prey of generalist predators (Pompanon et al. 2012; Piñol et al. 2014a). NGS outputs several thousand to millions of sequences per PCR product, allowing for the direct characterization of dozens of samples, and can also potentially reveal many consumed species simultaneously (Pompanon et al. 2012). However, NGS technologies are not perfect: universal primers used in species-rich mixtures are likely to have more affinity for some species than for others (Piñol et al. 2014a). Additionally, the quality of metagenomic data is limited by the reference library available. Many species, particularly arthropods, have not been

barcoded and therefore identification of individuals in the gut contents can be limited to order and family.

Piñol et al. (2014a) were the first to use Ion Torrent NGS to determine the gut contents of the arthropod generalist predator, a linyphiid spider. This study used primers for the insect mitochondrial cytochrome oxidase (COI) region and reported over two million raw reads, revealing libraries rich in Collembola, Lepidoptera, Diptera, and Nematoda (Piñol et al. 2014a). Using the COI region as a molecular marker is common in many evolutionary studies (Lunt et al. 1996); the complete sequence is ~1,537 bp, and has been shown to be highly conserved in all aerobic organisms thus far (Gennis 1992). These primers have worked on most insects from a variety of geographic regions (Zeale 2010; Bohmann 2011; Piñol et al. 2014a; Gomez-Polo et al. 2015). In a later paper, Gomez-Polo et al. (2015) used the same primers to sequence hemipteran gut contents using the Ion Torrent platform as well. Because all "universal" primers used in environmental samples may have more affinity for some species than others (Pompanon et al. 2012; Piñol et al. 2014a), applying a complementary methodical approach for analyzing a species' diet is necessary.

Stable Isotope Analysis

Natural abundance stable isotope analysis (SIA) is a complementary approach for understanding the diet of arthropod predators. These studies use naturally occurring differences in isotopic signature to determined trophic position and diet of organisms (Hood-Nowotny & Knols 2007). Nitrogen ratios (^{15}N:^{14}N; $\delta^{15}N$) are typically measured for trophic level analysis while carbon ratios (^{13}C:^{12}C; δ^{13}) are measured for prey/diet matching signature (Post 2002; Hood-Nowotny & Knols 2007). For example, Wise et al. (2006) were able to determine prey of two generalist predators, carabids and spiders, using SIA to examine the effectiveness of biocontrol on a farm in Kentucky. Recent improvements in technology such as SIA have

revealed carabids are not always predacious. For example, McNabb et al. (2001) documented an herbivorous diet in a carabid suggested by $\delta^{15}N$ and $\delta^{13}C$ patterns that was thought to be omnivorous. Interestingly, Gratton & Forbes (2005) were able to show a difference in stable isotopes between male and female coccinellid beetles (*Coccinella septempunctata*), suggesting different metabolic rates can be correlated with gender. Mestre et al. (2013) concluded from SIA that predators that are more than one trophic level above abundant prey species display intraguild predation. Furthermore, natural abundance SIA of $\delta^{15}N$ and $\delta^{13}C$ can provide a complementary tool for measuring trophic positions and energy flow through arthropod communities. The use of both NGS and SIA techniques to decipher the diet of carabids could be of notable and complementary use, especially in systems that have been extensively studied.

Hawaiian Carabidae

The Hawaiian Islands are an important area of study because of the low species-level diversity and high endemism distributed throughout the island chain (Liebherr & Polhemus 1997). The archipelago is the most isolated on Earth, nearly 3,700 km (2,300 mi) from the nearest continent (Clague 1996). The islands are formed over a stationary hot spot located below the middle of the Pacific plate, each island forming as the plate slowly moves northwestward due to plate tectonics (Clague 1996). This hotspot has formed a series of Hawaiian Islands in a geological sequence composed of shield volcanoes.

The geological formation of the Hawaiian Islands has led to a unique pattern of colonization and diversification for many endemic and indigenous species, colonizing the "high" islands first, beginning with Kauaʻi, then "jumping down" the island chain and proliferating with in each newly formed island (Price & Clague 2002). This pattern of diversification from older to younger islands, known as the "progression rule" (Percy et al. 2008) can be seen in

Drosophilidae (Kaneshiro & Boake 1987), *Metrosideros* trees (Percy et al. 2008), land snails (Cooke et al. 1960), *Tetragnatha* spider species (Gillespie et al. 1997), and carabid beetles (Liebherr 2000), where species display independent evolution of similar morphotypes on different islands.

As few as 230 to 250 ancestors are founders for more than 5,000 native Hawaiian insects (Nishida 1992; Miller & Eldredge 1995; Zimmerman 2001), with single island endemism nearly 100% in many taxa such as Drosophilidae (Diptera) and Curculionidae (Coleoptera) (Nishida 1992). The insect order Coleoptera contains over 1,600 species in Hawai‘i, 1,200 of them are endemic, comprising about 20% of the indigenous and endemic insects (Zimmerman 2001). The ground beetle family, Carabidae, is one of the most extensively developed of Hawai‘i’s native fauna, containing 225-recorded endemic species (Zimmerman 2001). Only five of the 225 endemic species can be found on more than one island, therefore nearly 98% of Hawaiian Carabidae are single-island endemics, most being further restricted to a portion of a given island (Zimmerman 2001). Carabids are the numerically dominant insects in most habitats of Hawai‘i, having undergone extensive adaptive radiations (Liebherr & Zimmerman 2000; Zimmerman 2001).

The carabid species endemic to Hawai‘i can be classified in to three tribes: Platynini, Psydrini, and Bembidiini; each carabid tribe in Hawai‘i is represented by a single genus: *Blackburnia* Sharp, *Mecyclothorax* Sharp, and *Bembidion* Latrielle, respectively (Liebherr 2009). *Blackburnia* Sharp is precinctive to Hawai‘i, *Mecyclothorax* Sharp to the Austral-Pacific, and *Bembidion* Latrieille is globally distributed (Liebherr 2009). All three genera of native ground beetles are most diversely represented in tropical montane forest habitats (Liebherr 2009), although some may be found in shrublands (Liebherr & Krushelnycky 2007), riparian corridors

(Liebherr & Short 2006), alpine zones (Krushelnycky et al. 2005), and caves (Liebherr & Samuelson 1992). Species found in the tropical montane forests exhibit extensive adaptation to the localized habitat, having restricted geographic ranges primarily due to the duration of these habitats during climatic cycling for millions of years (Southwood 1977).

The genus *Blackburnia* has undergone extensive radiation on all of the high islands. It is the most extensively studied genus of Carabidae in Hawai‘i, with detailed species-level taxonomy (Liebherr & Polhemus 1997). The first *Blackburnia* are estimated to have arrived during the Miocene epoch, about 20-30 million years ago (Zimmerman 2001; Liebherr 2006), the same time as the arrival of *Drosophila* species (Russo et al. 1995). Of the 129 species of *Blackburnia*, 117 of them have vestigial wing flaps instead of functional metathoracic wings (Liebherr 2000), much like other carabids throughout the world (Darlington 1943). *Blackburnia* are most easily collected by beating vegetation such as *Cibotium* tree ferns and *Astelia* axils (Liebherr & Zimmerman 2000).

The genus *Blackburnia* has been further divided into four subgenera: *Protocaccus, Colpocaccus, Blackburnia, and Metromenus* (Liebherr & Zimmerman 2000). *Copocaccus* is of particular interest, for it the most generalized taxa of the genus, they are all flight-capable and display the perfect example of the “progression rule” (Liebherr 2006). *Copocaccus* is the most basal and least derived clade, comprised of only four species: *B. positcata* of Kaua‘i, *B. tantalus* of O‘ahu, *B. lanaiensis* of Maui Nui, and *B. hawaiiensis* of Hawai‘i. Strong evidence suggests that *B. tantalus* was extirpated by the introduction of invasive ants and has not been recorded since the 1940’s (Liebherr & Polhemus 1997). *Blackburnia hawaiiensis* is one of five endemic *Blackburnia* species to Hawai‘i Island and can be found on all five volcanoes. *B. hawaiiensis* is

often moisture limited and abundant in the montane forest, foraging and mating on plant surfaces at night (Liebherr & Zimmerman 2000).

Because *B. hawaiiensis* is widely distributed on young substrate to fully developed forest, it represents an appealing study subject for the use of current molecular and stable isotope techniques. Liebherr (pers. com. 2014) has analyzed the gut contents of these beetles with a high resolution microscope, describing remnants of which he believes to be spiders, caterpillars, and fruiting bodies of moss mats. *Blackburnia* larvae live with adults from May to October (Liebherr & Zimmerman 2000), presumably consuming Lepidoptera larvae (Bridwell 1918). These observations lead us to investigate if *B. hawaiiensis* is a carnivorous top-predator of the montane forest.

To my knowledge no other study has applied the NGS technology of the Ion Torrent to arthropods of the Hawaiian montane forest, specifically, Carabidae of Hawai‘i. Likewise, to my knowledge no SIA has been conducted on Hawaiian arthropods; this research is unique and vital in a place where efforts to conserve ecosystem function are hindered by the lack of basic taxonomic and ecological data (Howarth & Mull 1992; Medeiros et al. 2013). This research will aim to provide a baseline of how the entire arthropod community may adjust to the increasing anthropogenic changes and invasive species. *Blackburnia hawaiiensis* may prove to be a valuable indicator species as well as a top-predator in the montane forest once the feeding ecology is understood.

Chapter II

Food Web Analysis of Hawai‘i Island’s *Blackburnia hawaiiensis* (Coleoptera: Carabidae) Using Next-generation Sequencing and Stable Isotope Techniques

INTRODUCTION

Preservation of biodiversity is the primary concern of conservation (Rojas 1992), and the maintenance of biodiversity is dependent on knowledge of a system’s ecology. The study of food webs is necessary in order to understand the energy flow among organisms within the community, further defining the ecosystem. Diet analysis of a species in a given environment can improve understanding of community ecology and ecosystem functioning (Valentini et al. 2008; Pompanon et al. 2012). Particularly, the study of predator diet can reveal top-down influence on the entire community (Valentini et al. 2008). Furthermore, it is important to monitor ecosystems with high biodiversity endemism in order to mitigate the effects of invasive species and anthropogenic changes (Mittermeirer et al. 2011).

The tremendous endemism of Hawai‘i can be attributed to the extreme isolation of the archipelago. Initially recognized as a biodiversity hotspot (Myers et al. 1999), the Hawaiian Islands are becoming infamous as the endangered species hot spot of the United States (Dobson et al. 1997). The rapid decline of species in Hawai‘i has been occurring since the first colonization by humans (James & Burney 1997), and has escalated in recent years with increased anthropogenic effects such as climate change, habitat degradation, invasive species, and industrialization. Having evolved in the absence of many vertebrate predators, Hawaiian organisms face the threat of introduced species (Hadfield & Miller 1989). In order to preserve this precious biota, a better understanding of food webs and the ecosystems in which they have evolved is necessary. The study of Hawaiian insects is of particular interest, accounting for the

majority of the Hawaiian terrestrial biodiversity; Hawaiian arthropods fill a variety of ecological niches and provide many ecological services such as decomposition and pollination (Medeiros et al. 2013).

Trophic relationships are often difficult to observe, especially when considering small, rare, or cryptic species (Gomez-Polo et al. 2015). It is often difficult to assign trophic positions in arthropod food webs due to complexity, especially because most arthropod predators are generalists (Polis et al. 1989). Generalist predators are often subject to cannibalism and intraguild predation, eating not only herbivores but also other predators competing for the same resources (Sabelis 1992; McNabb et al. 2001; Mestre et al. 2013). However, the ability to understand food webs and diet in particular has greatly improved with advancements in modern technology (Mestre et al. 2013; Piñol et al. 2014b; Gomez-Polo et al. 2015). Traditional diet studies often included direct observation of scat or gut contents. However, this technique is only useful for organisms that consume large or indigestible substances leaving solid, identifiable remains (Hoogendoorn & Heimpel 2001). Until recently, genetic diet studies consisted of costly research designing prey-specific primers for Sanger sequencing.

Metagenomics, the genetic study of environmental samples, has surpassed the efficiency of Sanger sequencing and allows the identification of complex mixtures of DNA sequences (Shokralla et al. 2012). Next-generation sequencing (NGS) metagenomics has become a powerful tool for ecologists, allowing the examination of dietary breadth without the use of species-specific primers (Gomez-Polo et al. 2015). For example, Piñol et al. (2014a) used the Ion Torrent Personal Genome Machine (PGM) NGS platform to determine the diet of a linyphiid spider and Gomez-Polo et al. (2015) used the same platform to determine the diet of *Orius* spp. (Hemiptera: Anthocoridae). Although extremely effective, NGS has limitations including indels

and single nucleotide variance (Deagle et al. 2013; Piñol et al. 2014b; Thomas et al. 2014); therefore, employing a complementary technique to further understand an organism's diet and trophic position is necessary.

Stable isotope analysis has also become an increasingly favored tool by ecologists, enabling the characterization of trophic relationships among organisms (Mestre et al. 2013). Particularly, the stable nitrogen and carbon isotopes, $\delta^{15}N$ and $\delta^{13}C$, are used to characterize trophic interactions among organisms (Vander Zanden & Rasmussen 1999). This is possible because of fractionation, where the heavier isotope accumulates with increasing trophic levels, also known as trophic enrichment (Mestre et al. 2013). $\delta^{15}N$ accumulates at a much faster rate than $\delta^{13}C$, therefore $\delta^{15}N$ typically represents trophic position and $\delta^{13}C$ represents diet. Because arthropod food webs are relatively complex, the implementation of two techniques is necessary to provide complementary trophic and diet information.

This study is an autecology of the Hawaiian endemic carabid beetle *Blackburnia hawaiiensis*. Carabidae is one of the most extensively developed Hawaiian fauna with 225-described endemic species (Liebherr & Zimmerman 2000). *Blackburnia hawaiiesnsis* is of particular interest for a diet study because of its foraging capabilities inferred from its functioning metathoracic flight wings, a trait that most of its relatives in Hawai‘i have lost (Liebherr 2000). *Blackburnia hawaiiensis* is a numerically dominant predatory insect in the Hawaiian montane forest (Liebherr 2000), with the potential to be an indicator species of ecosystem health. Carabids are model indicator species because of their dependence on a balance of abiotic and biotic factors as well as their influence on trophic structure at various spatial scales (Lövei and Sunderland 1996). Carabids in Hawai‘i have been observed to respond positively to

habitat fragmentation through recolonization in comparison to other Coleopteran families (Ewing pers. com. 2015).

World-wide, carabids are commonly known to be opportunists, consuming a wide variety of prey items such as Diptera and Lepidoptera larvae, Collembola, and aphids (Borror & White 1970; Hagley et al. 1982; Fawki & Toft 2005). Using a high resolution microscope, Liebherr (pers. com. 2014) observed remnants of which he believes to be spiders, caterpillars, and fruiting bodies of moss mats in the gut contents of *Blackburnia*. Although carabids are known to be generalist predators (Allen 1979; Hagley et al. 1982; Bilde & Toft 1997; Ball & Bousquet 2001; Fawki & Toft 2005) several are phytophagus (Honek et al. 2003; Menalled et al. 2007) and little is known about the eating habits of Hawaiian arthropods in general. We combined the use of both NGS and SIA techniques to determine the diet and trophic position of *B. hawaiiensis*.

To the best of our knowledge, no other study has employed these two techniques on a single system and this is the first study to employ the combination of these techniques in the Hawaiian montane forest. This is not a comprehensive food web study but a demonstration of the potential in using these two techniques. Furthermore, these methods greatly aid in identifying the diet of consumers, untangling complex food webs and enhancing vital knowledge in a place where conservation efforts are hindered by a lack of basic taxonomic and ecological data (Howarth & Mull 1992; Medeiros et al. 2013). We use these techniques to test the hypothesis that *B. hawaiiensis* is a tertiary trophic carnivore with evidence of intraguild predation, eating a variety of available prey. Investigation of the diet of *B. hawaiiensis* is an important component to understanding the food web dynamics of the Hawaiian montane forest arthropod community.

METHODS

Site Description

Three sites on Hawai‘i Island were chosen for this study: Thurston (Nāhuku), Ka‘iholena, and Pu‘u Maka‘ala. Thurston (N19°24.822’; W155°14.222’) was formed from a Kīlauea volcano lava flow, Ka‘iholena (N19°11.319’;W155°35.127’) from a Mauna Loa flow, and Pu‘u Maka‘ala (N 19°32.795’; W 155°13.885’) from another Mauna Loa flow (Figure 1). These study sites were chosen to control as much as possible for abiotic factors with the three sites ranging in elevation between 1120-1210 m, a substrate age between 200-750 years old, having a mean annual temperature of 15.5-16 °C, and characterized as tropical montane forest (Table 1). Rainfall composition varied slightly between sites, Ka‘iholena and Thurston’s mean annual precipitation is 2,500-3,000 mm/yr and Pu‘u Maka‘ala is 4,500-5,700 mm/yr (Giambelluca et al. 2013). Sites were ground-truthed to select the most undisturbed areas that had low levels of human activity and invasive species. Sites were dominated by the tree *Metrosideros polymorpha* canopy and the tree fern *Cibotium* spp. understory with presence of *B. hawaiiensis.* The selection of plots was guided by LiDAR, ease of access, and local inspection.

Field Collection of Arthropods

Arthropods for both molecular and stable isotope techniques were collected along fence line for approximately 600 m in order to minimize forest disturbance. All collection took place in several trips during the beetle summer flux period of May-November 2014. Collections were restricted to the summer because this is when *B. hawaiiensis* is most prevalent and active in the forest, therefore making a major impact on the arthropod food web at this time. All arthropods were collected at ~3 m above ground in the lower forest arthropod community.

Stable Isotope Collection & Processing

Arthropods were collected by gently shaking the understory vegetation over a beating sheet for 10 seconds, and then specific arthropods that landed on the sheet were aspirated into snap-cap tubes. Focal taxa collection included adult *Nabis, Pagiopalus, Collembola, Leptogryllus, Laupala,* and *Cibotium* and *Metrosideros* leaf litter. Decomposing plant material was used instead of fresh leaf material to serve as a better baseline of the detritivore/ground arthropod food web. This procedure was repeated until sufficient prey was collected for SIA. Specimens were sorted by taxon and kept alive for 2-4 days to allow the digestive tract to clear in order to reduce the contamination of gut material in the samples (McNabb et al. 2001). Taxa were stored at -20°C for a maximum of one month before further processing. To prepare the specimens for SIA they were oven-dried at 60°C for at least 48 hours then placed in 8x5 mm tin capsules. Individuals were pooled until 0.5-1 mg dry-weight of arthropod and 1-2 mg of plant was achieved (typically 2 *Nabis,* 2 *Pagiopalus,* 20 *Collembola*, 3 *Leptogryllus,* 4 *Laupala*, and 4 *Cibotium* leaflets and 5 *Metrosideros* leaves). Isotopic compositions and $\delta^{15}N$ and $\delta^{13}C$ concentrations of each sample were measured using an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS; PN-150 Costech Auto Sampler and Elemental Analyzer with Finnegan Conflow III regulator and Isodat 3.0 software; Thermo Electron Corporation, San Jose, CA, USA) at the University of Hawai‘i at Hilo Analytical Laboratory (http://www.uhh.hawaii.edu/~analab/). Peach leaf standard (NIST 1547) was also run with the samples for quality control.

Genetic Collection & Processing

Arthropod samples necessary for genetic application were collected in snap-cap tubes by gently shaking vegetation on to a beating sheet. *Blackburnia hawaiiensis* were transferred within 30 minutes after collection to 2 ml screw-top micro stand tubes containing 95% ethanol (to prevent digestion of prey in the gut of the beetles) and then stored at -80°C in the lab later that day. Other arthropods for preliminary PCR and Sanger sequencing were transferred to 2 ml screw-top micro stand tubes containing 95% ethanol in the lab. All arthropods were identified and sexed under a Leica MZ-2500 binocular dissecting scope.

Molecular Techniques

Blackburnia hawaiiensis barcoding primer design

Platynini carabid-specific primers were designed using mitochondrial cytochrome-oxidase I (COI) barcode sequences obtained from GenBank for 68 Platynini species. Sequences were aligned in Geneious (Biomatters, Aukland, NZ). Conserved regions of DNA of the 68 available sequences were considered for potential primer synthesis. Primers for a number of appropriate binding sites were designed and tested in an approximated ~230-bp barcode region. Primers were tested and screened for amplification of femur samples from *B. hawaiiensis*. These primers, which will be referred to as the Platynini primers, were specifically designed to flank the 157-bp region of the "Zeale" primers, or ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale et al. 2010; Bohmann et al. 2011).

Polymerase Chain Reaction (PCR) and Sanger sequencing

The general invertebrate COI primers ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale et al. 2010; Bohmann et al. 2011; Piñol et al. 2014) or "Zeale" primers were selected to amplify 157-bp within the Folmer COI barcoding region (Folmer et al. 1994) via NGS of *B. hawaiiensis* gut

contents. These primers have been shown to amplify a wide range of arthropod orders (Zeale et al. 2010; Bohmann et al. 2011; Piñol et al. 2014). These primers were initially tested on Hawaiian taxa via PCR and Sanger sequencing to find the optimal annealing temperature for a wide range of Hawaiian arthropods. Optimal cycling was found at 95°C for 5 minutes, followed by 50 cycles of 95°C for 30 seconds, 51°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 7 minutes. The Platynini primers were used for PCR amplification and Sanger sequencing for later exclusion during analysis of the NGS data. Optimal *B. hawaiiensis* amplification with Platynini primers was performed at 95°C for 3 minutes, followed by 30 cycles of 95°C for 30 seconds, 53°C for 45 seconds, and 72°C for 45 seconds, and a final extension at 72°C for 5 minutes. Platynini primers were tested on 3 individuals from each site. Additionally, the LCO-HCO (Folmer et al. 1994) primers were used to cross-check the validity of the Platynini primers. These primers were tested and sequenced on two Kaʻiholena individuals, and one individual from Thurston and Puʻu Makaʻala.

DNA Extraction

Blackburnia hawaiiensis left meta-femur was removed for Sanger sequencing, and foregut, mid-gut, and hindgut was dissected for NGS. Observations were recorded on any physical remnants of arthropods in the gut when possible. DNA from dissected abdomens and femurs of *B. hawaiiensis* was homogenized and extracted with the Qiagen DNeasy Blood and Animal Tissue Kit (Qiagen Ltd, Crawley, UK) according to the manufacturer's protocol. The five best gut content extractions from each site were chosen for NGS, for a total of 15 individuals. Of the 15 individuals, 8 individuals were males and 7 individuals were female. An even spread of males and females from each site were used in order to compare sex (Kaʻiholena 2 males and 3 females; Thurston 3 males and 2 females; Puʻu Makaʻala 2 females and 3

females). Whole-body soaking extractions were conducted on focal taxa using the aforementioned kit, according to manufacturer's protocol. DNA quality was checked on a 1.5% agarose gel.

Next-generation Sequencing

Piñol et al. (2014) designed "Fusion Primers" with (1) the Ion Torrent adapter primer A linked to ZBJ-ArtF1c with Ion Torrent adapter trP1 linked to the reverse complement of ZBJ-ArtR2c (forward) and (2) the Ion Torrent adapter trP1 linked to the reverse complement of ZBJ-ArtR2c with Ion Torrent primer A linked to ZBJ-ArtF1c (reverse). The fusion primer method was employed on pilot data, with barcodes linked in between the A adapter and ZBJ-ArtF1c.

To decrease the cost of lengthy primers and increase the number of individuals barcoded per run, samples were prepared using the Ion Torrent recommendations for preparing amplicon libraries without fragmentation using the Ion Plus Fragment Library Sequencing Kit (Life Technologies) as per the alternative Ion Torrent recommendations for preparing amplicon libraries. This protocol was modified to reduce the bias of cycling and adjusted to lower throughput of DNA by using only 20 cycles for the initial PCR and reducing the end repair reagent volumes by 75% and the adapter ligation and nick repair reagent volumes by 50%.

Beetle gut genomic DNA was amplified in 50 µl reaction volumes containing 45 µl of Q5 High-Fidelity 2x Master Mix (New England Biolabs), 4 µl of template DNA, and 1 µl of Primer mix containing 10 µM ZBJ-ArtF1c and ZBJ-ArtR2c. PCR cycling was set at 95°C for 5 minutes, 20 cycles of 95°C for 45 seconds, 51°C for 45 seconds, 72°C for 45 seconds followed by a final extension at 72°C for 7 minutes. The PCR product was purified using Agencourt AMPure XP Reagent (Beckman Coulter).

The end-repair reaction was conducted using 15 µl of purified Amplicon DNA, 5 µl 5x End Repair Buffer (Ion Plus Fragment Kit), and 0.25 µl of the End Repair Enzyme (Ion Plus Fragment Kit). The end-repair reaction was incubated for 20 minutes at room temperature. Following end-repair, a second purification was conducted using the Agencourt AMPure XP Reagent.

Adapter and Barcode ligation and nick repair was conducted in the next step using 25 µl of purified, end-repaired DNA, 5 µl of 10X Ligase Buffer (Ion Plus Fragment Kit), 1 µl each of Ion P1 and Ion Xpress Barcode (Ion Xpress Barcode Adapter 1-16 Kit, Life Technologies), 1 µl dNTP mix (Ion Plus Fragment Kit), 24.5µl nuclease-free water, 1 µl DNA Ligase (Ion Plus Fragment Kit), and 4 µl nick repair polymerase (Ion Plus Fragment Kit). Ion Xpress Barcodes 1-5 were used for Kaʻiholena samples, 6-10 for Thurston samples, and 11-15 for Puʻu Makaʻala samples. Ligation and nick repair was conducted at 25°C for 15 minutes and 72°C for 5 minutes. Agencourt AMPure XP Reagent was used for clean-up at 1.2X per sample volume to optimize 200-300 base-read-library size.

I determined if library amplification was required by checking the quality and the quantity of the ligation on the Bioanalyzer 2100 using the DNA High Sensitivity Kit (Agilent Technologies) (See Appendix 1). Further amplification was required to increase the quantity of ligated DNA. Amplification contained 100 µl of Platinum PCR SuperMix High Fidelity (Ion Plus Fragment Kit), 5 µl Library Amplification Primer Mix (Ion Plus Fragment Kit), and 25 µl of unamplified library. PCR cycling included initial denaturation at 95°C for 5 minutes followed by 7 cycles of 96°C for 15 seconds, 58°C for 15 seconds, and 70°C for 1 minute. A final purification using the Agencourt XP AMPure reagent was done.

Purity of samples was checked (DNA High Sensitivity kit, Bioanalyzer 2100, Agilent Technologies) and quantified (Kappa Biosystems Ion Torrent Library Quantification Kit, quantitative real-time PCR) prior to creating an equi-molar pool of the 15-barcoded libraries (Appendix 2). The samples were then diluted, amplified (Template Hi-Q amplification, Ion One Touch 2 system), and sequenced on the Personal Genome Machine (PGM) using the Hi-Q Kit with the Ion Torrent 318v2 chip (Life Technologies) as described by the manufacturer (Life Technologies). The sequencing chemistry for 200-bp read length and version 4.4.3 of the Torrent Suite software was used for base calling (Life Technologies).

Data Analysis

Analysis of Stable Isotopes

The stable isotopic composition of element X is expressed as a difference in ratios and in parts per thousand:

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

where R_{sample} and $R_{standard}$ is the ratio of heavy to light isotope (^{13}C:^{12}C and ^{15}N:^{14}N) of the sample and standard (Craig 1953). Carbon isotope data were normalized to USGS 40 $\delta^{13}C$ vs VPDB = -26.4) and USGS 41 ($\delta^{13}C$ vs VPDB 37.6) (SD=0.2‰). Nitrogen isotope data were normalized to USGS 40 ($\delta^{15}N$ vs Air =-4.5) and USGS 41 ($\delta^{15}N$ vs Air -47.6) (SD=0.2‰).

Isotopic signals of primary consumers tend to vary less than primary producers (Vander Zanden & Rasmussen 1999), therefore $\delta^{15}N$ values were used to infer the trophic positions of consumers with Collembola as the baseline. Collembola have been documented to feed on plant litter, live plant tissue, bacteria, and fungi (Rusek 1998), and are likely the base of the arthropod

detrital food web. The enrichment of this food web is separate than that of plants in this system, and reflects the prevalence of soil microbial processes (Hyodo 2015). The trophic-level position of high-level predators/consumers was calculated as:

$$[(\delta^{15}N_{consumer}-{}^{15}N_{baseline}) /3.4] + 2$$

(McNabb et al. 2001; Cabana & Rasmussen 1996). The 3.4 represents the average Trophic Enrichment Factor (TEF) of animals, where a wide variety of animals gain +3.4‰ $\delta^{15}N$ with increasing trophic level (Post 2002). TEF can be referred to as fractionation/discrimination factors between food resources and consumers that can be obtained through feeding trials. The +2 allows the trophic position to be placed on the primary consumer scale.

The mean ± SE of each arthropod category was calculated across all sites and within each site. R version 3.0.2 was used for all further analysis. Two-way ANOVA was used to compare site and taxa for $\delta^{13}C$ and $\delta^{15}N$ separately. One-way ANOVA was used for individual $\delta^{13}C$ and $\delta^{15}N$ comparison of taxa across sites. If ANOVA yielded significant P-values, Tukey's honest significant difference (HSD) was used to determine significance among sites.

Bioinformatic Next-generation Sequencing Data Analysis

Torrent suite software sorted all sequences by barcode into separate files. Torrent Suite Plugin FileExporter version 4.4.3 was used to convert the output BAM files into human-readable FASTQ files. The primer sequence from the 5' and 3' ends were eliminated using cutadapt (Martin 2011). Cutadapt was also used to eliminate all sequences shorter than 100 bp in length. A simple sed (Unix programming language) script was used to correct phred scores (see www.phrap.com/phred/ for explanation) by one position for compatibility with other programs

for further manipulation. Galaxy tools (Cock et al. 2013) FASTQ quality filter and FASTQ quality trimmer were used to set the quality score specific to the Ion Torrent and discard reads with a mean quality score less than 20 (99% base call accuracy). FASTQ files were then converted to FASTA files to be clustered at 95% similarity via Cd-hit-est (Li & Godzik 2006). Clusters were then blasted (BLAST, McGinnis & Madden 2004) against a concatenated database containing the original *B. hawaiiensis* sequences obtained from Sanger sequencing with the Platynini primers, and the NCBI COI databases. I filtered all recovered sequences for haplotypes with <2 copies. Because of the limited Hawaiian arthropod data on GenBank, species matches were generally ignored and order or family information was retained. Results were visually inspected and verified in the Hawaiian arthropod database.

The Sorensen-Dice similarity coefficient was used to compare the similarity in taxa abundance between sites:

$$CI=2h/a + b$$

where *a* and *b* are the number of taxa in sites A and B, and *h* is the number of taxa shared by sites A and B, and CI is the Coincidence Index and ranges between 0 and 1 (Dice 1945; Sorensen 1948). Rarefaction curves were used to determine if the number of individuals sequenced properly characterized the diet of *B. hawaiiensis*. Analysis was conducted in excel by plotting the taxa counts in individual samples and calculating the coefficient of determination.

RESULTS

Stable Isotope Analysis

$\delta^{15}N$ was significantly different among sites for *B. hawaiiensis* (p=0.001), but was not different among sites in any of the other taxa (Tables 2, 3). Tukey's HSD revealed Thurston had a significantly lower $\delta^{15}N$ for *B. hawaiiensis* than the other two sites (p=0.001). Individual *B. hawaiiensis* samples were more clustered at Thurston than the other two sites (Figure 2). For $\delta^{13}C$, Collembola was significantly different across sites (p=0.005) with Collembola at Thurston having a higher $\delta^{13}C$ than Pu'u Maka'ala and Ka'iholena intermediate (p=0.005) (Tables 2, 3). *Cibotium* was significantly different in $\delta^{13}C$ across sites (p=0.032) with *Cibotium* at Thurston having the highest $\delta^{13}C$ as well (p=0.042) (Tables 2, 3). *Metrosideros* was significantly different in $\delta^{13}C$ across sites (p=0.037) with *Metrosideros* at Thurston having a lower $\delta^{13}C$ than Ka'iholena and Pu'u Maka'ala intermediate (p=0.045) (Tables 2, 3).

Based on average $\delta^{15}N$ values, *B. hawaiiensis* was consistently higher in terms of trophic level than all other taxa tested across all sites (Figure 3). *Blackburnia hawaiiensis, Pagiopalus, Nabis, and Laupala* all displayed predator trophic positioning and eating habits (Tables 4, 5). On average, *B. hawaiiensis, Nabis,* and *Pagiopallus* were more than one trophic-level position from Collembola, the primary consumer, across all three sites (Table 5). For $\delta^{13}C$, *B. hawaiiensis* was not significantly different than Collembola, *Laupala, Leptogryllus, Nabis,* and *Pagiopalus,* therefore these are all potential prey items for *B. hawaiiensis* (Table 5). There was no significant difference between Collembola and *Leptogryllus* $\delta^{13}C$ and $\delta^{15}N$, and both displayed similar primary consumer trophic levels. The two primary producers, *Metrosideros* and *Cibotium* also displayed no difference in $\delta^{13}C$ and $\delta^{15}N$.

The estimated trophic position of consumers was consistently lower across taxa at Thurston (Table 4). For the analysis of all taxa, site and taxa differed significantly in $\delta^{13}C$ and

$\delta^{15}N$ ($p<0.0001$) (Tables 6, 7). However, there was no significant interaction between site and taxa (Figures 4, 5). Thurston was significantly lower in $\delta^{15}N$ and higher in $\delta^{13}C$ in comparison to Kaʻiholena and Puʻu Makaʻala ($p<0.001$; $p<0.001$) (Tables 6, 7; Figures 4, 5).

Polymerase Chain Reaction and Sanger sequencing

Blackburnia hawaiiensis sequenced using the Platynini carabid-specific primers revealed no polymorphisms in DNA sequence among the three individuals in the three sites, therefore there is no evidence of population divergence in this ~230 bp region of COI mtDNA. These primers that are usable for any Platynini tribe sequencing were:

F:AGATATTGGAACWTTATATTTTATTTTTGG

R:ATCAAAAACTTATATTATTTATTCGAGG

When using the "Zeale" primers, an annealing temperature of 51°C was found to best amplify a wide range of Hawaiian Arthropods. These primers amplified *B. hawaiiensis*, *Drosophila heteroneura*, *Laupala* spp., Lepidoptera spp., *Leptogryllus* spp., *Nabis* spp., Nitidulidae, and *Triginidium* spp. taxa at 51°C.

Next-generation sequencing

The Ion Torrent run produced 4,477,826 DNA sequencing reads on the 318v2 chip with 63% loading (Figure 6). The mean read length was 211 bp (Figure 7). There was an even spread of sequences returned from each barcode, with a minimum number of reads at 199,987 and maximum of 270,275. After quality control 27% of reads were discarded from each sample. Of the remaining sequence reads 97% were blastable in the database. As expected, >99% of sequences belonged to the predator itself. Interestingly, 53 sequences were positive for *B. hawaiiensis* cytochrome oxidase B and were discarded in the final dataset. A number of

sequences matched *Agonum* spp., the closest relative to *B. hawaiiensis* in COI of Genbank. These sequences did not contain any obvious pattern and many appeared to be indels. It was concluded that these sequences were either nuclear pseudogenes or other *Blackburnia* species. There are 6 *Blackburnia* on Hawai‘i Island, but sequences are not available for COI. For the purpose of this study, these sequences were removed from the final dataset. Additionally, prey items were not amplified in 2 of the 15 gut content samples, one of which was male and the other female (Figure 8).

Overall, the majority of prey detected was from Amphipoda and *Zaprionus* (Diptera: Drosophilidae) (Table 8). Sequences included taxa from Arachnida, Coleoptera, Crustacea, Diptera, Lepidoptera, Neuroptera, and Orthoptera (Figure 9). Diptera was found in the diet across all three sites, *Zaprionus* in particular was a major diet preference. Crustacea was also found in the diet across all three sites, mostly in the order Amphipoda. Lepidoptera was found in the gut contents at Thurston and Pu‘u Maka‘ala but not Ka‘iholena (Figures 10-12). Orthoptera was detected in *B. hawaiiensis* gut contents at Pu‘u Maka‘ala and Ka‘iholena but not Thurston. Coleoptera was detected only in Ka‘iholena samples (Figure 12). The detection of Myrmeleontidae (Neuroptera), *Scymnus* (Coleoptera: Coccinellidae), and *Typhlodromus* (Acari: Phytoseiidae) sequences are all evidence of intraguild predation.

Ka‘iholena samples consistently contained combinations of Crustaca, Diptera, and Orthoptera (Figure 8, 12). Orthoptera was found in 4 of the 5 Ka‘iholena samples (Figure 8). Thurston samples were dominated by Crustacea and Diptera taxa. Sample 10 of Thurston had many more sequences than any other individual (Figure 8). Sample 10 had the highest abundance detected in all samples, containing 5 different orders, including Neuroptera, which was not found at any other site. Pu‘u Maka‘ala samples contained Diptera in all of the samples that prey items

were detected. Arachnida was found in the diet at Puʻu Makaʻala in sample 12 and at no other sites.

Taxa detected in male *B. hawaiiensis* gut contents included Crustacea, Diptera, Lepidoptera, Neuroptera, and Orthoptera (Figure 12). Taxa detected in female *B. hawaiiensis* gut content included Arachnida, Coleoptera, Crustacea, Diptera, and Orthoptera (Figure 13). Lepidoptera sequences were found in male samples and not females. Over 50% of the female gut content sequences were Diptera. Males displayed a more diverse diet than females (Figures 13, 14). Rarefaction curve coefficients of determination for Puʻu Makaʻala, Kaʻiholena, and Thurston were 0.77, 0.82, and 0.48, respectively.

DISCUSSION

Knowledge of feeding ecology can ultimately improve the effectiveness of land management, and conservation of the environment as a whole. The construction of high-resolution food webs is important for understanding community structure and stability, particularly because anthropogenic effects on the community are often diet linked (Fry 1996). Although arthropod food webs are complex and difficult to assign trophic levels (Polis et al. 1989), we can begin to understand trophic position by using more than one technique to untangle these food webs. In this study, I was able to identify specific food sources, detect trophic position, and reveal evidence of intraguild predation in *B. hawaiiensis* through both NGS and SIA. Furthermore, both techniques affirmed the hypothesis that *B. hawaiiensis* is a high-level consumer and generalist predator. This beetle may play a major role in the Hawaiian montane arthropod food web, and the conservation of this species is important for the stability of the arthropod community.

The Use of Complementary Techniques

NGS techniques provide an invaluable tool for determining diet of a predator species (Pompanon et al. 2012) and SIA is a powerful tool for constructing food webs (Boecklen et al. 2011). I have demonstrated that these techniques could be used together to more clearly determine the diet of an understudied species, improving our understanding of food web networks across multiple communities. In SIA, $\delta^{13}C$ allows the estimation of diet and $\delta^{15}N$ allows the estimation of trophic position, while NGS determines both diet and trophic position by evaluating prey DNA fragments. The synergy of these techniques is beneficial for elucidating the feeding ecology of organisms that are difficult to rear in the lab such as *B. hawaiiensis*. The

combination of both techniques allows the enhancement of each other's results. For example, intraguild predation was implied by the SIA, but confirmed in the detection of predacious species in NGS.

The opportunistic feeding habits of carabids can be better categorized through NGS than SIA technology, as seen in the range of prey sequences detected from amphipods to Coccinellidae. Prey sequences were obtained from the majority of the individuals, whereas to our knowledge, no other diet study has used individual barcodes on individual samples in NGS. By barcoding individuals, one can gain a better understanding of the proportion of species consumed by a single beetle within the range of sequencing detection. Furthermore, I was able to detect specific taxa in the diet of individual *B. hawaiiensis*, providing insights into their generalist behavior. Interestingly, sequences from the order Hemiptera were not detected, which is a major group in the arthropod food web. However, the "Zeale" primers were tested on *Nabis* (Hemiptera: Nabidae) and were capable of Hemipteran amplification. SIA revealed *Nabis* as a possible prey item of *B. hawaiiensis*, and the shortcomings of the NGS sample size were negated by the additional technique.

SIA provides insight of energy movement in the food web that NGS cannot. For example, the significant differences in $\delta^{13}C$ of both Collembola and *Cibotium* between Puʻu Makaʻala and Thurston may suggest that Collembola are feeding directly on *Cibotium*. These data are expected because Collembola have been characterized as feeding on plant litter, live plant tissue, bacteria, and fungi (Rusek 1998). The consistent $\delta^{13}C$ concentrations across all three sites indicate all of the taxa in the SIA study are potential food sources for *B. hawaiiensis*. However, $\delta^{13}C$ ratios do not always reliably reflect diet because of trophic plasticity of a species. For example, seasonal differences in prey availability will affect the diet of opportunistic foragers such as *B.*

hawaiiensis. The addition of NGS can account for trophic plasticity by elucidating prey item fragments at time of collection.

Differences among sites were detected using both NGS and SIA. Thurston was consistently different than Puʻu Makaʻala and Kaʻiholena in the SIA. There were moderate differences among sites in the NGS data according to the Sorensen-Dice similarity index (Kaʻiholena-Thurston 0.4, Thurston-Puʻu Makaʻala 0.375, Kaʻiholena-Puʻu Makaʻala 0.4). The genetic results may also reveal significant differences in available prey items at Thurston, considering the prey species richness at this site is contributed from a single sample (Figure 8). These consistent differences among sites may be due to differences in vegetation composition. Thurston's understory is more open than Puʻu Makaʻala and Kaʻiholena (J.Y. Yim pers. obs. 2015) and appears to be between Phase 2 and 3 of primary succession (Kitayama et al. 1995; Mueller-Dombois & Boehmer 2013). Phase 2 is characterized by a persisting *Cibotium* layer while a closed, dense shrub layer of *Cibotium* forms in Phase 3. The pace of primary succession is dependent on several factors such as substrate age, rainfall, microclimate, and proximity to older lava flows. However, exact substrate ages for Kaʻiholena and Puʻu Makaʻala are unknown and Puʻu Makaʻala receives higher amounts of annual rainfall than the other two sites, thus decreasing the availability of nitrogen in the soil (Austin & Vitousek 1998).

Thurston is also notorious for invasive species such as feral ungulates, Kahili ginger (*Hedychium gardner*) and faya tree (*Morella faya*). Although much of these invasive species have been removed, a simplified food web could be remnant of the site's history. Particularly, the presence of the nitrogen-fixing faya tree could alter the typically nitrogen negative deficient ecosystems of Hawaiʻi (Vitousek et al. 1987). The SIA data suggests a trophic level may be absent at Thurston, or it may contain fewer omnivores than at Kaʻiholena and Puʻu Makaʻala.

The consistently lower estimated trophic levels at Thurston can be seen in all of the consumer taxa measured (Table 4). Overall, minimal differences existed between Puʻu Makaʻala and Kaʻiholena, thus these sites can serve as replicates of native montane wet forest.

Interestingly, the presence of Lepidoptera sequences in male samples and not female samples may implicate different foraging habits, particularly different microhabitats. Gratton & Forbes (2005) and Ekbom (1992) both saw differences in Coleoptera male and female diet, where females may be consuming different prey items for reproductive purposes. *Blackburnia hawaiiensis* occurs in a variety of forest microhabitats such as the forest floor and numerous arboreal situations (Liebherr 2000). The subgenus *Colpocaccus* are thought to have no spatial constraints, with a possible seasonal pattern, being observed on vegetation and along streams during springtime and aggregated under logs in the winter (Liebherr & Zimmerman 2000). Sampling of more individuals and male and female collection localities would strengthen this conclusion of different microhabitat foraging. SIA of male and female samples could further quantify differences in male and female diets.

Although these techniques are complementary, they are not comparable because the samples were collected at the same time and the temporal scales are different. Stable isotopes are the result of a natural accumulation over a life span, while the NGS samples are representations of what the organism ate within a few days, depending on the digestion rate (half-life) of the prey item. Additionally, holometabolous insects retain the stable isotope signatures of prey eaten at both the larval and adult stages. However, the life span of carabids is generally 1-2 years (Lövei & McCambridge 2001), therefore temporal scales are not as relevant in comparison to vertebrates. Multiple genetic samples of *B. hawaiiensis* throughout the year for several years could provide a comparable assessment to SIA. Mestre et al. (2013) were able to detect seasonal

variation in isotopic signatures of arthropods, revealing a short temporal scale in both predators and primary consumers. Further investigation is necessary for understanding the temporal scale of these techniques in Hawaiian montane arthropods.

Limitations and Future Studies

Comprehensive food web studies are often limited by cost, making it difficult to observe all species within an ecosystem. Orthoptera was detected by both techniques, while 8 taxa were considered for SIA and 15 taxa were revealed by NGS. This may suggest that an adequate amount of prey items were analyzed in SIA to complement the NGS data. However, the sequencing of more individuals is necessary in order to properly characterize the diet of *B. hawaiiensis* (Figure 15). By barcoding individual samples I was able to track the contribution of prey sequences to individuals, and reveal two samples contained no prey sequences. This may be due to numerous factors such as sample handling, extraction technique, or simply because the carabids did not feed recently enough before collection. In order to minimize differences among samples, I suggest a standardization of collection time for future diet studies. Because carabids are nocturnal, nighttime collection using only one method could improve results. Additionally, arthropod abundance and vegetation survey data would ameliorate our understanding for the differences in both SIA and NGS among sites.

High-throughput diet sequencing often results in consistent sequence proportions but improper diet estimates (Deagle et al. 2013). Therefore, due to factors such as digestion bias, primer amplification bias, food preference bias, and stringency of bioinformatic parameters the NGS data cannot be analyzed as abundance (Deagle et al. 2010; Deagle et al. 2013; Thomas et al. 2013). Deagle et al. (2013) showed that minor prey items eaten frequently would appear to be

a more significant part of the diet in genetic studies. The probability of prey amplification is also dependent on the annealing temperature, and some arthropod orders may have been preferentially amplified. For example, in preliminary testing, Hawaiian *Tetragnatha* spiders were found to have a lower optimal annealing temperature than *Leptogryllus* (46°C and 52°C, respectively). Additionally, the forward "Zeale" primer was found to be a weak match for the Hymenoptera order, containing a cytosine at the first position of the 3' end instead of a guanine.

Feeding studies conducted in the lab would be beneficial to both NGS and SIA. Lab studies could reveal the suitability of the "Zeale" primers and their ability to detect arthropod prey items in the gut contents of *B. hawaiiensis.* By using a controlled experiment, the temporal scale of NGS could be determined for *B. hawaiiensis* as well as the accuracy of the primers to amplify certain prey and the proportion of prey amplified after digestion. Gomez-Polo et al. (2015) determined prey decay rates through the use of controlled feeding studies to monitor food ingested by *Orius majusculus* (Hemiptera: Anthocoridae) in relation to hours after feeding. Thomas et al. (2013) developed methods to obtain tissue and digestion correction factors by sequencing both control materials of known composition and dietary samples. By using feeding studies, correction factors could be used to better categorize diet proportions. Feeding studies are also beneficial for determining the proper TEF for estimating trophic position. The 3.4‰ TEF used in this study is an average value from multiple ecosystem studies (Post 2002), and *B. hawaiiensis* must be raised in the lab in order to obtain proper TEF. Although lab experiments would be beneficial to this project, *B. hawaiiensis* has never been successfully raised in the lab. During my 5-month attempt to raise these beetles in the lab, *B. hawaiiensis* preferred cannibalism to diced mealworm larvae for meals. Leibherr (2000) has raised a closely related species in the *Colpocaccus* subgenus, but a self-perpetuating colony was not established. The

investigation of *B. hawaiiensis* diet is limited by its ability to be raised in the lab, highlighting the use of less unequivocal techniques.

SIA is limited by the necessity to obtain all possible prey items. The SIA results indicate that *B. hawaiiensis* is not consuming *Cibotium* or *Metrosideros*, but the addition of moss material would aid in determining if *B. hawaiiensis* is a polyphagous consumer. Genetic data using plant-specific primers on *B. hawaiiensis* gut contents could confirm these results. Stable isotope mixing models such as MixSIR (Moore & Semmens 2008) or SIAR (Parnell et al. 2010) are also limited by inclusion of all possible food sources and larger sample sizes. These models are used to determine the food source proportion of the diet in consumers such as *B. hawaiiensis*. These models also require several types of additional data such as a larger sample size and the proper TEF (Phillips et al. 2014) and include many assumptions; therefore the use of NGS in addition to SIA is more advantageous.

Numerous modifications to the protocol for NGS metagenomics exist to reduce predator-sequencing biases including gut dissection, minimal initial PCR cycles, and the use of blocking primers. Several studies (Piñol et al. 2014a,b; Gomez-Polo et al. 2014) have used the brute-force approach with 40 initial PCR cycles, resulting in over 99% predator sequences. In this study, gut dissections and the reduction of PCR cycles to 20 cycles did not significantly decrease predator amplification in comparison to the pilot study. In Piñol et al. (2014b) the main consequence of reducing PCR cycles was an increase in the proportion of the less well-amplified taxa. Therefore, regardless of the number of initial PCR cycles rare-sequences were consistently amplified. Blocking primers are host-specific primers modified with a C3 spacer at the 3' end of the forward universal primer to inhibit DNA amplification of the host (Vestheim & Jarman 2008; Deagle et al. 2013; Gomez-Polo et al. 2014). In conjunction with the "Zeale" primers, blocking

primers have not proven to be necessary on the Ion Torrent PGM system (Gomez-Polo et al. 2014; Piñol et al. 2014b). For example, Piñol et al. (2014b) found that the use of blocking oligonucleotides was less than one order of magnitude effective. After the alignment of the COI arthropod sequences available in Genbank, it was concluded that blocking primers could not be used for *B. hawaiiensis* COI. Because this region is highly conserved (Table 9), significant proportions of all insect orders examined would not have been detected if the blocking-primer method was used.

The use of an additional target region and/or the use of longer amplicons could improve sequence identification to genus or species level. Certain orders such as Lepidoptera are very similar across family in the "Zeale" COI region, and the sequencing of another region such as ITS2 would be required in order to identify some fragments past the order level (Piñol et al. 2014a). For example, the families Proxidae and Bombycidae of Lepidoptera are not found in Hawai‘i but their sequences are highly similar to other families found in the region; therefore these sequences could only be identified to order. Anslan & Tedersoo (2015) suggest using ITS2 as an alternative barcoding region for Collembola. However, using nuclear DNA for barcoding purposes of mixed samples can result in intra-individual sequencing length polymorphisms and co-amplification of non-animal DNA (Anslan & Tedersoo 2015). COI is also the accepted heavily used arthropod barcoding region (Wilson 2012), and straying from this region could lead to more difficulty with the arthropod barcoding databases available. Perhaps the use of longer amplicons could prove to be fruitful, but the Ion Torrent sequencer is presently limited to 400 bp. Sequencing length with the Ion Torrent should be increasing to 500 bp within the next year (Fernando Rivadavia pers. com. 2015). However, short amplicons may be necessary to ensure amplification of highly degraded DNA in gut contents (Piñol et al. 2014a). Therefore,

sequencing longer amplicons presents a paradoxical approach where digestion bias may limit the read length.

Sequencing to species level is also limited by database availability and the knowledge of the local arthropod fauna (Piñol et al. 2014a). Approximately 90% of the described insects in the world lack reference DNA barcodes (Virgilio et al. 2012). Local arthropod databases are currently in the making, and availability of these data should help to assign sequences to species level. For example, the 'modified mouth-parts' Drosophilidae of Hawai'i are highly studied with sequences available in Genbank, allotting the identification to genus level in this study: *Zaprionus* (Leblanc et al. 2009; O'Grady & DeSalle 2008). However, the species list of Hawai'i's arthropod fauna is continually growing and is limited by funding and personnel. Hawai'i is also a hot spot for introduction of invasive species while new native species are being described simultaneously. Therefore, it is difficult to keep a full checklist of all established arthropod species in Hawai'i. Better record keeping of arthropod collection sites combined with the sharing of GPS coordinates and mapping will prove to be beneficial in the future. According to Medeiros et al. (2013), an invertebrate database is being developed by the State of Hawai'i Department of Land and Natural Resources, Division of Forestry and Wildlife including geographic locality. The availability of these data in combination with a reference sequence database specific to Hawai'i could greatly improve the accuracy of this study. However, these types of studies deal with highly degraded DNA, and short fragments may still not be identifiable to species level.

Implications for the use of Hawaiian Carabidae as an Indicator Species

In conclusion, these results demonstrate *B. hawaiiensis* is a predatory beetle in the Hawaiian Montane forest, and a potential indicator species for these environments. Because *B. hawaiiensis* is a high trophic level consumer and a generalist predator, it is likely to have top-down influence on the entire arthropod community. Additionally, Hawaiian carabid beetles have been documented to be consumed by native birds such as the ʻAmakihi, ʻElepaio, and Hawaiʻi Creeper (Banko et al. 2015). Because of their influence on trophic structure at various spatial scales, carabids could be used as a linkage between the arthropod and vertebrate food webs of Hawaiʻi. There is an absence of large vertebrates in Hawaiian ecosystems, consequently this linkage between food webs may be of particular importance. *Blackburnia* are specifically moisture limited (Liebherr & Zimmerman 2000), and could be used as an environmental indicator species of rainfall. Beetles were scarce in Summer 2014 and I did not detect them at many sites with historic collection localities such as Puʻu O ʻUmi, ʻAlili Springs, and Laupāhoehoe. Rainfall was significantly higher in Summer 2015, and *B. hawaiiensis* was sighted at Puʻu O ʻUmi and Laupāhoehoe once again. Therefore, the abundance of *B. hawaiiensis* could be directly correlated with weather conditions.

Hawaiian carabids are sensitive to the introduction of invasive species, particularly ants (Krushelnycky & Gillespie 2010) and isopods (Liebherr & Zimmerman 2000). These carabids are also only found in relatively undisturbed forest, therefore they can reflect the influence of invasive species and indicate the status of environmental condition. Presence and absence data of Hawaiian carabids, particularly *B. hawaiiensis*, could indicate ecosystem health. Furthermore, the monitoring of these beetles may aid in the conservation of Hawaii's biodiversity.

TABLES

Table 1. Site parameters for elevation, precipitation, temperature, substrate age, and coordinate localities. Annual temperature data is from Giambelluca et al. (2014) and rainfall data is from Giambelluca et al. (2013).

Site	**GPS Coordinates**	**Elevation (m)**	**Precipitation (mm)**	**Temperature (ºC)**	**Substrate Age (Yr)**
Kaʻiholena	N19°11.319' W155°35.127'	1062	2500-3000	15	200-750
Puʻu Makaʻala	N 19°32.795' W155°13.885'	1162	4500-5700	15	200-750
Thurston	N19°24.822' W155°14.222'	1175	2500-3000	16	200-750

Table 2. $\delta^{13}C$ and $\delta^{15}N$ isotope values (± 1SE) of *B. hawaiiensis*, *Pagiopalus*, *Nabis*, *Laupala*, *Leptogryllus*, Collembola, *Metrosideros*, and *Cibotium*. Taxa are listed in estimated trophic position decreasing from top to bottom. Number of samples analyzed (n) of each taxa are listed in order from left to right as Kaʻiholena, Puʻu Makaʻala, and Thurston. Post-hoc Tukey's HSD analysis for significantly different sites are denoted by a superscript, means that do not share a letter are significantly different. SE not available when n=1 and denoted by *.

	$\delta^{13}C$			$\delta^{15}N$		
Taxa	Kaʻiholena[a]	Puʻu Makaʻala[a]	Thurston[b]	Kaʻiholena[a]	Puʻu Makaʻala[a]	Thurston[b]
B. hawaiiensis (n= 4, 5, 4)	-26 ± 0.65	-27.2 ± 0.48	-26.2 ± 0.14	-1.3 ± 0.35[a]	-0.9 ± 0.23[a]	-3.0 ± 0.35[b]
Pagiopalus (n=4 ,4, 5)	-27.7 ± 0.68[a]	-27 ± 0.40[a]	-26.2 ± 0.10[a]	-3.4 ± 1.23	-2.2 ± 0.38	-3.2 ± 0.51
Nabis (n=3, 4, 4)	-26.3 ± 0.78	-26.2 ± 0.16	-25.7 ± 0.28	-1.8 ± 0.44	-2.7 ± 0.38	-3.1 ± 0.26
Laupala (n=4, 5, 5)	-27.3 ± 0.82	-26.9 ± 0.23	-26.5 ± 0.25	-3.6 ± 0.57	-3.4 ± 0.15	-4.8 ± 0.52
Leptogryllus (n=1, 4, 3)	-25.2 ± *	-25.7 ± 0.37	-25.6 ± 0.28	-5.4 ± *	-4.6 ± 0.24	-6.2 ± 0.54
Collembola (n=3, 3, 3)	-26.7 ± 0.12[a,b]	-27.2 ± 0.10[a]	-26.0 ± 0.22[b]	-5.5 ± 0.32	-6.5 ± 0.05	-5.9 ± 0.30
Metrosideros (n=3, 3, 3)	-29.4 ± 0.06[a]	-29.5 ± 0.21[a,b]	-30.0 ± 0.07[b]	-7.1 ± 0.32	-5.9 ± 0.16	-7.1 ± 0.82
Cibotium (n=3, 3, 3)	-30.6 ± 0.84[a,b]	-30.7 ± 0.17[a]	-28.5 ± 0.07[b]	-5.4 ± 1.06	-6.5 ± 0.31	-6.7 ± 0.17

Table 3. One-way ANOVA results of each taxon comparing stable isotopes $\delta^{13}C$ and $\delta^{15}N$ across sites. *Blackburnia hawaiiensis* was significantly different in $\delta^{15}N$ (p=0.0012). *Cibotium, Metrosideros*, and Collembola were significantly different across sites in $\delta^{13}C$ (p=0.0215; 0.0375; 0.0053). Significant P-values are bolded.

	$\delta^{15}N$			**$\delta^{13}C$**		
Taxa	DF	F-value	P-value	DF	F-value	P-value
B. hawaiiensis	2; 10	14.09	**0.0012**	2; 10	2.229	0.158
Pagiopalus	2; 9	0.748	0.501	2; 9	2.673	0.123
Laupala	2; 11	3.223	0.0791	2; 11	0.755	0.493
Cibotium	2; 6	1.063	0.402	2; 6	6.489	**0.0316**
Nabis	2; 7	3.324	0.089	2; 7	1.758	0.233
Metrosideros	2; 6	1.579	0.281	2; 6	5.959	**0.0375**
Leptogryllus	2; 5	4.657	0.0721	2; 5	0.253	0.786
Collembola	2; 6	3.71	0.0894	2; 6	1.419	**0.0053**

Table 4. Estimated trophic level of consumers at each site. Taxa are listed in estimated trophic position decreasing from top to bottom. Trophic position across all sites was estimated using $[(\delta^{15}N_{consumer}-^{15}N_{baseline}) /3.4] + 2$ with Collembola as the baseline (McNabb et al. 2001; Cabana & Rasmussen 1996).

Taxa	Ka‘iholena	Pu‘u Maka‘ala	Thurston
B. hawaiiensis	3.4	3.5	2.9
Nabis	3.2	3.0	2.9
Pagiopalus	2.8	3.1	2.8
Laupala	2.7	2.8	2.4
Leptogryllus	2.2	2.4	1.9

Table 5. Average $\delta^{13}C$ and $\delta^{15}N$ isotope values (± 1SE) across sites and estimated trophic position for *B. hawaiiensis, Pagiopalus, Nabis, Laupala, Leptogryllus, Collembola, Metrosideros, and Cibotium.* Number of samples analyzed (n) of each taxa are listed. Taxa are listed in estimated trophic position decreasing from top to bottom. Trophic position across all sites was estimated using $[(\delta^{15}N_{consumer}-{}^{15}N_{baseline}) /3.4] + 2$ with Collembola as the baseline (McNabb et al. 2001; Cabana & Rasmussen 1996). Significantly different means determined by Tukey's HSD are denoted by a superscript, means that do not share a letter are significantly different.

Taxa	Trophic Position	$\delta^{13}C$	$\delta^{15}N$
B. hawaiiensis (n= 13)	3.3	-26.5 ± 0.30^{a,b}	-1.7 ± 0.30^{a}
Pagiopalus (n=13)	3.0	-26.9 ± 0.27^{b}	-3.0 ± 0.42^{b,c}
Nabis (n=11)	2.9	-26.0 ± 0.16^{a,b}	-2.6 ± 0.25^{a,b}
Laupala (n=14)	2.6	-26.9 ± 0.25^{b}	-4.0 ± 0.29^{c,d}
Leptogryllus (n=8)	2.2	-25.6 ± 0.20^{a}	-5.3 ± 0.35^{d,e}
Collembola (n=9)	Baseline	-26.6 ± 0.19^{a,b}	-6.0 ± 0.19^{e}
Metrosideros (n=9)	---	-29.6 ± 0.12^{c}	-6.7 ± 1.79^{e}
Cibotium (n=9)	---	-29.9 ± 0.44^{c}	-6.2 ± 0.38^{e}

Table 6. Two-way ANOVA results comparing $\delta^{15}N$ across site, taxa and their interaction. Significant P-values are bolded.

	Df	Sum Sq	Mean Sq	F-value	P-value (>F)
Site	2	16.979	8.489	9.1340	< **0.0004**
Taxa	7	258.612	36.945	39.7495	< **2.2e-16**
Site * Taxa	14	17.594	1.257	1.3521	0.2044
Residuals	62	57.625	0.929		

Table 7. Two-way ANOVA results comparing $\delta^{13}C$ across site, taxa, and their interaction. Significant P-values are bolded.

	Df	Sum Sq	Mean Sq	F-value	P-value (>F)
Site	2	10.395	5.1976	8.9974	< **0.0004**
Taxa	7	164.274	23.4677	40.6243	< **2.2e-16**
Site * Taxa	14	14.691	1.0494	1.8165	0.0560
Residuals	62	35.816	0.5777		

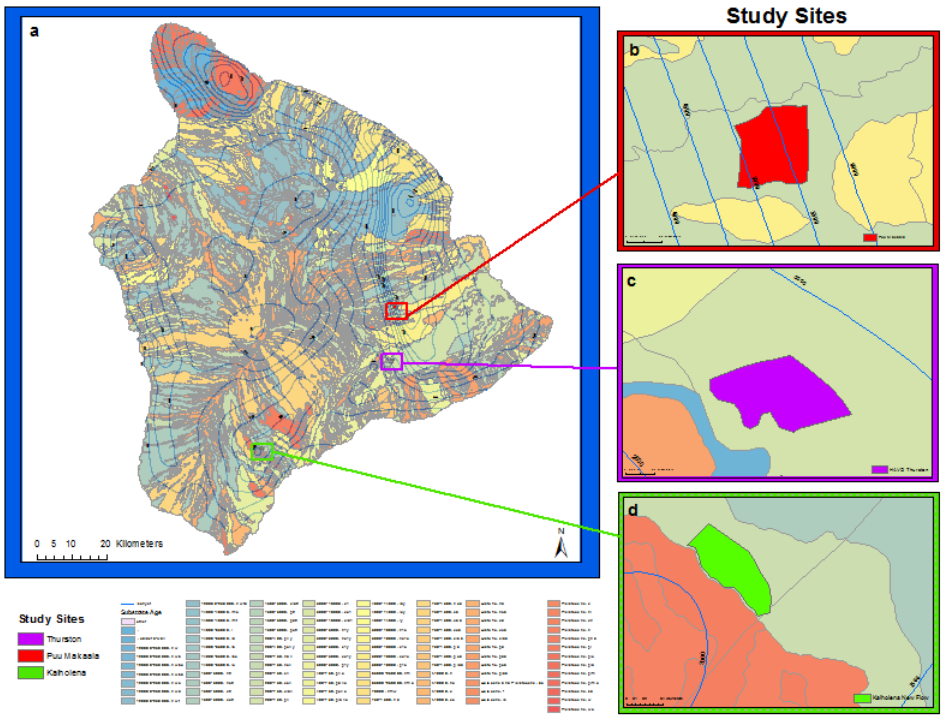
Table 8. Percent of prey sequences produced on the Ion Torrent PGM sequencer. Classification is according to BlastN hits, however, due to a shortage of the Hawaiian arthropod reference library, family, genus, and species is unreliable is most cases. The Bishop Museum's Hawaiian Arthropod Checklist was used to verify sequences (http://data.bishopmuseum.org/HBS/checklist/query.asp?grp=Arthropod). Unfortunately, specific site location of taxa listed is not yet available. These blast hits matched the checklist to the species level: *Lucilla sericata*; to genus: *Zaprionus, Schistocerca, Symus,* and *Typhlodromus*; to family: Psychodidae, Sepsidae, Nitidulidae, Papilionidae, Nymphalidae, Sessidae, and Myrmeleontidae. The families Lithodidae, Niphargidae, Bombycidae, and Proxidae have not been listed in Hawai'i, however, it is assumed that these sequences represent the most closely related family in that taxa. The Decapoda results are most likely a variation of the Amphipoda sequences or other Crustacea. Verified taxonomy is in black, blast hits that could not be verified are in gray. Taxa listed in alphabetical order.

Class	Order	Family	Genus and/or Species	Percentage of Prey Sequences
Arachnida	Acari	Phytoseiidae	*Typhlodromus pyri*	2%
Crustacea	Amphipoda	Niphargidae	*Niphargus fontanus*	20%
Crustacea	Decapoda	Lithodidae	*Paralomis elongata*	2%
Insecta	Coleoptera	Coccinellidae	*Scymnus coccivora*	2%
Insecta	Coleoptera	Nitidulidae	*Meligethes subaeneus*	3%
Insecta	Diptera	Calliphoridae	*Lucilia sericata*	5%
Insecta	Diptera	Drosophilidae	*Zaprionus sepsoides*	32%
Insecta	Diptera	Psychodidae	*Lutzomyia trapidoi*	4%
Insecta	Diptera	Sepsidae	*Zuskamira inexpectata*	2%
Insecta	Lepidoptera	Bombycidae	*Rondotia meciana*	2%
Insecta	Lepidoptera	Nymphalidae	*Magneuptychia ocypete*	4%
Insecta	Lepidoptera	Papilionidae	*Charaxes galawadiwosis*	2%
Insecta	Lepidoptera	Proxidae	*Corcyra cephalonica*	2%
Insecta	Lepidoptera	Sesiidae	*Pyropteron umbriferum*	3%
Insecta	Neuroptera	Myrmeleontidae	*Myrmeleon immanis*	2%
Insecta	Orthoptera	Acrididae	*Schistocerca albolineata*	15%

Table 9. Estimate of evolutionary divergence between consensus sequences of Folmer COI 658 bp region of arthropods from GenBank. Lepidoptera is split into two files due to the large number of sequences. The number of base differences per sequence from between sequences are shown. All positions containing gaps and missing data were eliminated. There were a total of 73 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

	Coleoptera	**Odonota**	**Hemiptera**	**Psocoptera**	**Collembola**	**Hymenoptera**	**Tricoptera**	**Orthoptera**	**Neuroptera**	**Lepidoptera A**	**Lepidoptera B**	**Diptera**
Coleoptera		1	1.4	1.9	1.4	1	0	0	1.3	1	1	0
Odonota	1		1.6	1.9	1.0	1.4	1	1	1.3	1.4	1.4	1
Hemiptera	2	3		1.9	1.9	1.6	1.4	1.4	1.8	1.6	1.6	1.4
Psocoptera	4	4	4		2.1	2.1	1.9	1.9	1.4	2.1	2.1	1.9
Collembola	2	1	4	5		1.7	1.4	1.4	1.6	1.7	1.7	1.4
Hymenoptera	1	2	3	5	3		1	1	1.6	0	0	1.0
Tricoptera	0	1	2	4	2	1		0	1.3	1.	1	0
Orthoptera	0	1	2	4	2	11	0		1.3	1.	1	0
Neuroptera	2	2	4	2	3	13	2	2		1.6	1.6	1.3
Lepidoptera A	1	2	3	5	3	30	1	1	3		0	1
Lepidoptera B	1	2	3	5	3	0	1	1	3	0		1
Diptera	0	1	2	4	2	0	0	0	2	1.0	1	

FIGURES



Figures 1a-d. (a) Study sites on Hawai‘i Island including substrate ages and rainfall isohyets. **(b)** Pu‘u Maka‘ala **(c)** Thurston **(d)** Ka‘iholena. Substrate layers were created by USGS (Wolfe & Morris 1996) and precipitation layer created by Rainfall Atlas Hawai‘i (Giambelluca et al. 2013). Ka‘iholena and Pu‘u Maka‘ala were formed from a Mauna Loa flow and Thurston was formed from a Kīlauea flow.

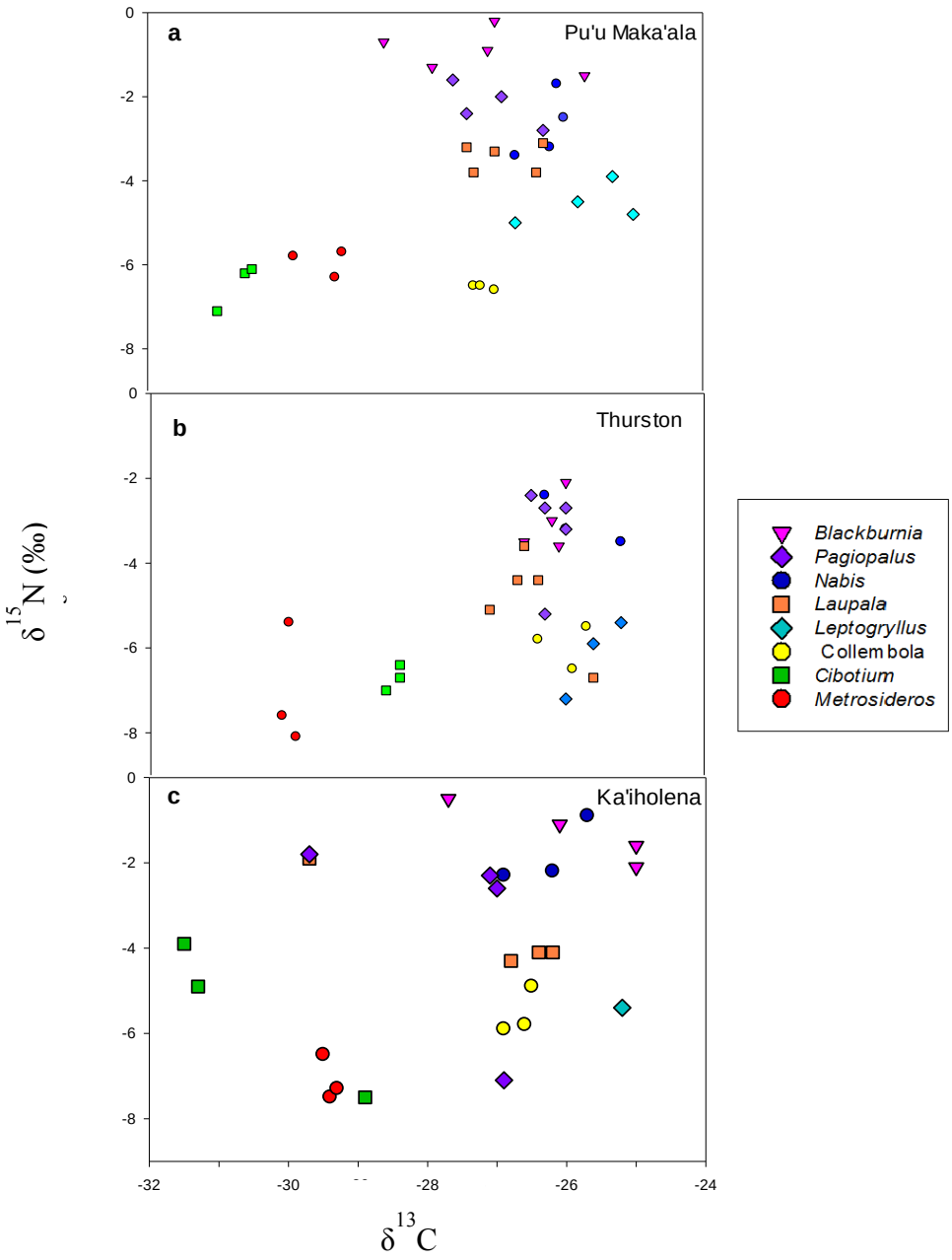


Figure 2 (a-c) Individual $\delta^{13}C$ and $\delta^{15}N$ bi-plots for arthropod samples collected from **(a)** Puʻu Makaʻala, **(b)** Thurston, and **(c)** Kaʻiholena. The trophic and dietary range of each species is shown in comparison with other species. *Pagiopalus*' trophic position varies drastically at Kaʻiholena. *B. hawaiiensis* is consistently at the highest trophic level across sites. *B. hawaiiensis* is less variable in $\delta^{15}N$ at Thurston than the other two sites.

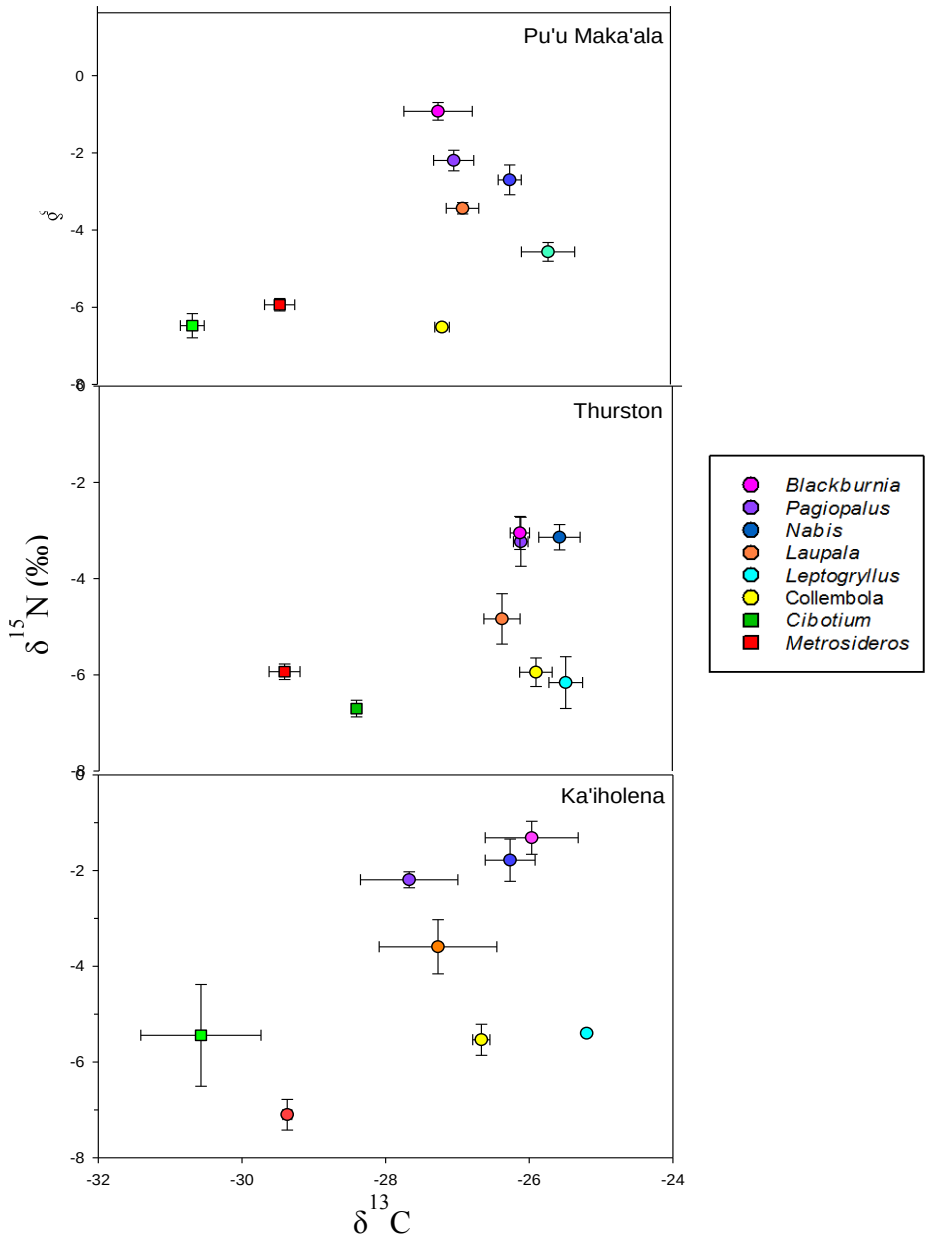


Figure 3 (a-c). $\delta^{13}C$ and $\delta^{15}N$ (mean ± 1SE) bi-plots of arthropod samples collected from **(a)** Puʻu Makaʻala, **(b)** Thurston, and **(c)** Kaʻiholena. Degree of fractionation significantly differed between taxa and site for both $\delta^{15}N$ and $\delta^{13}C$ (Two-way ANOVA $P<0.001$). $\delta^{15}N$ significantly differed across sites in *B. hawaiiensis* at Thurston (Tukey's HSD Thurston-Kaʻiholena P=0.007 Thurston-Puʻu Makaʻala P=0.001). $\delta^{13}C$ significantly differed across sites in *Cibotium* (P=0.03), *Metrosideros* (P=0.03), and Collembola (P=0.005) at Thurston (Tukey's HSD Thurston-Kaʻiholena P=0.05 Thurston-Puʻu Makaʻala P=0.04; Thurston-Kaʻiholena P=0.04 Thurston-Puʻu Makaʻala P=0.07; Thurston-Kaʻiholena P=0.05 Thurston-Puʻu Makaʻala P=0.004). Collembola served as a better baseline than *Cibotium* and *Metrosideros.*

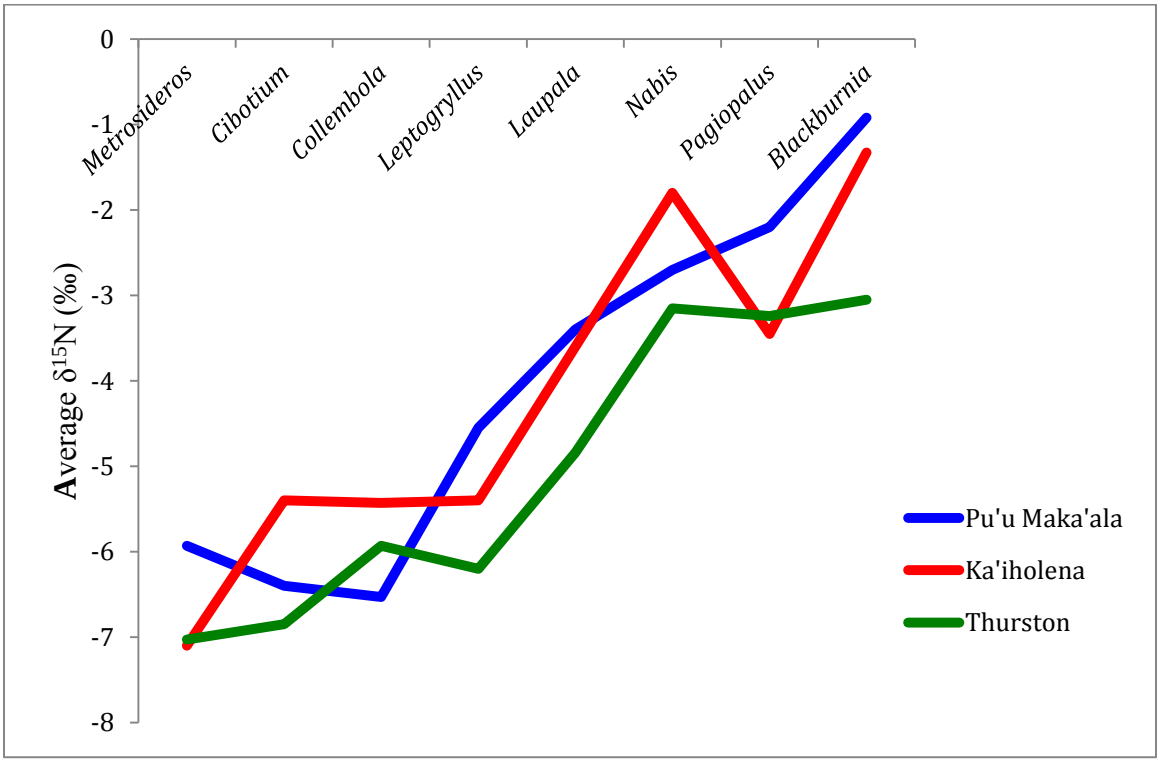


Figure 4. Interaction plot of taxa $\delta^{15}N$ values and sites for the three study sites: Puʻu Makaʻala indicated in blue, Kaʻiholena indicated in red, and Thurston indicated in green. Taxa are listed in increasing trophic level. Thurston is consistently lower in $\delta^{15}N$ than Puʻu Makaʻala and Kaʻiholena.

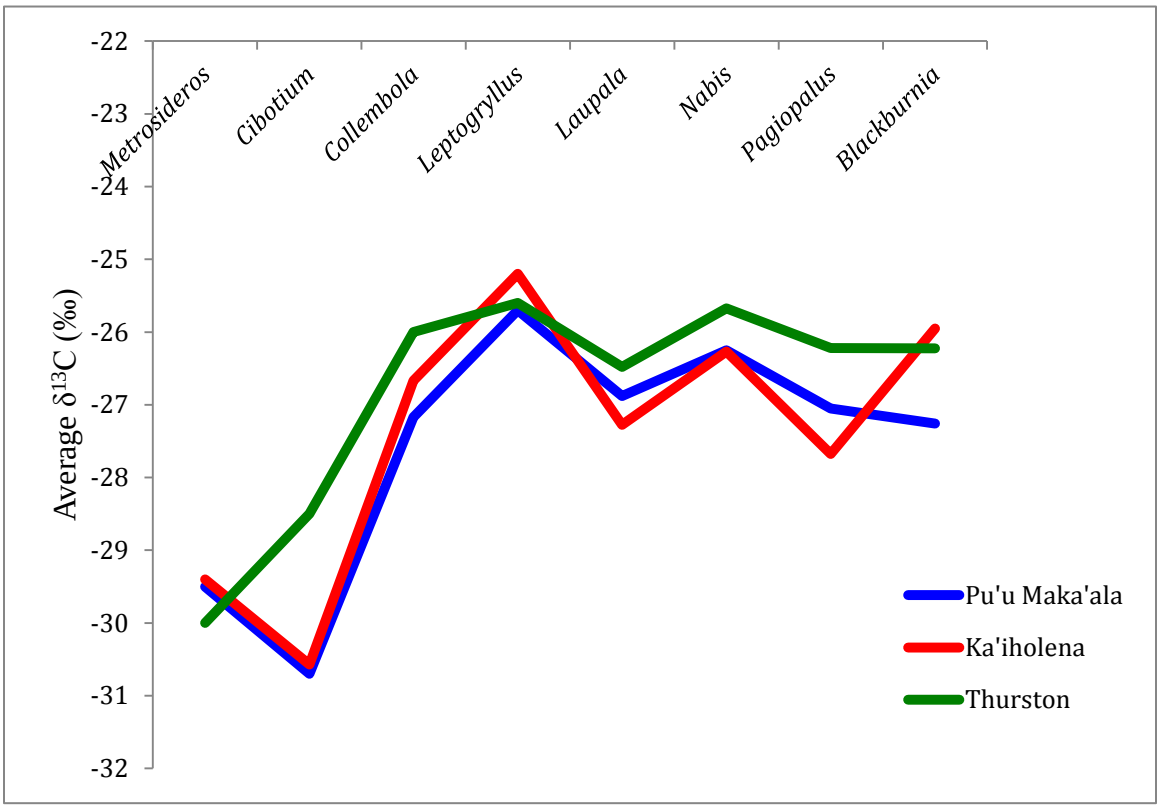


Figure 5. Interaction plot of taxa $\delta^{13}C$ values and sites for the three study sites: Puʻu Makaʻala indicated in blue, Kaʻiholena indicated in red, and Thurston indicated in green. Taxa are listed in increasing trophic level. Thurston is consistently higher in $\delta^{13}C$ than Puʻu Makaʻala and Kaʻiholena. All arthropods have similar $\delta^{13}C$ values.

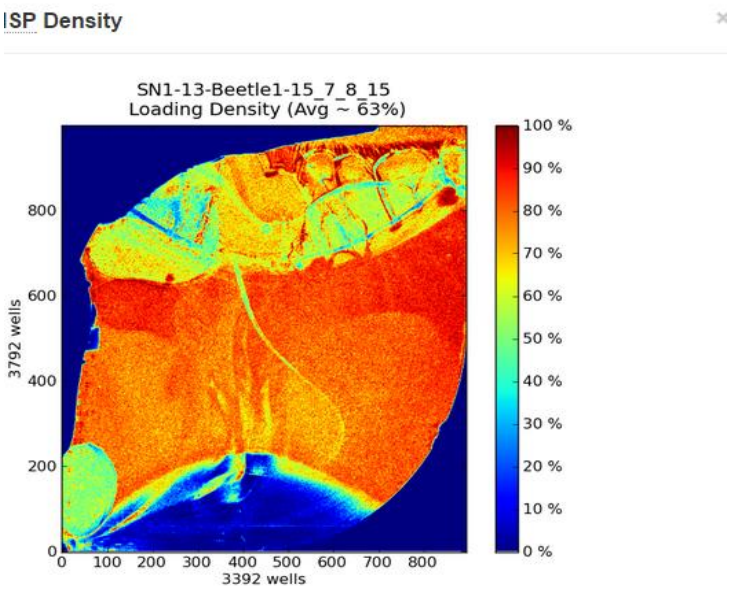


Figure 6. Ion Torrent 318v2 Chip loading density. ISP loading was 63%, 100% enrichment, 74% clonal, with a final library of 86% for a total of 4,477,826 reads.

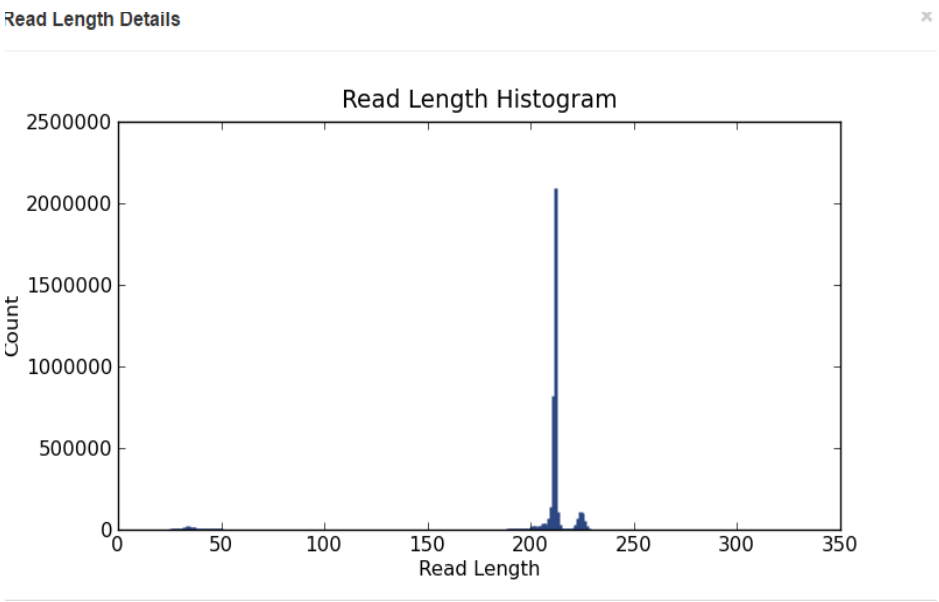


Figure 7. Histogram of total Ion Torrent PGM reads showing the amplified product in base pairs (x-axis) by the number of sequences produced (y-axis). The majority of reads were in the targeted region ~220 bp seen in the second peak.

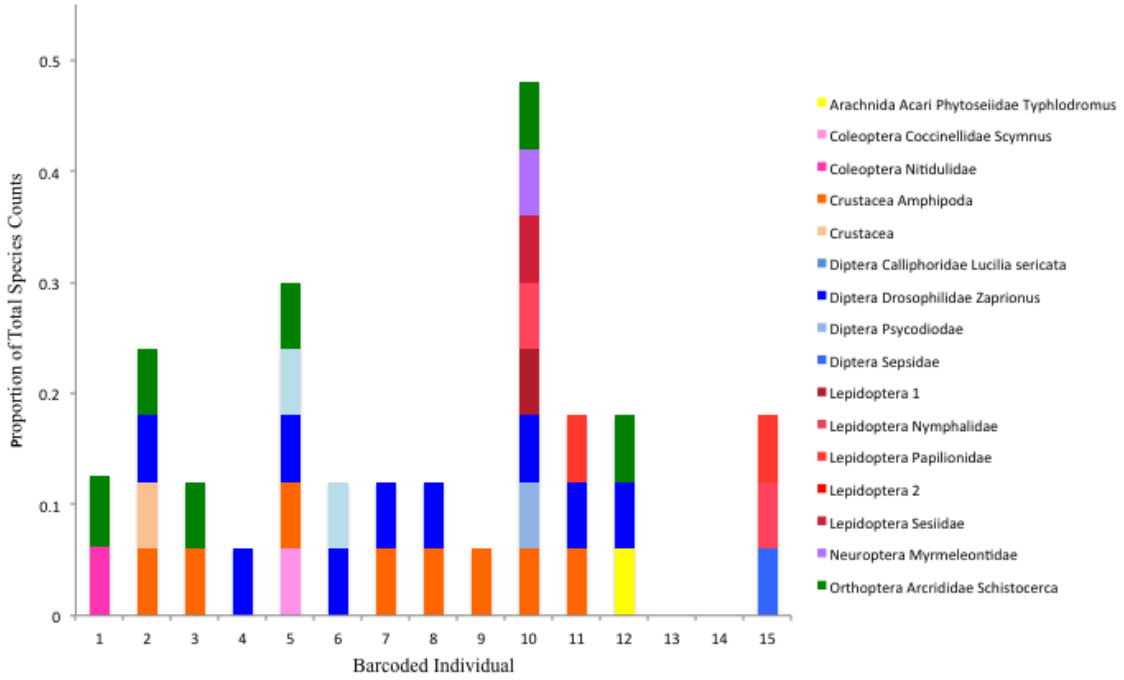


Figure 8. Taxa richness detected in each barcoded *B. hawaiiensis* gut content sample obtained from the analysis of the Ion Torrent data. Samples 1-5 are from the Kaʻiholena site, samples 6-10 are from the Thurston site, and samples 11-15 are from the Puʻu Makaʻala site. Samples 13 and 14 had no prey species found in the gut contents.

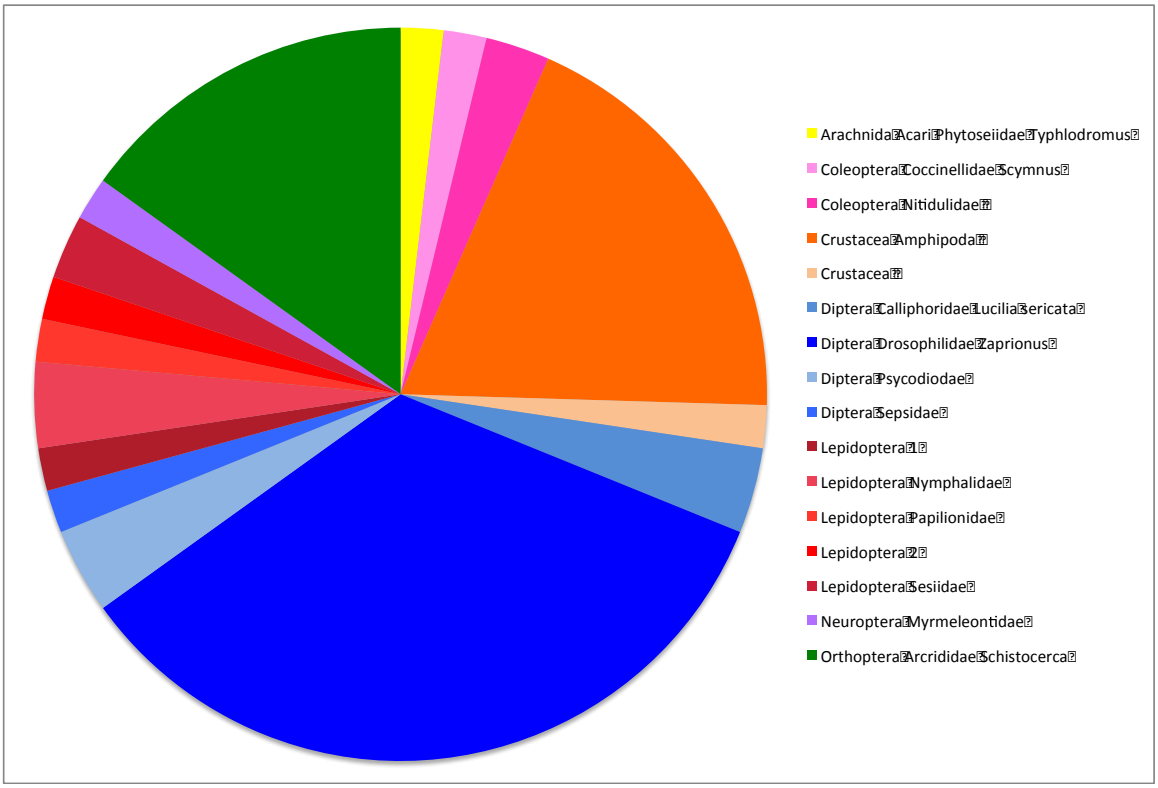


Figure 9. Prey sequences detected in *B. hawaiiensis* gut contents across all three sites via the Ion Torrent. Sequences included taxa from Arachnida, Coleoptera, Crustacea, Diptera, Lepidoptera, Neuroptera, and Orthoptera. Colors are organized by class for those taxa not in Insecta and order for those Insecta.

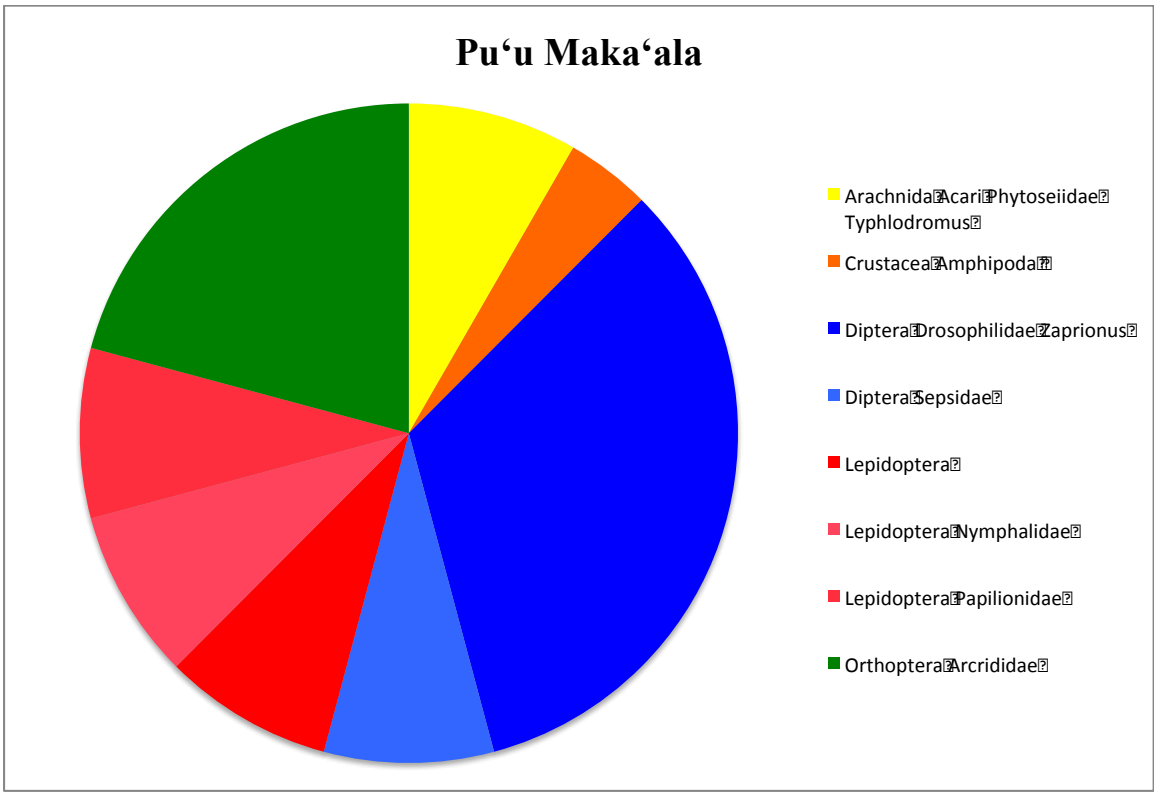


Figure 10. Gut content prey sequences of *B. hawaiiensis* at Pu‘u Maka‘ala detected via the Ion Torrent. Taxa included Arachnida, Crustacea, Diptera, Lepidoptera, and Orthoptera. Colors are organized by class for those taxa not in Insecta and order for those Insecta.

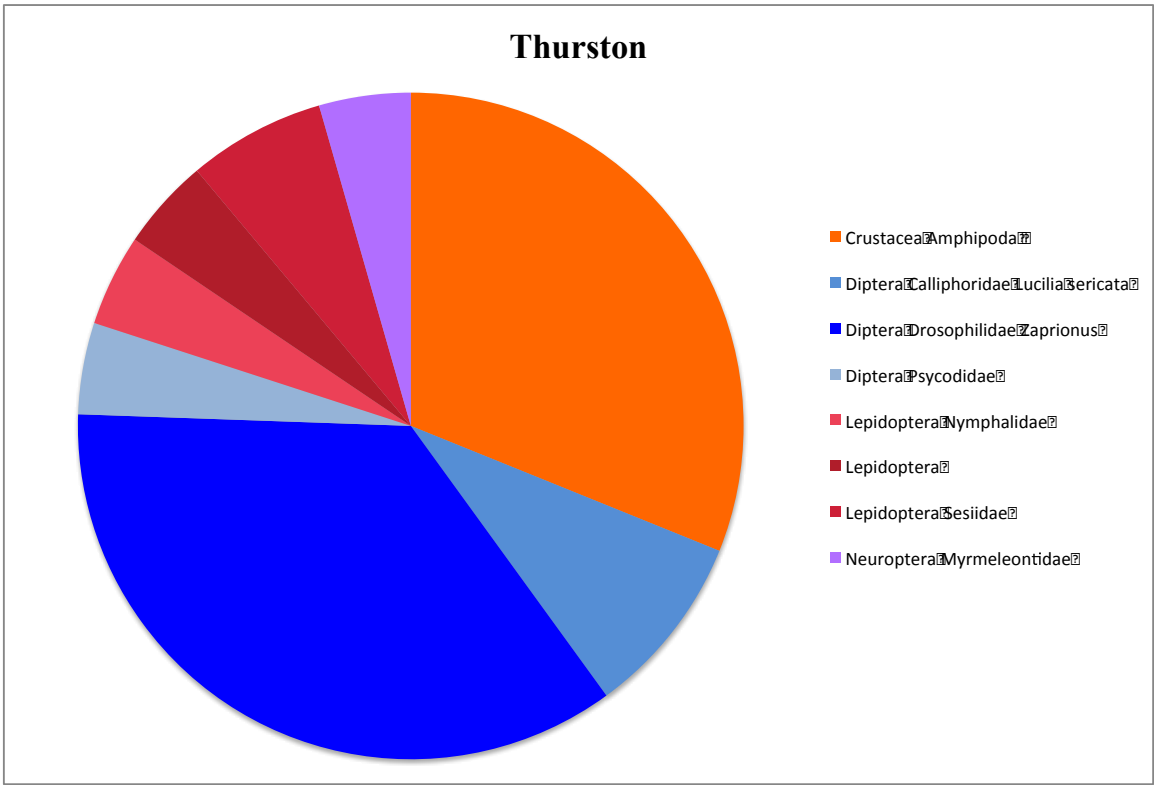


Figure 11. Gut content prey sequences of *B. hawaiiensis* at Thurston detected via the Ion Torrent. Taxa included Crustacea, Diptera, Lepidoptera, and Neuroptera. Colors are organized by class for those taxa not in Insecta and order for those Insecta.

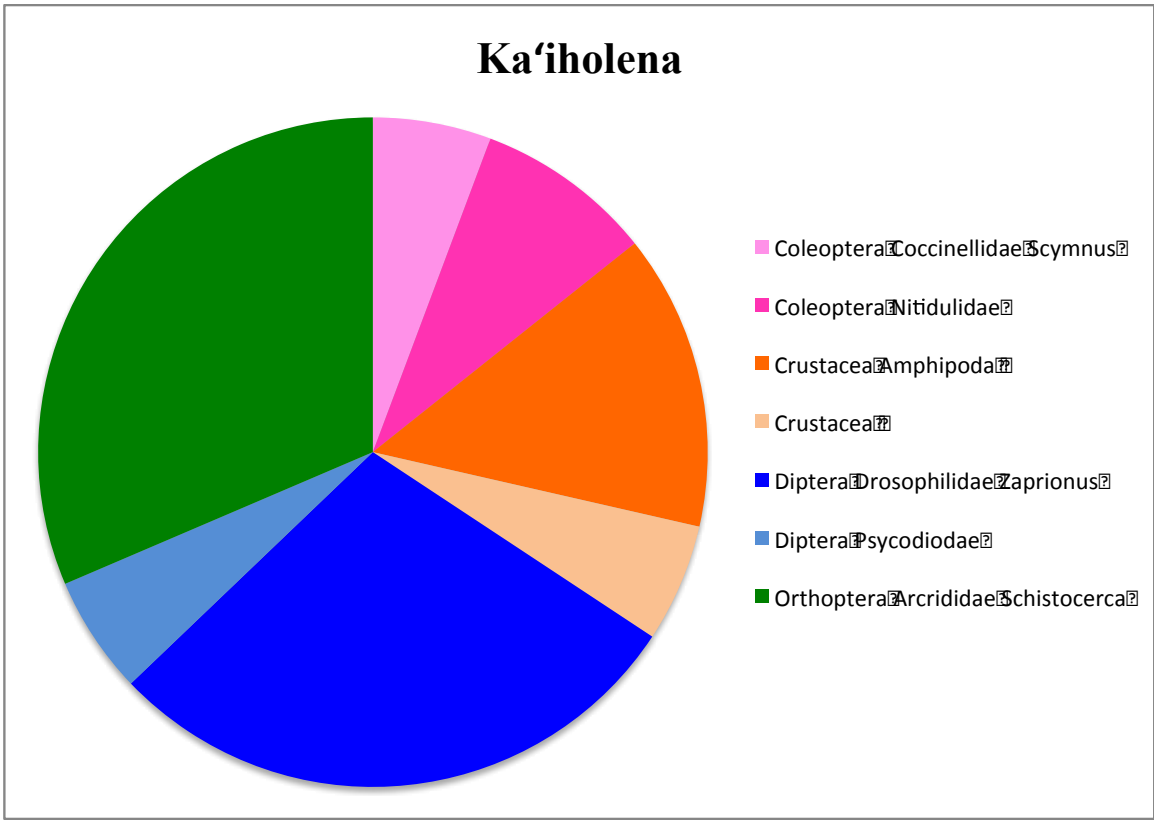


Figure 12. Gut content prey sequences of *B. hawaiiensis* at Ka‘iholena detected via the Ion Torrent. Taxa included Coleoptera, Crustacea, Diptera, and Orthoptera. Colors are organized by class for those taxa not in Insecta and order for those Insecta.

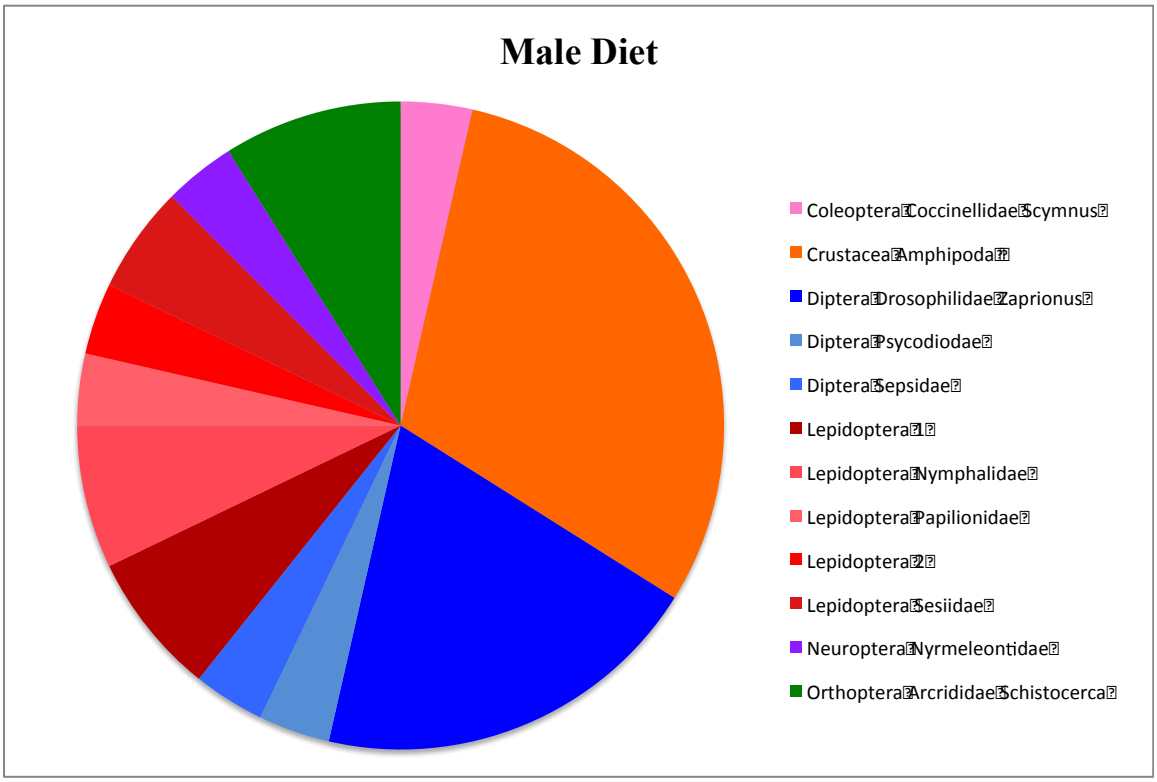


Figure 13. Gut content prey sequences found across all three sites in the *B. hawaiiensis* male individuals. Eight male individuals were sequenced and 7 contained prey sequences. Lepidoptera was found in nearly a quarter of the sequences, and not found in the female diet. Taxa included Coleoptera, Crustacea, Diptera, Lepidoptera, Neuroptera, and Orthoptera. Colors are organized by class for those taxa not in Insecta and order for those Insecta.

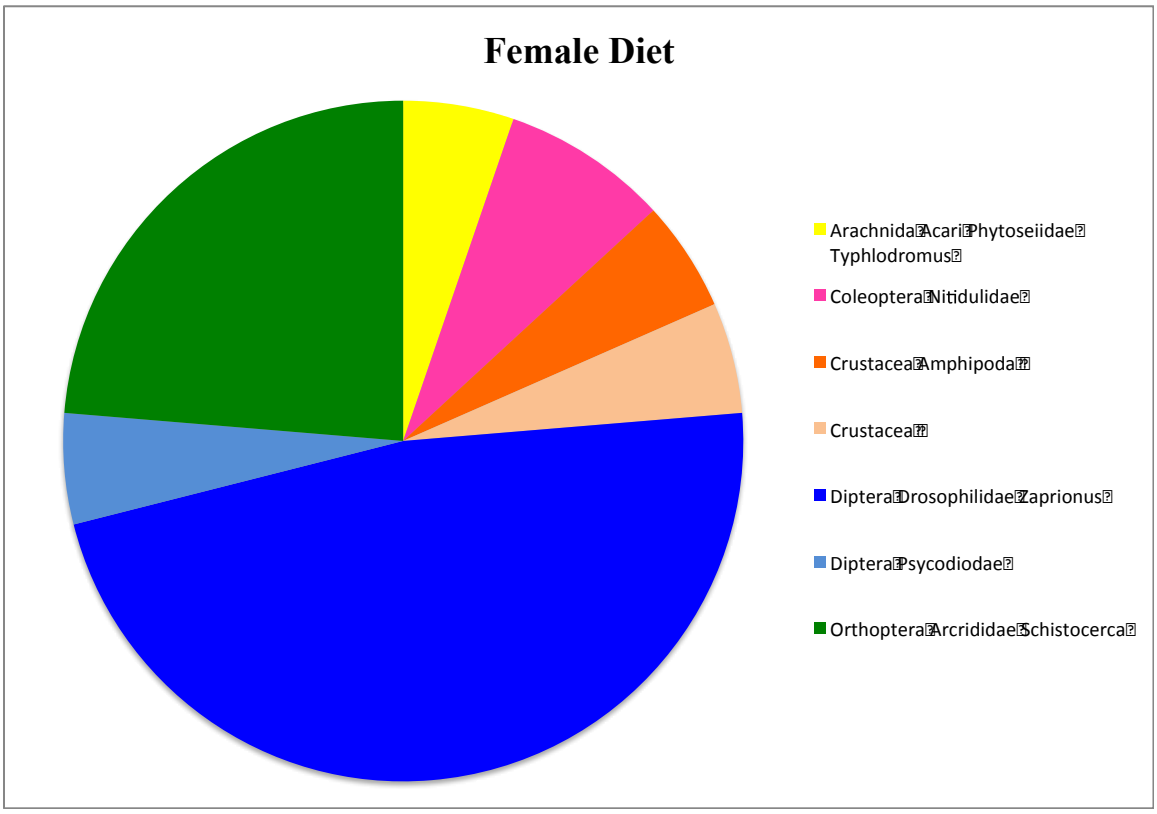


Figure 14. Gut content prey sequences found across all three sites in the *B. hawaiiensis* female individuals. Seven female individuals were sequenced, and 6 contained prey sequences. Diptera was found in over 50% of the prey sequences. Taxa included Arachnida, Coleoptera, Crustacea, Diptera, and Orthoptera. Colors are organized by class for those taxa not in Insecta and order for those Insecta.

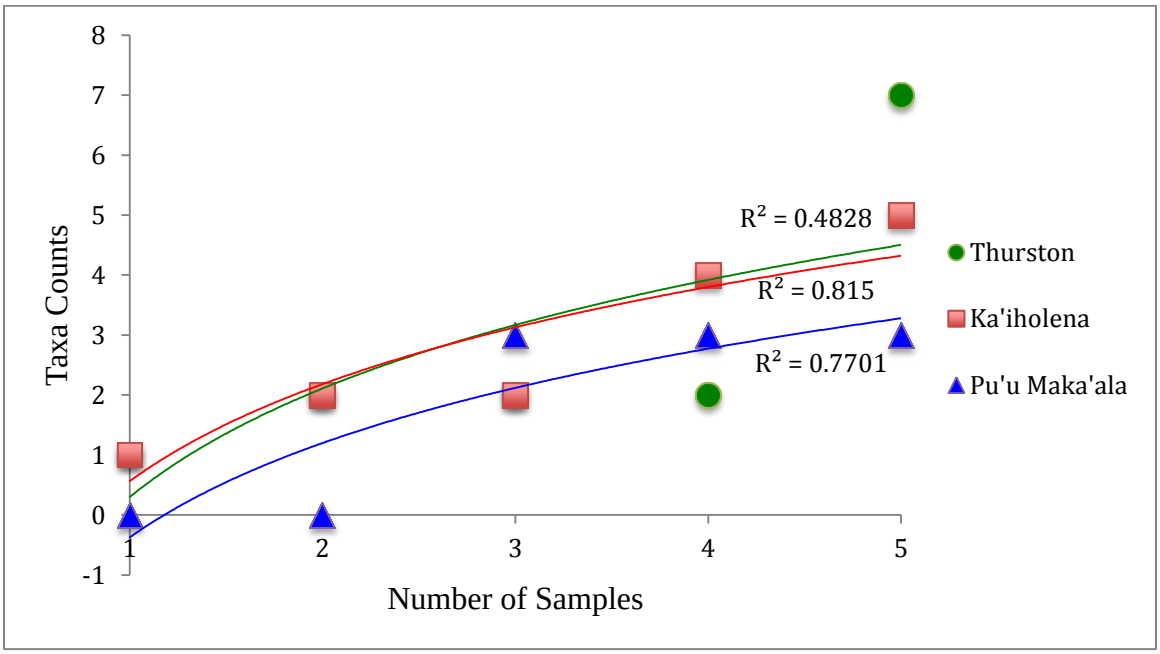
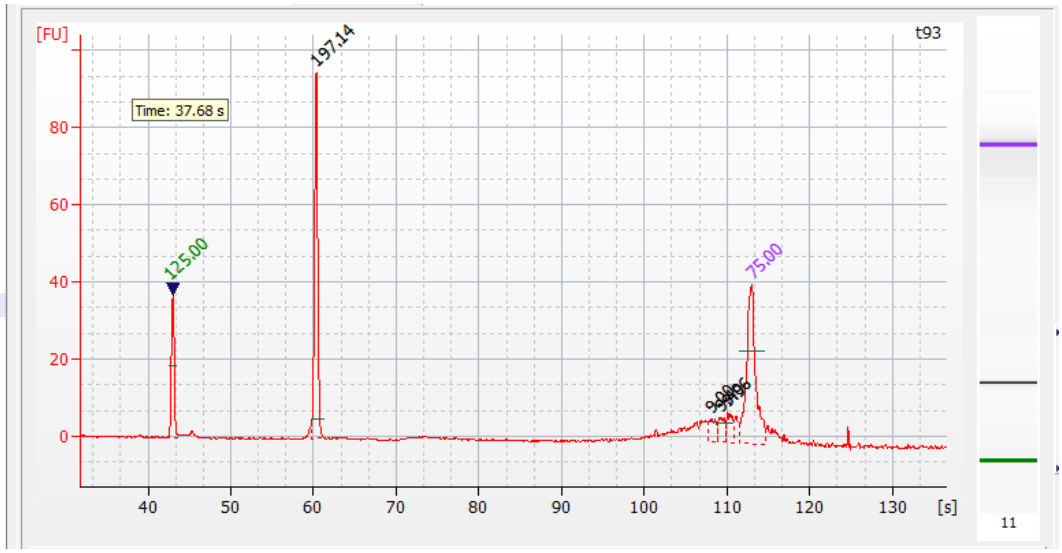
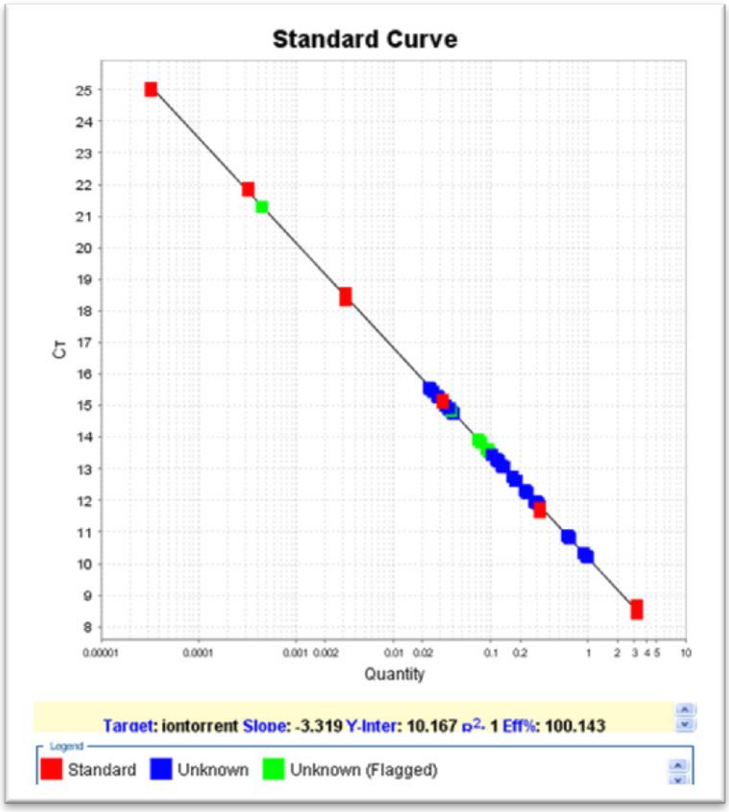


Figure 15. Rarefaction analyses of species counts per gut content sample at the three study sites. Pu‘u Maka‘ala indicated in blue (R^2=0.77), Ka‘iholena indicated in red (R^2=0.82), and Thurston indicated in green (R^2=0.48).

APPENDICES



Appendix 1. Example of acceptable Bioanalyzer results for use on the Ion Torrent system. X-axis is DNA in pM and y-axis is seconds. Peaks shown are as follows: lower standard marker, test DNA in question, and upper standard marker.



Appendix 2. Example of qPCR standard curve used to determine optimal DNA loading for emulsion PCR. X-axis is quanitity of DNA and Y-axis is temperature melting point. Red dots are the standard, blue dots the unknown DNA, and green are flagged unknown DNA.

LITERATURE CITED

Allen, J.A. 1988. Frequency-dependent selection by predators. Philosophical Transitions of the Royal Society of London, Series B 319: 486-503.

Allen, R.T. 1979. The occurrence and importance of ground beetles in agricultural and surrounding habitats. Pages 485-505 *in*: Erwin, T.L., G.E. Ball, and D.R. Whitehead (eds.). *Carabid Beetles: Their Evolution, Natural History and Classification*. Dr. W. Junk Publishing, The Hague-Boston-London.

Anslan, S. and L. Tedersoo. 2015. Performance of cytochrome c oxidase subunit I (COI), ribosomal DNA Large Subunit (LSU) and Internal Transcribed Spacer 2 (ITS2) in DNA barcoding of Collembola. European Journal of Soil Biology 69: 1-7.

Austin, A. T. and P. M. Vitousek. 1998. Nutrient dynamics on precipitation gradient in Hawai‘i. Oecologia 113: 519-529.

Ball, G. E., and Y. Bousquet. 2001. CARABIDAE Latreille. Pages 32-132 *in* Arnett, R. H. Jr. and M. C. Thomas (eds.). *American Beetles* Volume 1 (Archostemata, Myxophaga, Adephaga, Polyphaga: Staphylinifornia). CRC Press, Boca Raton, FL, USA.

Banko, P.C., R. W. Peck, K.W. Brinck, and D. L. Leonard. 2015. Richness, diversity, and similarity of arthropod prey consumed by a community of Hawaiian forest birds. Hawai‘i Cooperative Studies Unit Technical Report HCSU-066.

Baum, J.K. and B. Worm. 2009. Cascading top-down effects of changing oceanic predator abundances. Journal of Animal Ecology 78: 699-714.

Bilde, T. and S. Toft. 1997. Consumption by carabid beetles of three cereal aphid species relative to other prey types. Entomophage 42: 21-32.

Blake, S., G.N. Foster, G.E.J. Fischer, G.L. Ligertwood. 1996. Effects of management practices on the ground beetle faunas of newly established wildflower meadows in southern Scotland. Annales Zoologici Fennici 33: 139-147.

Boecklen, W. J., C.T. Yarnes, B.A. Cook, A. C. James. 2011. On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution, and Systematics 43: 411-440.

Bohmann, K., A. Monadjem, C. Lehmkuhl Noer, M. Rasmussen, M. R. K. Zeale, E. Clare, G. Jones, E. Willerslev, and M. Thomas P. Gilbert. 2011. Molecular dietary analysis of two African free-tailed bats (molossidae) using high throughput sequencing. PLoS One 6:e21441.

Borror, D. J. and R. E. White. 1970. *A Field Guide to the Insects of American North of Mexico*. Houghton Mifflin Company, Boston.

Bridwell, J. C. 1918. Notes on the habits of *Brosconymus optatus* Sharp (Carabidae). Proceedings of the Hawaiian Entomological Society 3: 391-392.

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

Cabin, R.J. 2013. *Restoring Paradise: Rethinking and Rebuilding Nature in Hawai'i*. University of Hawai'i Press, Honolulu, Hawai'i.

Clague, D.A. 1996. The growth and subsidence of the Hawaiian-Emperor volcanic chain. Pages 35-50 *in* Keast, A. and S.E. Miller (eds). *The origin and Evolution of Pacific Island Biotas, New Guinea to Eastern Polynesia: Patterns and Processes.* SPB Academic Publishing, Amsterdam.

Cock P.J.A., B. A. Grüning, K. Paszkiewicz, and L. Pritchard. 2013. Galaxy tools and workflows for sequence analysis with applications in molecular plant pathology. PeerJ 1:e167 https://dx.doi.org/10.7717/peerj.167

Cooke, C.J. Montague, and Y. Kondo. 1960. Revision of Tornatellinidae and Achatinellidae (Gastropoda, Pulmonata). B.P. Bishop Museum Bulletin 221:1-303.

Craig, H. 1953. The geochemistry of the stable isotopes of carbon. Geochimica et Cosmochimica Acta 3:53-92.

Darlington, P.J., Jr. 1943. Carabidae of mountains and islands: data on the evolution of isolated faunas, and on atrophy of wings. Ecological Monographs 13: 37-61.

Davies, K.F. and C.R. Margules. 1998. Effects of habitat fragmentation on carabid beetles: experimental evidence. Journal of Animal Ecology 67: 460-471.

Deagle, B. E., A. C. Thomas, A. K. Shaffer, A. W. Trites, and S. N. Jarman. 2013. Quantifying sequence proportions in DNA-based diet study using Ion Torrent amplicon sequencing: which counts count? Molecular Ecology Resources 13: 620-633.

Deagle, B.E., A. Chiaradia, J. McInnes, S.N. Jarman. 2010. Pyrosequencing fecal DNA to determine diet of little penguins: is what goes in what comes out? Conservation Genetics 11: 2039-2048.

Dice, L. R. 1945. Measures of the amount of ecologic association between species. Ecological Society of America 26: 297-302.

Dinter, A. 2009. Intraguild predation between erigonid spiders, lacewing larvae and carabids. Journal of Applied Entomology 122: 165-167.

Dobson, J. P., W. M. Roberts, D. S. Wilcove. 1997. Geographic distribution of endangered species in the United States. Science 275: 550-553.

Dufrene, M. and P. Legendre. 1997. Species assemblages and indicator species: he need for a flexible asymmetrical approach. Ecological Monographs 67:345-366.

Ekbom, B. 1992. Diet, fecundity and egg size in some polyphagous predatory carabid beetles. Entomologia Experimentalis et Applicata 65: 129-140.

Eyre, M.D. and M.L. Luff. 1990. A preliminary classification of European grassland habitats using carabid beetles. Pages 227-236 *in* Stork, N.E. (ed.). *The Role of Ground Beetles in Ecological and Environmental Studies.* Intercept, Andover, U.K.

Fawki, S. and S. Toft. 2005. Food preferences and the value of animal food for the carabid beetle *Amara similata* (Gyll.) (Col., Carabidae). Journal of Applied Entomology 129: 551-556.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294-299.

Fry, B. 2006. *Stable Isotope Ecology*. Springer Science+Business Media, NY, USA.

Gennis, R.B. 1992. Site-directed mutagenesis studies on subunit I of the aa3-type cytochrome c oxidase of *Rhodobacter sphaeroides*: a brief review of progress to date. Biochimica et Biophysica Acta 1101: 184-187.

Giambelluca, T.W., Q. Chen, A.G. Frazier, J.P. Price, Y.-L. Chen, P.-S. Chu, J.K. Eischeid, and D.M. Delparte. 2013. Online Rainfall Atlas of Hawai'i. Bulletin of the American Meteorological Society 94: 313-316, doi: 10.1175/BAMS-D-11-00228.1.

Giambelluca, T.W., X. Shuai, M.L. Barnes, R.J. Alliss, R.J. Longman, T. Miura, Q. Chen, A.G. Frazier, R.G. Mudd, L. Cuo, and A.D. Businger. 2014. Evapotranspiration of Hawai'i. Final report submitted to the U.S. Army Corps of Engineers—Honolulu District, and the Commission on Water Resource Management, State of Hawai'i.

Gillespie, R.G., H.B. Croom, G.L. Hasty. 1997. Phylogenetic relationships and adaptive shifts among major clades of Tetragnatha spiders (Araneae: Tetragnathidae) in Hawai'i. Pacific Science 51: 380-394.

Gomez-Polo, P., O. Alomar, C. Castane, T. Aznar-Fernandez, J.G. Lundgren, J. Piñol, and N. Agusti. 2015. Understanding trophic interactions of *Orius spp*. (Hemiptera:Anthocoridae) in lettuce crops by molecular methods. Pest Management Science doi 10.1002/ps.3989.

Gratton, C. and A. E. Forbes. 2005. Changes in δ13C stable isotopes in multiple tissues of insect predators fed isotopically distinct prey. Oecologia 147: 615-624.

Hadfield, M.G. and S. E. Miller. 1989. Demographic studies on Hawai‘i’s endangered tree snails: *Partulina proxima*. Pacific Science 43: 1-16.

Hagley, E.A.C., N.J. Holliday, and D.R. Barber. 1982. Laboratory studies of the food preferences of some orchard carabids (Coleoptera: Carabidae). The Canadian Entomologist 114: 431-437.

Hajibabaei, M. S. Shokralla, X. Zhou, G.A.C. Singer, and D.J. Baird. 2011. Environmental barcoding: a next-generation sequencing approach for biomonitoring applications using river Benthos. PLoS ONE 6:e17497.

Harrison, J.F., H. Arthur Woods, and S.P. Roberts. 2012. *Ecological and Environmental Physiology of Insects*. Oxford University Press, Oxford, New York.

Hatteland, B.A. 2010. Predation by carabid beetles (Coleoptera, Carabidae) on the invasive Iberian slug *Arion lusitanicus*. Dissertation for the Degree Philosophiae Doctor, University of Bergen, Norway.

Heimpel, G.E., J.A. Rosenheim, and M. Mangel. 1997. Predation on adult *Aphytis* parasitoids in the field. Oecologia 110:346-352.

Holling, C.S. 1978. *International series on applied systems analysis: Adaptive environmental assessment and management. Vol. 3*. John Wiley and Sons, New York.

Honek, A. Z. Martinkova, and V. Jarosik. 2003. Ground beetles (Carabidae) as seed predators. European Journal of Entomology 100: 531-544.

Hood-Nowotny, R. and B. G. J. Knols. 2007. Stable isotope methods in biological and ecological studies of arthropods. Entomologia Experimentalis et Applicata 124: 3-16.

Hoogendoorn, M. and G. E. Heimpel. 2001. PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. Molecular Ecology 10: 2059-2067.

Howarth, F.G. and W.P. Mull. 1992. *Hawaiian Insects and Their Kin.* University of Hawai‘i Press, Honolulu, Hawai‘i.

Hyodo, F. 2015. Use of stable carbon and nitrogen isotopes in insect trophic ecology. Entomological Science 18: 295-312.

James, H.F. and D. A. Burney. 1997. The diet and ecology of Hawai‘i’s extinct flightless waterfowl: evidence from coprolites. Biological Journal of the Linnean Society 62: 279-297.

Jaskula, R. and A. Sosynska-Maj 2011. What do we know about winter active ground beetles (Coleoptera, Carabidae) in Central and Northern Europe? Zookeys 100: 517-532.

Kaneshiro, K.Y. and Boake, C.R.B. 1987. Sexual selection and speciation: Issues raised by Hawaiian Drosophilids. Trends in Ecology and Evolution 2: 207-211.

Kaneshiro, K. Y. and F. C. Val. 1977. Natural hybridization between a sympatric pair of Hawaiian Drosophila. The American Naturalist 11:897-902.

King, R.A., I. P. Vaughan, J.R. Bell, D. A. Bohan, and W.O.C. Symondson. 2010. Prey choice by carabid beetles feeding on an earthworm community analyzed using species- and lineage-specific PCR primers. Molecular Ecology 19: 1721-1732.

Kitayama, K., D. Mueller-Dombois, P. M. Vitousek. 1995. Primary succession of Hawaiian montane rain forest on a chronosequence of eight lava flows. Journal of Vegetation Science 6: 211-222.

Kremen, C., R. K. Colwell, T. L. Erwin, D. D. Murphy, R. F. Noss, and M. A. Sanjayan. 1993. Terrestrial Arthropods: Their Use in Conservation Planning. Conservation Biology, 7: 796-808.

Krushelnycky, P.D., and R.G. Gillespie. 2010. Correlates of vulnerability among arthropod species threatened by invasive plants. Biodiversity Conservation 19: 1971-1988.

Krushelnycky, P.D., R.G. Gillespie, L.L. Loope, and J.K. Liebherr. 2005. Rediscovery and uncertain future of high-elevation Haleakala carabid beetles (Coleoptera). Pacific Science 59: 399-410.

Larsson, S.G. 1939. Development type and development times of Danish carabids. Entomologiske Meddelelse 20: 227-560.

Leblanc, L. P. M. O'Grady, D. Rubinoff, S. L. Montgomery. 2009. New immigrant Drosophilidae and a Checklist of the established immigrant species. Proceedings of the Hawaiian Entomological Society 41: 121-127.

Lee, J. C. and D. L. Edwards. 2011. Impacts of predatory carabids on below- and above-ground pests and yield in strawberry. Biocontrol doi 10.1007/s10525-011-9425-z.

Li, W. and A. Godzik. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics Applications Note 22: 1658-1659.

Liebherr, J.K. 2009. Native and alien Carabidae (Coleoptera) share Lanaʻi, an ecologically devastated island. The Coleopterists Bulletin 63:383-411.

Liebherr, J.K. 2006. Hawaiian *Blackburnia* beetles (Coleoptera: Carabidae, Platynini): Patterns of specialization with implications for conservation. Mitt. Dtsch. Ges. Allg. Angew. Ent. 15:57-62.

Liebherr, J.K. 2000. The unity of characters: ecological and morphological specialization in larvae of Hawaiian Platynine Carabidae (Coleoptera). Invertebrate Taxonomy 14: 931-940.

Liebherr, J.K. and P.D. Krushelnycky. 2007. Unfortunate encounters? Novel interaction of native *Mecyclothorax,* alien *Trechus obtusus* (Coleoptera: Carabidae), and Argentine ant (*Linepithema humnile,* Hymenoptera: Formicidae) across a Hawaiian landscape. Journal of Insect Conservation 11:61-73.

Liebherr, J.K. and D. A. Polhemus. 1997. Comparisons to the century before: the legacy of R.C.L. Perkins and Fauna Hawaiiensis as the Basis for a long-term ecological monitoring program. Pacific Science 51:490-504.

Liebherr, J.K. and G.A. Samuelson. 1992. The first endemic troglobitic carabid beetles in Hawaiian lava tubes (Coleoptera: Carabidae). Pan-Pacific Entomologist 68:157-168.

Liebherr, J.K. and A.E.Z. Short. 2006. *Blackburnia riparia*, new species (Coleoptera:Carabidae, Platynini): a novel element in the Hawaiian riparian insect fauna. Journal of the New York Entomological Society 114:1-16.

Liebherr J.K. and E.C. Zimmerman. 2000. *Insects of Hawai'i : Vol. 16 Hawaiian Carabidae (Coleoptera) Part 1: Introduction and Tribe Platynini*. University of Hawai'i Press, Honolulu, Hawai'i.

Lövei, G. L. and McCambridge. 2001. Adult mortality and minimum lifespan of the ground beetle *Harpalus affinis* (Coleoptera: Carabidae) in New Zealand. New Zealand Journal of Zoology 29: 1-4.

Lövei, G. L. and K. D. Sunderland. 1996. Ecology and behavior of ground beetles (Coleoptera: Carabidae). Annual Review of Entomology 41:231-256.

Luff, M., M.D. Eyre, and S.P. Rushton. 1992. Classification and prediction of grassland habitats using ground beetles (Coleoptera, Carabidae). Journal of Environmental Management 35:301-315.

Lunt, D.H., D.X. Zhang, J.M. Szymura, and G.M. Hewitt. 1996. The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. Insect Molecular Biology 5: 153-165.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. Technical Notes EMBnet.Journal 17:10-12.

McGinnis, S. and T. L. Madden. 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Research 32: doi 10.1093/narlgkh435.

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.

Medeiros, M. J., J. A. Eiben, W. P. Haines, R. L. Kaholoolaa, C. B. A. King, P. D. Krushelnycky, K. N. Magnacca, D. Rubinoff, F. Starr, and K. Starr. 2013. The importance of insect monitoring to conservation actions in Hawai‘i. Proceedings of the Hawaiian Entomological Society 45: 149-166.

Menalled, F.D., R.G. Smith, T. Dauer, and T. B. Fox. 2007. Impact of agricultural management on carabid communities and weed seed predation. Agriculture, Ecosystems and Environment 118: 49-54.

Mestre, L. J. Piñol, J. A. Barrientos, X. Espadler, 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 http://dx.doi.org/10.1016/j.baae.2013.05.001.

Miller, S.E. and L.G. Eldredge. 1995. Numbers of Hawaiian species: Supplement 1. Bishop Museum Occasional Papers 45:8-17.

Mittermeirer, R.A., W.R. Turner, and F.W. Larsen. 2011. Global Biodiversity Conservation: the critical role of hotspots. Pages 3-7 *in* Zachos, F.E. and J.C. Habel (eds.). *Biodiversity Hotspots: Distribution and Protection of Conservation Priority Areas*. Springer-Verlag, Berlin Heidelberg.

Moore, J.W. and B.X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters 11: 470-480.

Mueller-Dombois, D. and H. J. Boehmer. 2013. Origin of the Hawaiian rainforest and its transition states in long-term primary succession. Biogeosciences 10: 5171-5182.

Murphy, D.D. and B.D. Noon. 1991. Coping with uncertainty in wildlife biology. Journal of Wildlife Management 55: 773-782.

Myers, N. and A. H. Knoll. 2001. The biotic crisis and the future of evolution. PNAS 98: 5389-5392.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 1999. Biodiversity hotspots for conservation priorities. Nature 403: 853-858.

Niemela, J., Y. Haila, E. Halme, T. Pajunen, and P. Puntilla. 1989. The annual activity cycle of carabid beetles in the southern Finnish taiga. Annual Zoological Fennici 26: 35-41.

Niemela, J., D. Langor, and J.R. Spence. 1993. Effects of clear-cut harvesting on boreal ground-beetle assemblages (Coleoptera: Carabidae) in western Canada. Conservation Biology 7: 551-561.

Nishida, G.M. 1992. *Hawaiian terrestrial arthropod checklist*. Biological Survey, Bishop Museum. Honolulu, HI.

O'Grady, P. and R. DeSalle. 2008. Out of Hawaii: the origin and biogeography of the genus *Scaptomyza* (Diptera: Drosophilidae). Biological Letters 4: 194-199.

Parnell, A. C., R. Inger, S. Bearhop, and A.L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. PLoS ONE 5: e9672. doi:10.1371/journal.pone.0009672. PMID:20300637.

Pearson, D.L. and F. Cassola. 1992. World-wide species richness patterns of tiger beetles (Coleopera: Cicindelidae): indicator taxon for biodiversity and conservation studies. Conservation Biology 6: 376-391.

Pedigo, L.P. 1999. *Entomology and Pest Management* 3rd Edition. Prentice Hall, Upper Saddle River, New Jersey.

Percy, D.M., A.M. Garver, W.L. Wagner, HF. James, C.W. Cunningham, S.E. Miller, and R.C. Fleisher. 2008. Progressive island colonization and ancient origin of Hawaiian *Metrosideros* (Myrtaceae). Proceedings of the Royal Society B 275:1479-1490.

Phillips, D. L., R. Inger, S. Bearhop, A. L. Jackson, J. W. Moore, A. C. Parnell, B. X. Semmens, and E. J. Ward. 2014. Best practices for use of stable isotope mixing models in food webs studies. Canadian Journal of Zoology 92: 823-835.

Pierce, B. A. 2012. *Genetics: A conceptual approach* 4th Edition. W.H. Freeman and Company, New York.

Piñol, J., V. San Andres, E.L. Clare, G. Mir, and W.O.C. Symondson. 2014a. A pragmatic approach to the analysis of diets of generalist predators: the use of next-generation sequencing with no blocking probes. Molecular Ecology Resources 14: 18-26.

Piñol, J., G. Mir, P. Gomez-Polo, and N. Agusti. 2014b. Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. Molecular Ecology Resources doi:10.1111/1755-0998.12355.

Polis, G. A., C.A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild predation: Potential competitors that eat each other. Annual Review of Ecological Systematics 20: 297-300.

Pompanon, F. B.E. Deagle, W.O.C. Symondson, D.S. Brown, S.N. Jarman, and P. Taberlet. 2012. Who is eating what: diet assessment using next generation sequencing. Molecular Ecology 21:1931-1950.

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

Powell, W., M. P. Walton, and M. A. Jervis. 1996. Populations and communities. Pages 223-292 *in* M. Jervis and N. Kidd (eds.). *Insect natural enemies: practical approaches to their study and evaluation.* Chapman & Hall, London, UK.

Price, D.K., S. Sounder, T. Russo-Tait. 2013. Sexual Selection, Epistasis and Species Boundaries in Sympatric Hawaiian Picture-winged Drosophila. Journal of Insect Behavior doi 10.1007/s10905-013-9404-6.

Price, J.P. and D.A. Clague. 2002. How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. Proceedings of the Royal Society of London 269: 2429-2435.

Raino, J. and J. Niemela. 2002. Ground beetles (Coleoptera: Carabidae) as bioindicators. Biodiversity and Conservation 12: 487-506.

Rojas, M. 1992. The species problem and conservation: what are we protecting? Conservation Biology 6: 170-178.

Rusek, J. 1998. Biodiversity of Collembola and their functional role in the ecosystem. Biodiversity and Conservation 7: 1207-1219.

Russo, C.A.M., N. Takezaki, and M. Nei. 1995. Molecular phylogeny and divergence times of Drosophilid species. Molecular Biological Evolution 12: 391-404.

Sabelis, M. W. 1992. Predatory arthropods. Pages 225-264 *in* M.J. Crawley (ed.) *Natural Enemies, the population biology of predators, parasites and diseases*. Blackwell Scientific, Oxford.

Shokralla, S. Spall, J.L. J.F. Gibson, and M. Hajibabaei. 2012. Next-generation sequencing technologies for environmental DNA research. Molecular Ecology 21:1794-1805.

Sorensen, T.J. 1948. A method of establishing groups of equal amplitude in plant sociology based on similarity of species and its application to analyses of the vegetation

on Danish commons. Biologiske Skrifter/Kongelige Danske Videnskabernes Selskab 5: 1-34.

Southwood, T.R.E. 1977. Habitat, the template for ecological strategies? Journal of Animal Ecology 46:337-365.

Stork, N.E. 1987. Adaptations of arboreal ground beetles to life in trees. Acta Phytopathologica et Entomologica Hungarica 22: 273-291.

Stork, N.E. 1993. How many species are there? Biodiversity and conservation 2:215-232.

Tamura K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725-2729.

Thiele, H.-U. 1977. *Carabid beetles in their environments*. Springer-Verlag, Berlin, Germany.

Thomas, A. C. S. N. Jarman, K. H. Haman, A. W. Trites, and B. E. Deagle. 2014. Improving accuracy of DNA diet estimates using food tissue control materials and an evaluation of proxies for digestion bias. Molecular Ecology 23: 3706-3718.

Triltsch, H. 1997. Gut contents in field sampled adults of *Coccinella septempunctata* (Col: Coccinellidae). Entomophaga 43: 125-131.

Triplehorn, C.A. and N.F. Johnson. 2005. *Study of Insects* 7th Edition. Brooks/Cole Cengage Learning, Belmont, California.

Valentini, A., F. Pompanon, and P. Taberlet. 2008. DNA barcoding for ecologists. Trends in Ecology and Evolution 24: 110-117.

Vander Zanden, M.J. and J.B. Rasmussen. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of the aquatic consumers. Ecology 80: 1396-1404.

Vestheim, H. and S.N. Jarman. 2008. Blocking primers to enhance PCR amplification of rare sequences in mixed samples – a case study on prey DNA in Antarctic krill stomachs. Frontiers in Zoology 5: 12.

Virgilio, M., K. Jordaens, F. C. Breman, T. Backeljau, M. De Meyer. 2012. Identifying insects with incomplete DNA barcode libraries, African fruit flies (Diptera: Tephritidae) as a test case. PLoS ONE 7: e31581.

Vitousek, P.M., L.R. Walker, L.D. Whiteaker, D. Mueller-Dombois, and P. A. Matson. 1987. Biological invasion by Myrica faya alters ecosystem development in Hawaii. Science 238: 802-804.

Wilson, J. J. 2012. DNA Barcodes for Insects. Pages 17-46 *in* John Kress, W. and D. L. Erickson (eds.). *DNA Barcodes Methods and Protocols*. Humana Press, NY.

Wise, D. H., D. M. Moldenhauer, and J. Hallaj. 2006. Using stable isotopes to reveal shifts in prey consumption by generalist predators. Ecological Applications 16:865-876.

Withgott, J. and S. Brennan. 2008. *Environment: The Science Behind the Stories*. Pearson Education, Inc., Boston, Massachusetts.

Wolfe, E. W. and J. Morris. 1996. Geologic Map of the Island of Hawaii. U.S. Geological Survey, Geologic Investigations Series Map I-2524-A.

Zeale, M.R.K., R. K. Butlin, G. L. A. Barker, D. C. Lees, and G. Jones. 2010. Taxon-specific PCR for DNA barcoding arthropod prey in bat feces. Molecular Ecology Resources 11:236-244.

Zimmerman, E.C. 2001. *Insects of Hawai'i, Vol. 1*. University of Hawai'i Press, Honolulu, Hawai'i.