Diet Analysis of Hawai'i Island's *Blackburnia hawaiiensis* (Coleoptera: Carabidae) using Stable Isotopes and High-Throughput Sequencing[1]

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Abstract: Determining the diet of arthropods can be difficult due to their small size and complex food webs, especially in Hawai'i, where knowledge of arthropod predator–prey interactions is sparse. The diet of the Hawai'i Island-endemic carabid beetle, *Blackburnia hawaiiensis* Sharp (Coleoptera: Carabidae) is of particular interest because of its peculiar arboreal behavior and metathoracic flight wings. Our study objective was to determine the diet of *B. hawaiiensis* in replicated, geographically separated locations by using two different yet complementary laboratory techniques: natural abundance stable isotope analysis (SIA) and high-throughput sequencing (HTS). Overall, *B. hawaiiensis* had a greater average $\delta^{15}N$ and similar $\delta^{13}C$ compared to the other arthropods sampled in this study and HTS data revealed Diptera and Lepidoptera sequences in the beetle's gut contents. These results are consistent with *B. hawaiiensis* being classified as a generalist predator. The combination of SIA and HTS are important methods for determining the diet of species within complex food webs, particularly for species that are difficult to observe in nature.

Keywords: food web, metabarcoding, Hawai'i, carabid, *B. hawaiiensis*

Understanding the diet of organisms can elucidate conservation needs and inform management. However, trophic relationships are often difficult to observe, especially when considering small, rare, or cryptic species (Gomez-Polo et al. 2015). Traditional diet studies often include direct observation of scat or gut contents, although these techniques are limited to organisms that consume indigestible structures leaving solid, identifiable remains (Hoogendoorn and Heimpel 2001). Specifically, arthropod diets and trophic positions are difficult to assign not only because of their small size but also because many are generalists, therefore creating complex food webs (Polis et al. 1989). Additionally, generalist predators can partake in 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).

With complex food webs, stable isotope analysis (SIA) can be a helpful tool for characterizing trophic relationships among organisms (Mestre et al. 2013). Particularly, $\delta^{15}N$ and $\delta^{13}C$ are well documented to be useful for assessing trophic position, where the heavier isotope accumulates with

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successive trophic levels (Cabana and Rasmussen 1996, Post 2002, Johnston et al. 2018, Kennedy et al. 2018, Kennedy et al. 2019). In order to properly estimate trophic positions in a given food web the establishment of an isotopic baseline in that given system is necessary but often difficult to discern (Post 2002, Kristensen et al. 2016). In addition, the variability in measurements in species-rich, complex food webs can make it difficult to assign trophic levels (Mestre et al. 2013). Therefore, it can be advantageous to employ a complementary technique such as the use of genetic metabarcoding of gut contents, which can yield higher prey resolution.

The ability to elucidate food webs through genetics has greatly improved with recent technological advancements (Mestre et al. 2013, Piñol et al. 2014*b*, Gomez-Polo et al. 2015). Metabarcoding, the genetic study of environmental samples utilizing universal primers and high-throughput sequencing (HTS), facilitates the identification of complex mixtures of DNA sequences (Shokralla et al. 2012). HTS 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. (2014*a*) used HTS to determine the diet of linyphiid spiders, Gomez-Polo et al. (2015) determined that of a hemipteran, and Krehenwinkel et al. (2017*a*) determined the diet of grass spiders. Although effective for determining potential prey items, HTS has limitations such as sequencing error and incomplete reference libraries (Deagle et al. 2013, Piñol et al. 2014*b*, Thomas et al. 2014). Because of the limitations of HTS, combining SIA for a time-integrated trophic structure may be advantageous, particularly for complex arthropod food webs (Vander Zanden et al. 1997).

The integrated approach of using both SIA and HTS allows for a better understanding of the feeding ecology of organisms that are difficult to rear in the lab and observe in nature. Employing these two techniques provides complementary information with two lines of diet evidence at different resolutions (Carreon-Martinez and Heath 2010, Whitaker et al. 2019). Stable isotope measurements are the result of a natural accumulation over time, while HTS identifies prey eaten within a few days or hours prior to collection.

Blackburnia hawaiiensis Sharp (Coleoptera: Carabidae) is a Hawai'i Island endemic, numerically dominant predatory insect of the Hawaiian montane forest (Liebherr and Zimmerman 2000); therefore understanding the beetle's diet could have conservation and management implications. The Hawaiian carabids are a particularly diverse fauna with 402 described endemic species descended from three colonization events: 140 *Blackburnia* (Platynini), 239 *Mecyclothorax* (Moriomorphini), and 23 *Bembidion* (Bembidiini) (Liebherr and Zimmerman 2000, Liebherr 2008*a*, Liebherr 2008*b*). Of the 140 *Blackburnia*, only 23 species in the subgenus *Colpocaccus* possess fully functioning flight wings. *Blackburnia hawaiiensis* is of interest for a diet study because it is the only *Blackburnia* on Hawai'i Island with fully functioning flight wings, allowing for increased foraging capabilities (Liebherr and Zimmerman 2000).

Worldwide, carabids are commonly considered to be opportunists, consuming a wide variety of prey items such as Diptera and Lepidoptera larvae, Collembola, and aphids (Borror and White 1970, Hagley et al. 1982, Fawki and Toft 2005). Liebherr (pers. com. 2014) observed remnants of spiders, caterpillars, and fruiting bodies of moss mats in the gut contents of *Blackburnia* spp. Although carabids are usually assumed to be generalist predators (Allen 1979, Hagley et al. 1982, Ekbom 1992, Bilde and Toft 1997, Ball and Bousquet 2001, Fawki and Toft 2005) several are phytophagous (Honek et al. 2003, Menalled et al. 2007) and oligophagous (Hatteland et al. 2011, Brandmayr and Brandmayr 2013), such as *Scaphinotus*, which feed exclusively on snails (Ober et al. 2011).

Our objective was to determine the diet of *B. hawaiiensis*, testing the hypothesis that this beetle is a generalist predator, consuming more than one arthropod order. Ultimately, we intended to enhance basic knowledge of Hawaiian arthropods where conservation efforts are hindered by a lack of taxonomic

and ecological data (Howarth and Mull 1992, Medeiros et al. 2013). We tested this hypothesis by analyzing the $\delta^{15}N$ and $\delta^{13}C$ isotope values of these beetles and other taxa collected in the same microhabitat, and by using genetic metabarcoding on the dissected gut contents of *B. hawaiiensis*.

MATERIALS AND METHODS

Site Description

Three geographically separated, replicated sites on Hawai'i Island were chosen for this study: Nāhuku (N 19° 24′; W 155° 14′), Ka'iholena (N 19° 11′; W 155° 35′), and Pu'u Maka'ala (N 19° 32′; W 155° 13′). Substrates at Nāhuku consist of Kīlauea volcanic eruptions and Ka'iholena and Pu'u Maka'ala were formed from Mauna Loa eruptions. The selection of sites was guided by Light Detection and Ranging (LIDAR), ease of access, and local inspection to control for abiotic factors: elevation range of 1120–1210 m, a substrate age of 200–750 years, and a mean annual temperature of 15.5–16 °C. The three sites were also characterized as tropical montane forest with a *Metrosideros polymorpha* canopy, tree fern (*Cibotium* spp.) subcanopy, and active populations of *B. hawaiiensis* (Figure 1). Annual rainfall was slightly higher at one of the sites; the mean annual precipitation of Ka'iholena and Nāhuku is 2,500–3,000 mm/yr while Pu'u Maka'ala is 4,500–5,700 mm/yr (Giambelluca et al. 2013). Sites were ground-truthed to select areas with low human and feral ungulate disturbance and predominantly native vegetation.

Arthropods for both molecular and stable isotope techniques were collected along fence lines for approximately 600 m in order to minimize damage to delicate native vegetation during the study. Most arthropod collections took place at dusk and dawn during several trips May–November 2014, which is the dry season when *B. hawaiiensis* is most prevalent and active in the forest (Liebherr pers. com. 2014). Supplemental SIA collection occurred in Summer 2015 to increase sample sizes. All arthropods were collected at ~1.3 m above ground in the lower forest arthropod community. Arthropods were collected by gently shaking brown *Cibotium* fronds over a beating sheet for 10 s and aspirating the arthropods into snap-cap tubes.

Arthropod Collection for Stable Isotope Analyses

Focal taxa selection for stable isotope analyses was based on the following criteria: observed in the same microhabitat as *B. hawaiiensis* (brown *Cibotium* leaves), common to all three sites, and easily identifiable in the field based on morphological characteristics. Taxa included adult *Nabis* (Hemiptera: Nabidae), *Pagiopalus* (Araneae: Philodromidae), Collembola, *Leptogryllus* (Orthoptera: Gryllidae), *Laupala* (Orthoptera: Gryllidae), and *Cibotium* (Cyatheales: Cibotiaceae) and *Metrosideros* (Myrtales: Myrtaceae) leaf litter. Specimens were sorted by taxon and kept alive for 2–4 days in order to allow their digestive tracks to clear, reducing 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 ≤48 hrs, pulverized, then placed in 8 × 5 mm tin capsules. Individuals were pooled until 0.5–1 mg dryweight of arthropod and 1–2 mg of plant material was sampled (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) by the first author 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.

Blackburnia Collection for Genetic Analyses of Gut Contents

Blackburnia hawaiiensis were transferred within 30 min after collection to 2 ml screw-top micro stand tubes containing 95% ethanol

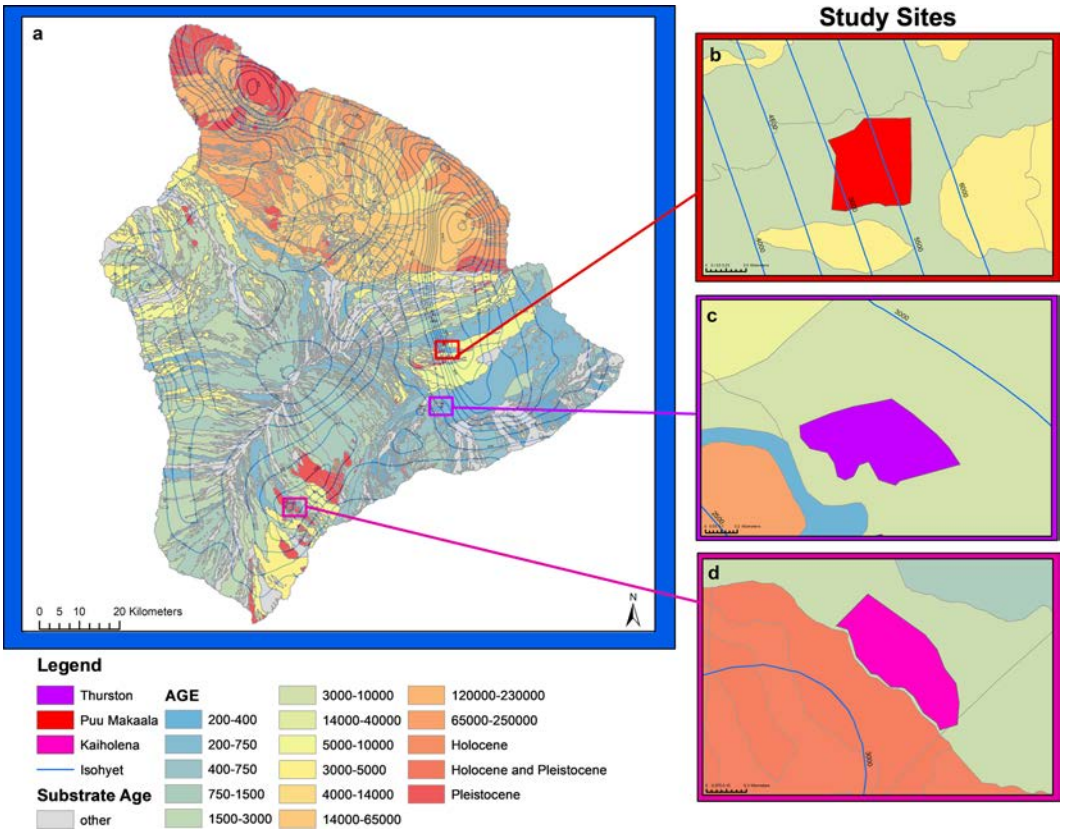

FIGURE 1. (*A*) Study sites on Hawai'i Island including substrate ages and rainfall isohyets. (*B*) Pu'u Maka'ala. (*C*) Nāhuku. (*D*) Ka'iholena. Substrate layers were created by USGS (Sherrod et al. 2007) and precipitation layer created by Rainfall Atlas Hawai'i (Giambelluca et al. 2013). Ka'iholena and Pu'u Maka'ala were formed from a Mauna Loa volcanic eruption and Nāhuku was that of Kīlauea.

(to prevent digestion of prey in the gut of the beetles) and then stored at −80 °C until further processing. *Blackburnia hawaiiensis* were identified and sexed under a Leica MZ-2500 binocular dissecting scope according to Liebherr and Zimmerman (2000).

Molecular Techniques

Primer design and PCR — A reference sequence for *B. hawaiiensis* was created by first designing Platynini carabid-specific primers using mitochondrial cytochrome-oxidase I (COI) barcode sequences obtained from GenBank for 68 Platynini species. Sequences were aligned in Geneious v. 8.1.6 (Biomatters, Aukland, NZ). Conserved regions of DNA flanking the 157-bp region of the Zeale primers (ZBJ-ArtF1c and ZBJ-ArtR2c) for gut content metabarcoding (Zeale et al. 2010, Bohmann et al. 2011) of the 68 available sequences were considered for potential primer synthesis. The Platynini primers were designed to amplify a 230-bp barcode region of carabid mitochondrial DNA (**F**: AGATATTGGAACWTTATATTTTATTTTTGG, **R**: ATCAAAAACTTATATTATTTATTCGAGG). *Blackburnia hawaiiensis* samples were amplified using: initial denaturation at 95 °C (3 min); 30 cycles of 95 °C (30 s), 53 °C (45 s); and 72 °C (45 s), with a final extension at 72 °C (5 min). Platynini primers were tested on 3 individuals from each site, yielding identical sequences (Genbank accession No. MH733243).

The Zeale primers amplify a wide range of arthropod orders (Zeale et al. 2010, Bohmann et al. 2011, Piñol et al. 2014*a*). Preliminary testing of these primers on various Hawaiian taxa common to the environment in which *B. hawaiiensis* was collected, was conducted in order to find the optimal annealing temperature for a wide range of Hawaiian arthropods including *Drosophila heteroneura* (Diptera: Drosophilidae), *Laupala* spp., Lepidoptera spp., *Leptogryllus* spp., *Nabis* spp., *Eupetinus* and *Prosopeus* spp. (Coleoptera: Nitidulidae), and *Triginidium* spp (Orthoptera: Trigonidiidae). Optimal annealing temperature for all taxa was determined to be 51 °C and used for further HTS.

DNA extraction — For HTS, *B. hawaiiensis* foregut, midgut, and hindgut were dissected, homogenized, and extracted with the Qiagen DNeasy Blood and Animal Tissue Kit (Qiagen Ltd, Crawley, UK) according to the manufacturer's protocol. Five gut content extractions from each site were used for HTS, including 8 males and 7 females (Ka'iholena 2 males and 3 females; Nāhuku 3 males and 2 females; Pu'u Maka'ala 3 males and 2 females). DNA quality was checked on a 1.5% agarose gel.

High-throughput sequencing — Samples were prepared using the Ion Torrent recommendations for preparing amplicon libraries without fragmentation. The Ion Plus Fragment Library Sequencing Kit (Life Technologies) was used with minor modifications. All 15 samples were pooled into an equal-molar concentration of 15 ng/μl, with a 20-cycle initial PCR amplification to reduce the amplification bias. Additionally, the end repair reagent volumes were reduced by 75% and the adapter ligation and nick repair reagent volumes by 50% to compensate for the low throughput of DNA yields from gut extractions.

Sample purity was evaluated (DNA High Sensitivity kit, Bioanalyzer 2100, Agilent Technologies) and quantified (Qubit dsDNA HS Assay Kit, Qubit 3.0 Fluorometer, Life Technologies) between all library steps. Prior to sequencing, the Kappa Biosystems Ion Torrent Library Quantification Kit was used to determine the ideal concentration of template needed by quantitative real-time PCR. The samples were 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). All library preparation and Ion Torrent sequencing were performed by the first author at the University of Hawai'i at Hilo Evolutionary Core Genomics Laboratory.

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 $R_{standard}$ are 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‰).

R version 3.6.3 was used for all statistical analyses (R Core Team 2020). We fit linear mixed-effects models using the lmer function provided by the lme4 package for R (Bates et al. 2015) using site as a random effect with taxa as the predictor, and $\delta^{13}C$ and $\delta^{15}N$ as the response variables in their respective models. Post hoc tests for differences between model coefficients were done using the mltcomp package (Hothorn et al. 2008), comparing linear combinations of $\delta^{13}C$ and $\delta^{15}N$ *B. hawaiiensis* values to all other taxa. Logically constrained multiplicity adjustments were used to control multiple comparison P-values (Westfall et al. 1999).

High-throughput sequencing bioinformatic analysis — Torrent suite software sorted all sequences by barcode into separate BAM files,

which were then converted into FASTQ files using a simple stream editor (sed) script. Sequences were then filtered and clustered using USEARCH (Edgar 2010) according to (Krehenwinkel et al. 2017*b*). BLASTn was used to assign Operational Taxonomic Unit (OTU) clusters to order (McGinnis and Madden 2004) using a reference library containing NCBI COI databases concatenated with the COI *B. hawaiiensis* sequence, using a minimum similarity of 95%. Only order classification information was retained because of the limits of HTS such as sequencing accuracy and the limited reference database available.

TABLE 1

Average δ^{13}C and δ^{15}N Isotope Values (±1 SE) of Study Taxa. Number of Samples Analyzed (*N*) of Each Taxa is Reported Below. Taxa are Listed in Decreasing δ^{15}N from Top to Bottom

Taxa	δ^{15}N	δ^{13}C
B. hawaiiensis (*N* = 13)	−1.7 ± 0.30	−26.5 ± 0.30
Nabis (*N* = 11)	−2.6 ± 0.25	−26.0 ± 0.16
Pagiopalus (*N* = 13)	−3.0 ± 0.42	−26.9 ± 0.27
Laupala (*N* = 14)	−4.0 ± 0.29	−26.9 ± 0.25
Leptogryllus (*N* = 8)	−5.3 ± 0.35	−25.6 ± 0.20
Collembola (*N* = 9)	−6.0 ± 0.19	−26.6 ± 0.19
Metrosideros (*N* = 9)	−6.7 ± 1.79	−29.6 ± 0.12
Cibotium (*N* = 9)	−6.2 ± 0.38	−29.9 ± 0.44

RESULTS

Stable Isotope Analysis

Blackburnia hawaiiensis had the highest δ^{15}N of all taxa and similar δ^{13}C to all arthropods (Figure 2, Table 1). For both δ^{15}N and δ^{13}C, the linear mixed-effects models with taxa as the predictors were preferred over the null model (Table 2, Table 3). *Blackburnia hawaiiensis* δ^{15}N was not significantly different from that of *Nabis* ($P = .13$) and significantly

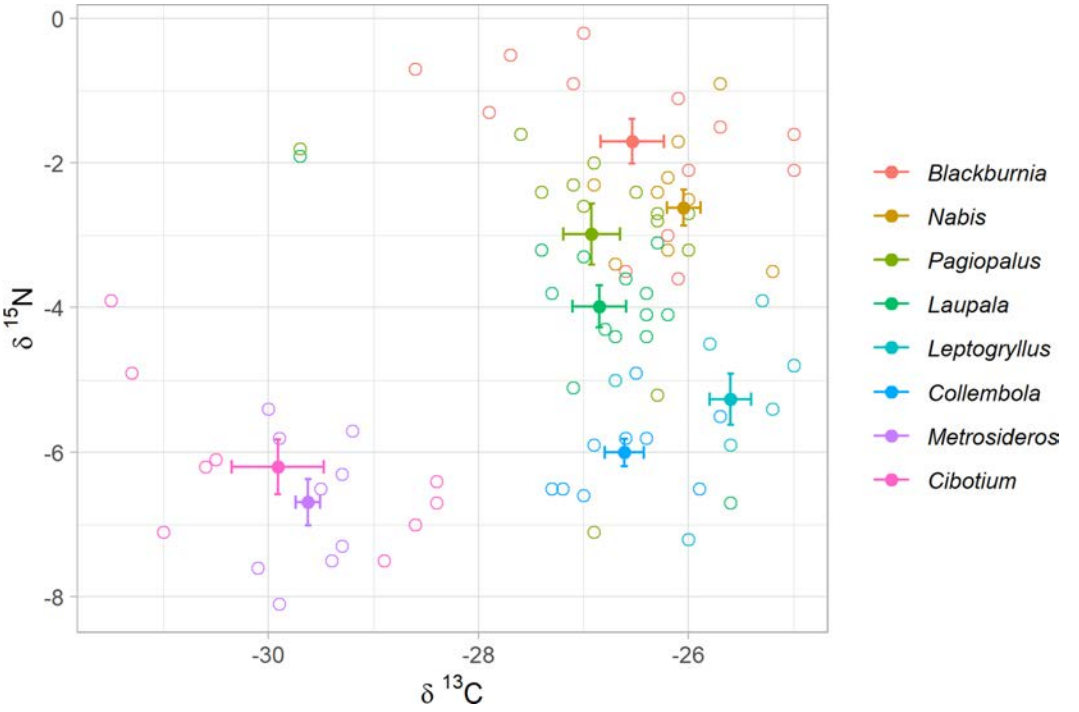

FIGURE 2. δ^{15}N and δ^{13}C isotope ratios of *B. hawaiiensis*, *Pagiopalus*, *Nabis*, *Laupala*, *Leptogryllus*, Collembola, *Metrosideros*, and *Cibotium*. Colored dots are mean ± 1 SE, and open dots are raw data points. Taxa are listed in descending δ^{15}N values.

TABLE 2

Multiple Comparisons of *B. hawaiiensis* to All Other Taxa Output from the Linear Mixed Effects Model Using Taxa as the Predictor and $\delta^{15}N$ as the Response with Site as a Random Effect ($\delta^{15}N \sim$ Taxa + −1 + (1 | Site)). Null Deviance 364.7 on 83 *df* Residuals, AIC 370.7. Alternative Deviance 241.0 on 76 *df* residuals, AIC 261.0. Random Effects Standard Deviation of 0.404, 86 Observations on 3 Groups (Sites)

B. hawaiiensis vs. Taxa	Estimate	Standard Error	*Z*-Value	*P*-Value
Nabis	0.876	0.390	2.245	.132
Pagiopalus	1.220	0.374	3.265	.007
Laupala	2.247	0.367	6.127	<.001
Leptogryllus	3.531	0.429	8.222	<.001
Collembola	4.275	0.413	10.354	<.001
Metrosideros	4.964	0.413	12.022	<.001
Cibotium	4.475	0.413	10.838	<.001

TABLE 3

Multiple Comparisons of *B. hawaiiensis* to All Other Taxa Output from the Linear Mixed Effects Model Using Taxa as the Predictor and $\delta^{13}C$ as the Response with Site as a Random Effect ($\delta^{13}C \sim$ Taxa + −1 + (1 | Site)). Null Deviance 326.7 on 83 *df* Residuals, AIC 332.7. Alternative Deviance 206.1 on 76 *df* Residuals, AIC 226.1. Random Effects Standard Deviation of 0.287, 86 Observations on 3 Groups (Sites).

B. hawaiiensis vs. Taxa	Estimate	Standard Error	*Z*-Value	*P*-Value
Nabis	−0.464	0.320	−1.451	.567
Pagiopalus	0.430	0.306	1.404	.603
Laupala	0.338	0.301	1.125	.803
Leptogryllus	−0.919	0.352	−2.612	.053
Collembola	0.091	0.338	0.268	1.000
Metrosideros	3.102	0.338	9.168	<.001
Cibotium	3.39	0.338	10.022	<.001

differed from all other taxa ($P = .007$ for *Pagiopalus*, $P < .001$ for all other taxa) (Table 2). *Blackburnia hawaiiensis* $\delta^{13}C$ was significantly different from both plant taxa, *Metrosideros* and *Cibotium* ($P < .001$), but was not significantly different from all other arthropods (Table 3).

High-Throughput Sequencing

The Ion Torrent run produced 4,477,826 DNA sequencing reads with 63% loading. The mean read length was 211 bp. Each barcode returned on average 254,811 ± 5,754.22 reads per barcode with a minimum number of reads at 199,987 and a maximum of 270,275. After USEARCH filtering, we recovered an average of 29,279 ± 871.841 SE reads per specimen, ranging from 21,912 to 34,077 reads. 0.05% of the usable sequences were prey; therefore, an average of 200 predator reads was generated for each prey sequence.

Prey items were amplified in eight of the 15 gut content samples, five females, and three males. The majority of prey reads belonged to Diptera (98%, 215 reads). Lepidoptera was found only in the gut contents of a single individual (2%, 5 reads).

DISCUSSION

Blackburnia hawaiiensis Isotopic Differences

Because $\delta^{15}N$ often indicates trophic level (Post 2002, Shiels et al. 2018), our $\delta^{15}N$ mixed-effects model suggests *B. hawaiiensis* is in a higher trophic category than all tested taxa except for *Nabis*, or damsel bugs. These data indicate *B. hawaiiensis* is on a similar trophic level to that of *Nabis*, which are considered generalist predators that feed on a variety of other insects (Lattin 1989, Stasek et al. 2018). On average, *B. hawaiiensis* $\delta^{15}N$ is more than 1.3 values higher than the other significantly different taxa (Table 1). Interestingly, $\delta^{15}N$ of *B. hawaiiensis* was significantly higher than *Pagiopalus*, a genus of running crab spider or Philodromidae, which have also been documented to consume a variety of arthropods (Huseynov 2008). In Hawai'i, stable isotopic studies have been conducted on native spiders, and Kennedy et al. (2018) in particular conducted research in close proximity to Pu'u Maka'ala. Kennedy et al.'s (2018) data suggest similar $\delta^{15}N$ for Tetragnathid and Ariamnes spiders and *B. hawaiiensis*.

Signatures of $\delta^{13}C$ tend to reflect the food source of an animal (Cabana and Rasmussen 1996, Post 2002, Hobson et al. 2010). Our linear mixed-effects model showed that

B. hawaiiensis $\delta^{13}C$ values were similar to all the arthropod taxa used in this study and therefore are all potential prey items for *B. hawaiiensis*. *Blackburnia hawaiiensis* $\delta^{13}C$ values were different from the tested plant material indicating that the beetle is unlikely to be consuming plant material. Additionally, our $\delta^{13}C$ data suggest that Collembola is a likely base for the Hawaiian montane forest arthropod food web, and better suited than *Metrosideros* and *Cibotium* for future SIA studies using mixing models.

Future research using Bayesian mixing models such as MixSIR (Moore and Semmens 2008) or SIAR (Parnell et al. 2010) would aid in determining the trophic position and spatial diet contributions of arthropod prey. In order to use these mixing models, feeding studies and larger sample sizes are necessary. Controlled feeding experiments of *B. hawaiiensis* for determining the trophic enrichment factor could be possible, as Liebherr (2000) successfully raised a closely related species, although a self-perpetuating colony was not established.

High-Throughput Sequencing Analysis

Our HTS analyses suggest that *B. hawaiiensis* is consuming at least two orders of arthropods: Diptera and Lepidoptera. However, these orders may not reflect the full spectrum of diet due to sample size ($N = 15$), noting that prey items were amplified in only eight of the individuals' gut contents. Interestingly, host amplicons were abundant despite reducing the amount of available host material through dissection and limiting the initial PCR to 20 cycles. Our results were similar to that of Piñol et al. (2014*a*) and Gomez-Polo et al. (2015), who both used the brute-force approach with 40 initial PCR cycles resulting in over 99% predator sequences. Interestingly, in Piñol et al. (2014*b*), reducing PCR cycles only increased the proportion of the less well-amplified taxa, not the presence of the taxa themselves.

High-throughput sequencing is a powerful and rapidly evolving technique but there are still many shortcomings that can make the data difficult to interpret. For example, the absence of site-specific reference databases and the potential for sequencing errors in HTS prevent the assigning of reads to species level. Blocking primers or host-specific primers modified with a C3 spacer at the 3′ end of the forward universal primer can also be used to reduce the number of predator sequences (Vestheim and Jarman 2008, Deagle et al. 2013, Gomez-Polo et al. 2015). However, blocking primers have not proven to be useful for arthropod predators that consume closely related organisms (Gomez-Polo et al. 2015, Piñol et al. 2014*b*). For example, Piñol et al. (2014*b*) found that the use of blocking oligonucleotides reduced predator sequence by less than one order of magnitude. After the alignment of the COI arthropod sequences available in Genbank, it became clear that this approach could not be used for *B. hawaiiensis* because high conservation of the COI target sequence would have led to significant underestimation of insect orders within the samples. The COI region is highly conserved and thus significant proportions of all insect orders examined may have been inappropriately blocked by blocking primers, as seen in Toju and Baba (2018). The addition of other barcoding genes such as 16S ribosomal RNA and ITS (Krehenwinkel et al. 2019), a Hawaiian arthropod reference library, and a larger sample size may improve genetic diet analyses and perhaps identify prey items that were not detected in this study.

Blackburnia hawaiiensis, A Generalist Predator

Knowledge of feeding ecology can ultimately improve the effectiveness of land management and conservation practices for species and ecosystems. In this study, we found evidence that *B. hawaiiensis* is a generalist predator that consumes at least two orders of arthropods, and has a trophic position similar to that of damsel bugs. Unfortunately, Diptera and Lepidoptera larvae were not abundantly collected through the beating method and were not included as potential prey items in the SIA. Liebherr and Zimmerman (2000) suggested that the foraging activity of

Hawaiian carabids is not limited by spatial constraints. The beetles can be found in moss mats, under logs and rocks, and on ʻōhiʻa trees and ʻieʻie vines (Pandanaceae: *Freycinetia arborea*) in addition to *Cibotium* fronds where *B. hawaiiensis* was collected in this study, a nod to their generalist eating habits (Liebherr and Zimmerman 2000). Indeed, larvae of both Diptera and Lepidoptera orders could be easily captured by a mobile predator in decaying woody tissue. Although further research is necessary to determine the full scope of *B. hawaiiensis* diet, the results from this study contribute to the limited but advancing Hawaiian arthropod diet ecology literature. The methodological approaches used in this study will be useful to document the natural history and diet of arthropods and could ultimately improve the study of trophic ecology.

AUTHOR CONTRIBUTIONS

KR, CPE, and DKP conceived and designed the experiments. KR and CPE conducted field work and identification of arthropods. KR performed the experiments, statistical analysis, and wrote the manuscript. KR, CPE, and DKP revised the manuscript.

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