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Stable Isotopes and Parasites Indicate Feeding Ecology in Florida, USA, Wading Birds

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Abstract.—We assessed $\delta^{13}C$ and $\delta^{15}N$ profiles and endoparasite community composition in Great Egrets (*Ardea alba*), Great Blue Herons (*A. herodias*), and White Ibis (*Eudocimus albus*) from four wildlife rescue centers (two mainland, two on islands in the Florida Keys) in south Florida, USA to elucidate feeding ecology. We detected among-species differences for $\delta^{15}N$ but not $\delta^{13}C$ and noted decreased $\delta^{13}C$ enrichment in Great Egrets and Great Blue Herons (but not White Ibis) from these centers. Parasite component community and infracommunity species richness were higher in Great Egrets and Great Blue Herons relative to White Ibis, and higher in birds of the same species from mainland centers. Multivariate analysis of parasite infracommunity structure detected co-occurring clusters of parasite taxa characteristic of Great Egrets and Great Blue Herons on the one part, and of White Ibis on the other; mainland Great Egrets and Great Blue Herons had similar parasite communities and clustered separately from conspecifics from the islands. We detected a significant (negative) correlation of infracommunity species richness with $\delta^{13}C$ but not $\delta^{15}N$. Lastly, parasite infracommunity Bray-Curtis similarity correlated significantly with stable isotope Euclidean distances. We conclude that the two approaches converge towards similar outcomes, providing complementary and consilient information on host feeding ecology. *Received 1 March 2020, accepted 16 July 2020.*

Key words.— $\delta^{13}C$, $\delta^{15}N$, feeding ecology, Great Blue Heron, Great Egret, parasites, wading birds, White Ibis

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Studies of foraging habits and dietary preferences typically use methods such as forced regurgitations, live observations of feeding, or *post-mortem* stomach content analysis (Marcogliese and Cone 1997; Inger and Bearhop 2008). These techniques can only provide a "snapshot" of dietary information integrated over a short period of time (i.e., up to a few hours; Marcogliese and Cone 1997; Inger and Bearhop 2008). Consequently, stable isotopes have been used to broadly characterize feeding preferences as they reflect longer-term assimilation of dietary items (Kelly 2000; Inger and Bearhop 2008). Tissues are synthesized at differing rates and the isotopic composition of new tissues reflects the diet of the individual at the time of tissue synthesis (Peterson and Fry 1987). Because different prey items may have distinctive isotopic signatures (DeNiro and Epstein 1978, 1981; Kelly 2000; Fry 2006), the values in an animal's tissue can be used to infer trophic ecology over a time frame of weeks to months (Peterson and Fry 1987; Bearhop *et al.* 2002; Hobson 2007; Inger and Bearhop 2008).

The most reported isotopes are carbon and nitrogen, with each isotope yielding slightly different information on an organism's diet. Stable carbon isotope ratios ($\delta^{13}C$) are used to determine the major sources of carbon in the diet. Carbon values vary among plants using different modes of photosynthesis; photosynthesis by C_4 and crassulacean acid metabolism (CAM) pathways results in lower carbon isotope fractionation than C_3 photosynthesis (Gannes *et al.* 1998). Furthermore, the $\delta^{13}C$ signatures of marine algae are higher than those of terrestrial plants (O'Leary 1981; Peterson and Fry 1987; Kelly 2000; Inger and Bearhop 2008). These differences make it possible to use isotopic signatures to trace the flow of carbon from specific producer pools to consumers (O'Leary 1981; Kelly 2000). On the other hand, stable nitrogen isotope ratios ($\delta^{15}N$) indicate trophic position within a food web: as trophic level increases, $\delta^{15}N$ values show a stepwise 3 to 4‰ enrichment (DeNiro and Epstein 1981; Hobson *et al.* 1994; Hebert *et al.* 1999; Herrera *et al.* 2003). Therefore, analyzing both carbon and nitrogen stable

isotopes in muscle tissue provides a more complete picture of an organisms feeding ecology, integrated over a period of weeks or months.

Parasite communities can also be used to infer dietary information because they reflect long-term trends in the diet and feeding ecology of their hosts: many parasites have complex life cycles that require development in at least one intermediate host as well as development in a definitive host in which sexual reproduction occurs. Different intermediate hosts and often paratenic hosts (hosts which participate in transmission but in which no parasite development occurs) may be involved in the life cycle of the parasite. Although some parasites directly penetrate their hosts (e.g., schistosomes), others are trophically transmitted: larval or juvenile stages infecting intermediate hosts must be ingested by the next host for the parasite life cycle to continue (Hoberg 1996; Marcogliese and Cone 1997; Lafferty *et al.* 2008). Endoparasites of birds are primarily transmitted in this manner and thus can reveal host feeding preference and even identify linkages within food webs (Marcogliese and Cone 1997; Dunne *et al.* 2013). Parasite communities reflect long-term trends in host feeding ecology, on a time scale of months or longer (Marcogliese and Cone 1997).

It follows that the combined use of stable isotope and endoparasite community analyses should theoretically provide complementary information on the diet and feeding ecology of organisms such as wading birds. While the combination of stable isotope (SI) and gut content analysis is well-established in the literature (Eloranta *et al.* 2015), fewer studies have compared SI ratios in parasites and their hosts, and only rarely in the context of studying host feeding ecology (Kanaya *et al.* 2019; Thieltges *et al.* 2019). This is unfortunate, as the combination of stable isotope and parasite community approaches has yielded interesting insights on host feeding ecology. For example, de la Vega *et al.* (2018) found that the switch from nursing to independent foraging on fish in harbor seals (*Phoca vitulina*) leads to a change in stable isotope values that coincides with the acquisition of trophically transmitted parasites. Similarly, stable isotopes revealed that shorthorn sculpin (*Myxocephalus scorpius*) consumed a wider variety of prey as they grew, which was reflected in a more diverse endoparasite community in older and larger fish (Dick *et al.* 2009). Stable isotopes and trophically transmitted parasites both allowed discrimination of planktivorous from piscivorous yellow perch (*Perca flavescens*; Johnson *et al.* 2004). Working with the same species, Bertrand *et al.* (2011) used a similar analysis of yellow perch parasite communities to confirm that variations in muscle $\delta^{13}C$ enrichment reflected their preferred foraging habitat rather than dietary preference for specific prey items such as zooplankton, allowing them to more accurately estimate adult yellow perch feeding range size. Welicky *et al.* (2018) and Demopoulos *et al.* (2015) used stable isotope analysis to demonstrate that parasites alter host feeding ecology in reef fishes.

Few studies have explicitly looked for correlations in stable isotope profiles and parasite communities to assess the extent to which these two types of information are complementary and consilient, i.e., converge towards similar conclusions regarding an organism's feeding habits. The bulk of the relevant literature concerns fishes, with little focus on birds. Although Aponte *et al.* (2014) used stable isotopes and gut contents to detect "trash" feeding in gulls (family Laridae) and noted a correlation between this behavior and decreased endoparasite diversity, the analysis of these two types of information on birds remained relatively unexamined.

Wading birds are commonly found in various freshwater and coastal ecosystems in south Florida, USA, ranging from inland Lake Okeechobee and Everglades southward into the islands of the Florida Keys. These bird species are critical contributors to ecological function, driving energy flow within ecosystems via predator-prey interactions and mediating energy flow between terrestrial and aquatic habitats (Polis and Hurd 1996; Mallory *et al.* 2010). Understanding the feeding ecology and dietary

preferences of wading birds thus provides additional information on trophic dynamics in the larger south Florida ecosystem. The wading birds in this study are from two families within the order Pelicaniformes: Great Egret (*Ardea alba*) and Great Blue Heron (*A. herodias*) in Family Ardeidae and White Ibis (*Eudocimus albus*) in Family Threskiornithidae. These birds occupy aquatic habitats from freshwater marshes and meadows to the shores of marine environments, and they have wide dietary preferences including other birds (particularly chicks), small mammals, and a range of aquatic food items including fish, amphibians, and invertebrates (Brooke and Birkhead 1991; Schreiber and Burger 2001; Lovette and Fitzpatrick 2016). Great Egrets forage predominately on fishes, crabs, and lizards (Hom 1983; Miranda and Collazo 1997; Frederick *et al.* 1999); Great Blue Herons consume primarily fishes (Kelsall and Simpson 1980; Powell 1983; Butler 1993); while the White Ibis diet ranges from fishes, crayfish, and insects to small lizards and vegetation (Kushlan and Kushlan 1975; Boyle *et al.* 2012).

The bird species within these two families have different foraging strategies impacting their prey selection and their resulting $\delta^{15}N$ signatures. White Ibis are tactile feeders that forage on small fish and crustaceans in shallow waters by probing their bill into substrates (Kushlan 1975, 1979; Frederick *et al.* 2009). For members of family Ardeidae, foraging is visual. Prey size is also dependent on bill length, so larger ardeid species are able to forage on a wider variety of prey items; as the largest ardeid species, the Great Blue Heron can forage on a larger range of fish (Hom 1983). In contrast, the narrow opening of the White Ibis bill presumably limits potential prey items to small, lower trophic level organisms with more depleted nitrogen signatures (Proctor and Lynch 1993).

The intent of this study was to improve the understanding of foraging ecology for three species of wading birds in south Florida using both stable isotope profiles and parasite communities, and to examine whether stable isotope and parasite community analyses yielded similar conclusions. We began by determining stable isotope ratios in bird tissues, followed by identifying and enumerating their endoparasites. We then used univariate and multivariate approaches to explore the extent to which the feeding ecology inferred from stable isotope profiles were statistically correlated with the species richness and composition of their corresponding endoparasite communities. Previous studies detected correlations between host trophic position inferred from gut contents and parasite species richness (e.g., Valtonen *et al.* 2010); we examined whether this might also be true for host stable isotope profile. Furthermore, recent studies have found that host trophic position is broadly predictive of parasite community similarity (e.g., Woodstock *et al.* 2020), so we examined whether we could detect a similar pattern among birds. We had the following specific objectives: 1) to test for differences in $\delta^{13}C$ and $\delta^{15}N$ ratios and endoparasite species richness among the three host study species and sampling localities; 2) to test for differences in parasite community similarity among host species and sampling location; and 3) to test for correlations between $\delta^{13}C$ and $\delta^{15}N$ ratios and endoparasite species richness and community similarity.

METHODS

Study Area and Field Collection

Bird specimens were obtained from four Florida wildlife rehabilitation centers. Two are located in the USA on the Florida mainland (South Florida Wildlife Center in Fort Lauderdale and Pelican Harbor Seabird Station in Miami), and two are located further south in the islands of the Florida Keys (Florida Keys Wild Bird Rehabilitation Center in Tavernier and Key West Wildlife Center in Key West; Fig. 1). Birds either died while receiving treatment at the wildlife centers or were euthanized upon admittance due to trauma. Due to the inherently opportunistic nature of our sample collection, we were unable to obtain individual life histories for the birds in our study.

Laboratory Processing

Stable isotopes.—Bird muscle samples were dried at 60°C for a minimum of 72 hours, ground and homogenized using a dental amalgamator (Wig-L-Bug, Crescent Dental Manufacturing Company), weighed to 0.6-0.8 milligrams (mg), and pelletized in tin cap-

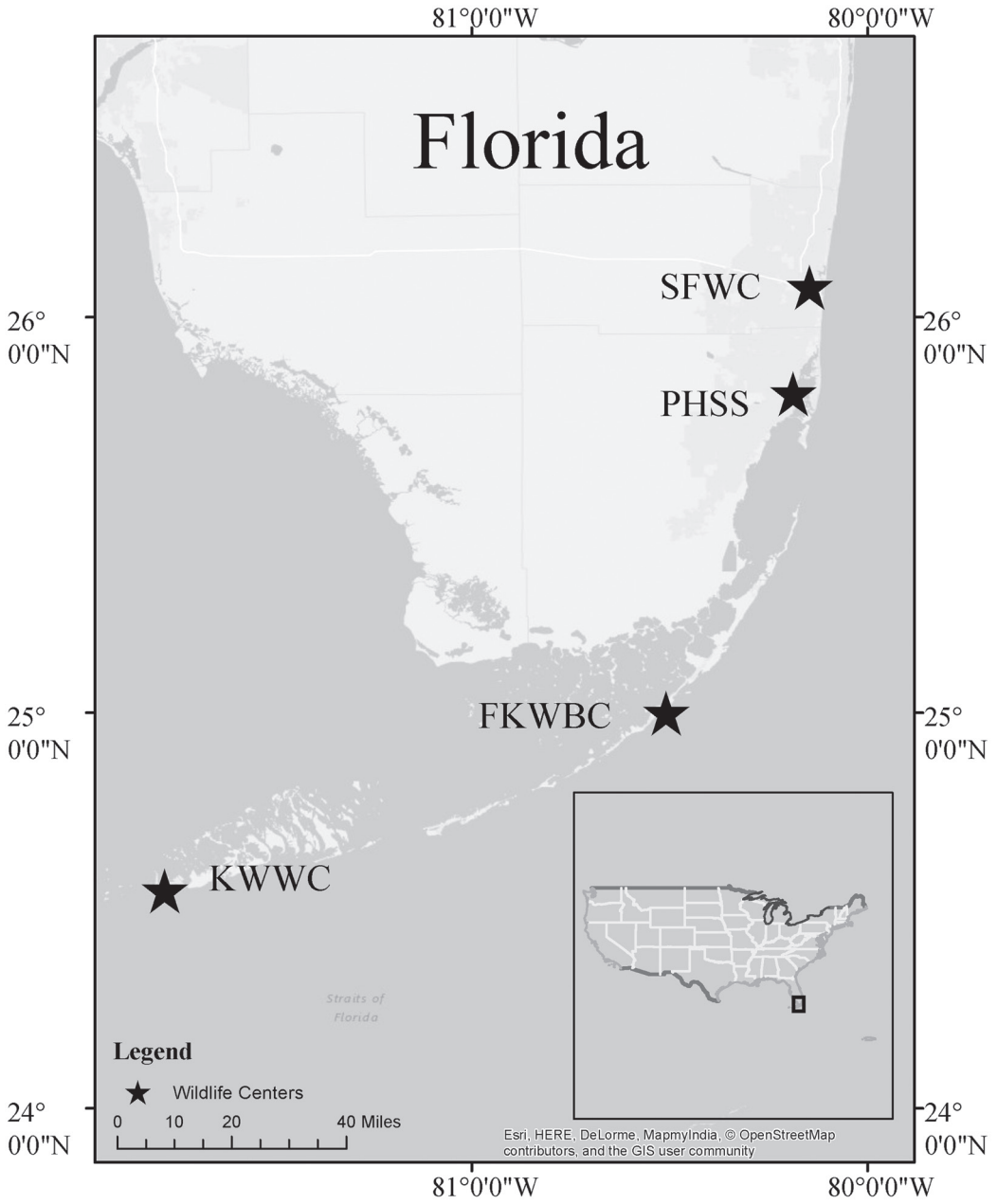

Figure 1. Map of wildlife centers that provided wading bird specimens: South Florida Wildlife Center (SFWC), Pelican Harbor Seabird Station (PHSS), Florida Keys Wild Bird Center (FKWBC), and Key West Wildlife Center (KWWC) in south Florida, USA.

sules for stable carbon and nitrogen isotope analysis. Stable isotope analyses for $\delta^{13}C$ and $\delta^{15}N$ were conducted at the Smithsonian Institution's Museum Conservation Institute in Suitland, Maryland, USA, using a Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to a Costech 4010 Elemental Analyzer (EA) via a Thermo Conflo IV (CF-IRMS). A set of standards was run every 10 - 12 samples and included USGS40 and USGS41 (L-glutamic acid) as well as Costech acetanilide. All samples and standards were run with the same parameters; this included an expected reproducibility of the standards $< 0.2‰$ (1σ) for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope values were expressed in terms of δ and reported in comparison to the respective standard reference materials, Pee Dee Belemnite (PDB) for carbon and at-

mospheric air (N_2) for nitrogen. The isotopic values were reported with the standard parts per thousand notation (‰):

$$\delta X = [(Rsample / Rstandard) - 1] * 1000$$

where X is the isotope being analyzed and R is the ratio of the heavy to light isotope.

Parasites.—Birds were dissected according to protocols adapted from McLaughlin (2001). Organs examined for parasites included the trachea, esophagus, stomach, liver, kidneys, and intestines. Platyhelminths (digeneans and cestodes) were fixed and stored in 70% ethanol, stained in acetocarmine, dehydrated in ethanol, cleared in clove oil, and mounted in Permount (Fisher Scientific) on glass slides. Nematodes were fixed and stored in a 70% ethanol/30% glycerol solution, progressively cleared in glycerol and semi-permanently mounted in glycerine jelly on glass slides (Pritchard and Kruse 1982; McLaughlin 2001). All parasites were subsequently identified to the lowest possible taxonomic level using standard taxonomic keys and species identifications from the literature. Infection rates for each parasite taxon were quantified in host populations using the metrics recommended by Bush *et al.* (1997): prevalence (percent of host population infected within a given parasite taxon), mean abundance (number of a given parasite taxon found in a host population, divided by the number of hosts examined), component community species richness (number of parasite taxa detected in a host population), and infracommunity species richness (mean number of parasite taxa detected in individual hosts).

Statistical Analyses

All univariate analyses were conducted using JMP (v. 12.1.0; SAS Institute Inc. 2015). We used ANOVA to test for differences in $\delta^{13}C$ and $\delta^{15}N$ values and parasite infracommunity species richness among host species, and between sampling locations, as preliminary analyses suggested that mainland and Florida Keys samples differed in their isotopic profiles. Prior to analysis, we confirmed that the statistical assumptions of ANOVA were met using Shapiro-Wilk's test for normality ($0.870 < W < 0.967$, all $P > 0.740$) and Levene's test for equality of variances (all $F < 1.331$, all $P > 0.271$). Tukey's HSD test was used to perform multiple comparisons when significant effects were detected. As explained above, previous studies detected correlations between trophic position (inferred from diet) and parasite species richness (Valtonen *et al.* 2010); we therefore tested for correlations between $\delta^{13}C$ or $\delta^{15}N$ values and parasite species richness using linear regression.

All multivariate analyses were conducted in PRIMER (v. 7.0.13; PRIMER-e; Quest Research Ltd. 2016). Stable isotope values were normalized and used to establish a pairwise Euclidean distance matrix for the stable isotope profiles of individual birds. Parasite abundances were fourth root transformed and used to establish a parasite community similarity matrix based on Bray-Curtis indices for all pairs of infracommunities (including a dummy variable). We used PERMANOVA to test for differences in parasite community similarity among host species and between sampling locations, using permutation of the residuals under a reduced model and the Type III sum of squares as recommended in Clarke and Gorley (2015). We used the Cluster and Similarity Profile routines in PRIMER to identify clusters of birds with similar parasite communities, and to identify co-occurring clusters of parasite taxa.

Lastly, following the procedure recommended by Clarke and Gorley (2015), we posited that if stable isotope profiles and endoparasite community provided similar information on the extent to which hosts differed in their feeding ecology, their corresponding similarity matrices would be correlated. Consequently, we used the *Relate* procedure (a modified Mantel test) in PRIMER to test for significant correlation between the structure of the Euclidean distance matrix for stable isotopes for all birds, and the corresponding Bray-Curtis similarity matrix for their parasite infracommunities. Statistical significance for all tests (univariate and multivariate) was assumed at $P \leq 0.05$.

RESULTS

Stable Isotopes

A total of 65 muscle samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ (Table 1). We observed variation in isotopic signatures, as $\delta^{13}C$ and $\delta^{15}N$ values ranged from -28.16 to -11.66‰ and 6.44 to

Table 1. Mean stable isotope ratios and endoparasite component community and infracommunity species richness for Great Egret (*Ardea alba*), Great Blue Heron (*A. herodias*), and White Ibis (*Eudocimus albus*) from south Florida, USA.

		Great Egret	Great Blue Heron	White Ibis
Isotope		$n = 18$	$n = 27$	$n = 21$
$\delta^{15}N$	$\bar{x}$ ± SD (‰)	9.56 ± 0.84	12.11 ± 1.23	7.49 ± 1.00
	Range (‰)	8.17 to 11.24	8.54 to 13.48	6.44 to 10.62
$\delta^{13}C$	$\bar{x}$ ± SD (‰)	-20.87 ± 3.85	-19.20 ± 5.54	-20.76 ± 2.21
	Range (‰)	-26.64 to -14.33	-28.15 to -11.66	-24.54 to -17.14
Parasite species richness		25	26	16
Infracommunity species richness	$\bar{x}$ ± SD	3.17 ± 1.98	3.00 ± 2.60	1.76 ± 1.09

13.78‰, respectively. The global ANOVA for $\delta^{15}N$ was significant ($R^2 = 0.699$, $F_{5, 65} = 27.946$, $P < 0.001$). Effect tests for both factors detected a significant effect of bird species ($F_{2, 65} = 58.576$, $P < 0.001$; all pairwise comparisons $P < 0.001$ per Tukey HSD test) but not location ($F_{1,64} = 0.131$, $P = 0.718$), nor for the species by location interaction term ($F_{2,64} = 1.557$, $P = 0.219$). The global ANOVA for $\delta^{13}C$ was also significant ($R^2 = 0.530$, $F_{5,65} = 13.519$, $P < 0.001$); however, we failed to detect any differences among species ($F_{2,65} = 0.205$, $P = 0.815$). We did detect differences among locations ($F_{1,64} = 35.345$, $P < 0.001$) as well as a significant species by location interaction ($F_{2,64} = 12.535$, $P < 0.001$): pairwise tests using Tukey's HSD found that the location factor was significant for Great Egrets and Great Blue Herons ($P \leq 0.040$), but not White Ibis ($P = 0.347$). Stable isotope values for all birds are presented by species and location in Fig. 2.

Parasites

We recovered endoparasites, including members of the Acanthocephala, Nemato-

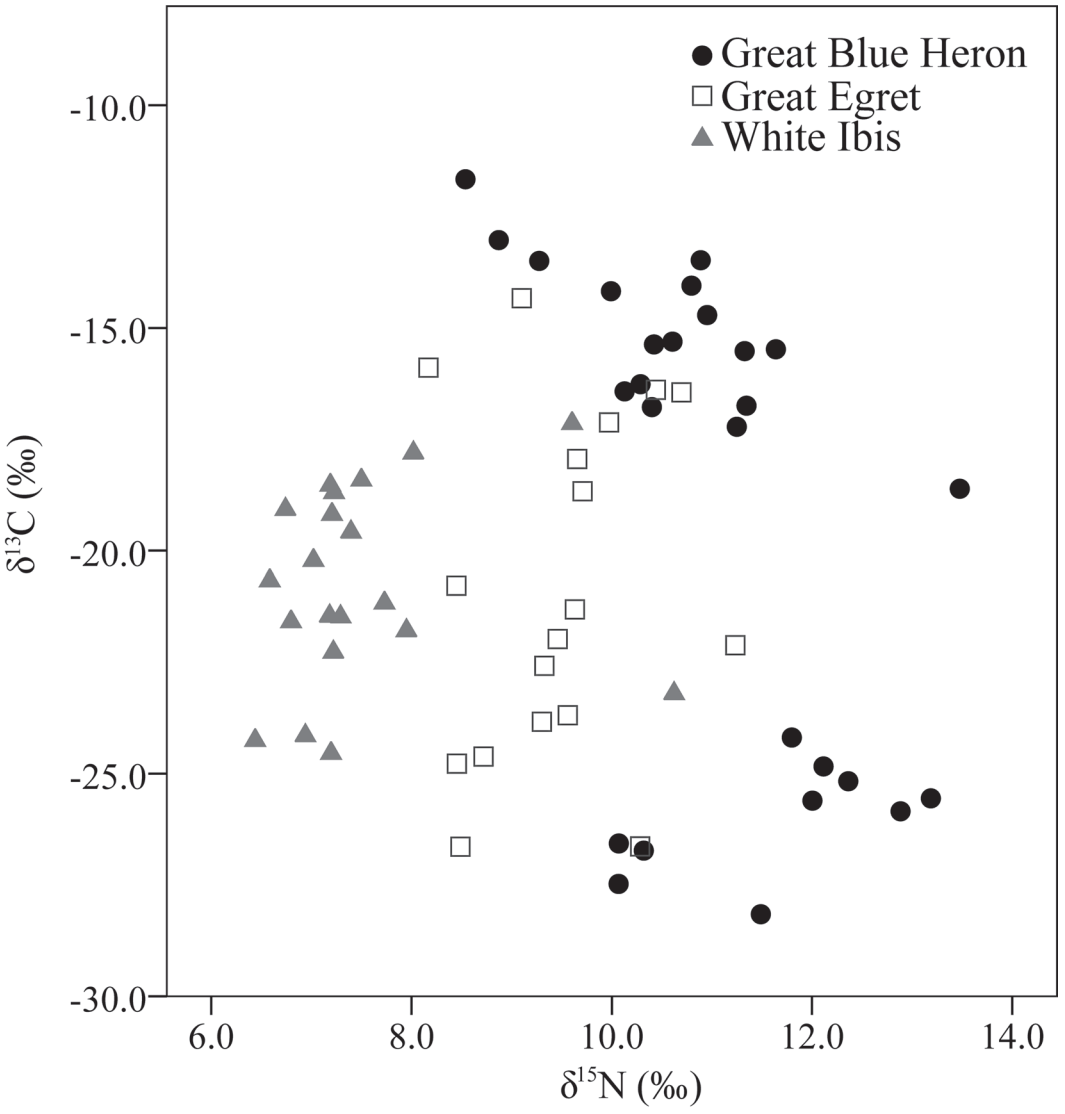

Figure 2. Relationship between $\delta^{13}C$ and $\delta^{15}N$ in three species of wading birds from south Florida, USA.

da, Cestoda and Trematoda, from 89.4% (n = 66) of the birds examined. Parasite data are summarized in Table 1 and Fig. 3; a full listing of the taxa and the basis for their identification are provided in Gumbleton (2018) and Gumbleton *et al.* (2019).

Overall, parasite component community species richness was similar in Great Egrets (25) and Great Blue Herons (26) but comparatively lower in White Ibis (21). The ANOVA model for infracommunity species richness was significant (R^2 = 0.218, $F_{5,64}$ = 3.296, P = 0.011), with significant effects noted among host species ($F_{2,64}$ = 4.826, P = 0.012) and between locations, albeit marginally ($F_{1,64}$ = 4.066, P = 0.048); the interaction term was not significant ($F_{2,64}$ = 2.721, P = 0.074). Subsequent pairwise testing using

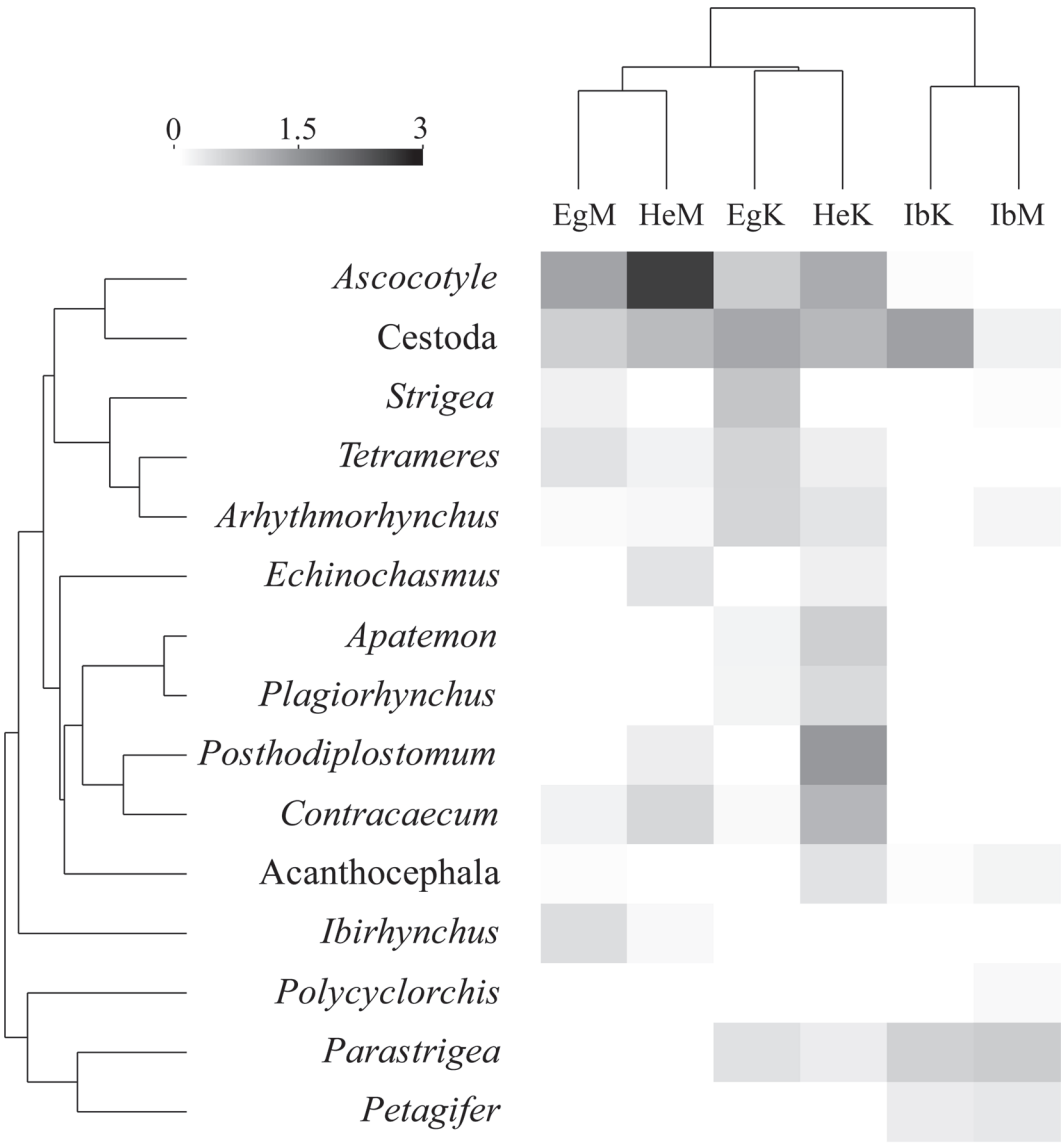

Figure 3. Shade plot depicting abundances of the parasite taxa detected in this study. The scale bar indicates the mean fourth root abundance for each taxon. Co-occurring parasite taxa are indicated by row clusters; host birds are clustered according to the similarity (Bray-Curtis) of their parasite communities. Hosts are labeled by species (Eg = Great Egret; He = Great Blue Heron; Ib = White Ibis) and by general location of the wildlife center which provided the samples (M: mainland; K: Florida Keys islands).

Tukey's HSD revealed that infracommunity species richness was significantly lower in White Ibis (1.76 ± 1.09) than in Great Egrets (3.17 ± 1.98) or Great Blue Herons (3.00 ± 2.60); infracommunity richness was also significantly higher in birds from mainland sources.

Overall, infracommunity Bray-Curtis similarity values ranged from 7.1 to 100% (33.2 ± 18.0%). PERMANOVA detected significant differences among host species and between sampling locations, as well as a significant species by location interaction (Table 2). Pairwise tests indicated that the three host species differed in their infracommunity similarity ($1.718 < t < 2.918$, all $P \leq 0.017$). Breaking down the interaction term, we detected significant differences in community similarity between sampling locations for Great Blue Herons ($t = 0.199$, $P = 0.008$) but not Great Egrets ($t = 1.251$, $P = 0.174$) or White Ibis ($t = 1.291$, $P = 0.182$). Hierarchical clustering revealed two patterns: first, there were co-occurring clusters of parasite taxa characteristic of Great Egrets and Great Blue Herons on the one part, and of White Ibis on the other; second, mainland Great Egrets and Great Blue Herons had similar parasite communities and clustered separately from conspecifics from the islands of the Florida Keys (Fig. 3).

Correlating Stable Isotopes and Parasites

Regression of infracommunity species richness against stable isotope profiles for individual birds detected a significant negative correlation with $\delta^{13}C$ ($R^2 = 0.358$, $F_{1,63} = 35.186$, $P < 0.001$) but not $\delta^{15}N$ ($R^2 = 0.048$, $F_{1,63} = 3.167$, $P = 0.080$) (Fig. 4). Lastly, parasite infracommunity Bray-Curtis similarity correlated significantly with stable isotope Euclidean distances (*Relate*; $\rho = 0.211$, $P = 0.001$); i.e., pairwise distances between samples in the parasite community matrix were significantly correlated with the corresponding distances between samples in the stable isotope matrix.

DISCUSSION

In this study, we noted significant differences in nitrogen isotopic signatures between Great Egrets and Great Blue Herons on the one part, and White Ibis on the other. Overall $\delta^{15}N$ values spanned from 6.44 to 13.48‰, a 7.04‰ range among all three wading bird species. Assuming a 3 to 4‰ increase in $\delta^{15}N$ per trophic level, our results indicate that the wading birds studied were likely feeding across one to two trophic levels (DeNiro and Epstein 1981; Hobson *et al.* 1994; Hebert *et al.* 1999; Herrera *et al.* 2003). Great Blue Herons were significantly enriched compared to the other two bird species, suggesting that Great Blue Herons are foraging at a trophic level higher, likely on larger fishes. Mean White Ibis $\delta^{15}N$ were the most depleted of the three species. Great Egrets displayed $\delta^{15}N$ values that were between White Ibis and Great Blue Herons indicating Great Egrets forage at an intermediate trophic level; this is not unexpected as Great Blue Herons mainly forage on fishes, White Ibis forage on crustaceans and other invertebrates, and Great Egrets consume both.

We failed to detect significant differences in carbon signatures among species. Interestingly, we did observe a significant difference in carbon signature in Great Egrets and

Table 2. Results of PERMANOVA test for differences in parasite infracommunity Bray-Curtis similarities among host species ((Great Egret (*Ardea alba*), Great Blue Herons (*A. herodias*), Great Blue Heron, White Ibis (*Eudocimus albus*)) and between sampling locations (mainland vs. Keys) from south Florida, USA.

Source	df	SS	Pseudo-*F*	*P*
Species	2	21560	5.424	0.001
Location	1	5638	2.837	0.012
Species x Location	2	8445	2.125	0.018
Residual	60	1.192×10^5		
Total	65	1.555×10^5		

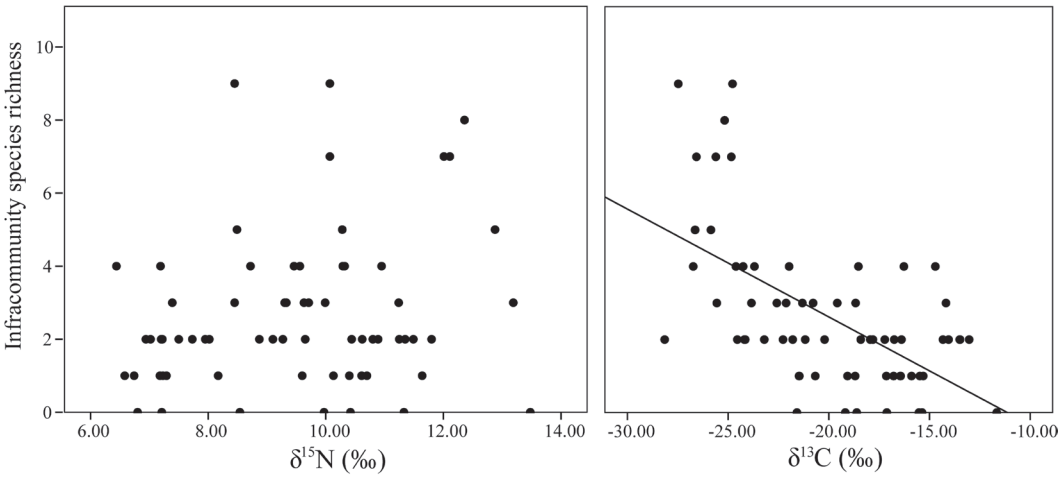

Figure 4. Scatterplot of the relationship between $\delta^{13}C$ and $\delta^{15}N$ and parasite infracommunity species richness in all three wading bird species (Great Egret, Great Blue Heron, White Ibis); note the significant negative correlation with $\delta^{13}C$ but not $\delta^{15}N$.

Great Blue Herons from mainland rescue centers and conspecifics from island centers located in the Florida Keys. Differences in $\delta^{13}C$ typically reflect the photosynthetic pathways of local plants (Smith and Epstein 1971; O'Leary 1981; Peterson and Fry 1987). The $\delta^{13}C$ of C_3 plants average -28‰ while C_4 plants, such as tropical plants and salt grasses, are approximately -13‰ (Peterson and Fry 1987). Enriched $\delta^{13}C$ signatures were observed in egrets and herons obtained from the Florida Keys wildlife centers relative to mainland centers, likely due to the diversity of C_3 plants (phytoplankton, algae, and seagrass beds) found within the Florida Keys. Previous studies have shown enriched $\delta^{13}C$ in seagrasses compared to other marine producers. Fourqurean *et al.* (2005) assessed the $\delta^{13}C$ of *Thalassia testudinum* collected throughout the Keys and obtained values ranging from -13.5‰ to -5.2‰. Campbell and Fourqurean (2009) noted average $\delta^{13}C$ of -6.2‰ for *Syringodium filiforme*, -8.6‰ for *Thalassia testudinum*, and -10.6‰ for *Halodule wrightii*. Variation in $\delta^{13}C$ suggests that birds obtained from the Keys rescue centers were likely foraging in an environment dominated by these relatively enriched marine producers. This effect was most apparent in Great Blue Herons, among which two distinct groups could be distinguished on the basis of their carbon isotope signatures, which corresponded to the location from which they were collected. Great Blue Herons from the island wildlife centers in the Keys had $\delta^{13}C$ values similar to that of C_3 plants found in the marine environment, while mainland Great Blue Herons had $\delta^{13}C$ values indicative of freshwater habitats and the abundance of C_4 plants in the Florida Everglades. In freshwater ecosystems, wide $\delta^{13}C$ variation can occur depending on the source of dissolved CO_2 in the water; $\delta^{13}C$ can be influenced by carbonate rock weathering, mineral springs, input from the atmosphere, or from respired plant matter, all prevalent in South Florida environments. The $\delta^{13}C$ for dissolved inorganic carbon may approach -20‰ when respired organic matter inputs are strong (Peterson and Fry 1987). Proximity of the Everglades and adjacent wetlands could provide such a rich source of depleted carbon. Stern *et al.* (2007) examined the source and turnover rates of carbon in the Florida Everglades using stable isotopic analysis of dissolved organic carbon, particulate organic carbon and dissolved inorganic carbon. The $\delta^{13}C$ from dissolved organic carbon displayed increasingly depleted values with increasing distance from the Everglades Agricultural Area, land designated for agricultural purposes in the northern Everglades. The depleted $\delta^{13}C$ is reflective of a diminished contribution of sugarcane agriculture

and an increase in the contribution of wetland vegetation to the dissolved organic carbon pool. The $\delta^{13}C$ signal of birds collected from mainland wildlife centers had similar values to those of dissolved organic carbon collected in the Everglades National Park, a relatively undeveloped region of south Florida with diverse habitats ranging from freshwater to marine.

We did not detect a similar difference in $\delta^{13}C$ signatures among White Ibis populations. Unlike Great Egrets and Great Blue Herons, White Ibis opportunistically forage on a wide variety of food sources including garbage (Dorn *et al.* 2011). The wide range in $\delta^{13}C$ exhibited by the White Ibis may be due to the consumption of food waste in which corn sweeteners and stabilizers are common ingredients, derived from C_4 plants. It is difficult to determine the impact of garbage on wading bird diet as stable isotope values of this refuse are difficult to predict and are likely to be diverse. Hobson (1987) used $\delta^{13}C$ to examine the contribution of marine and terrestrial protein to the diet of gulls (family Laridae). He noted that assessing terrestrial contributions to North American gull diets was difficult because garbage contains a broad spectrum of food types. However, we doubt that anthropogenic sources (e.g., offshore sewage outfalls) had much impact on $\delta^{13}C$; such sources are typically associated with enrichment in $\delta^{15}N$ due to increases in ammonium and nitrate concentrations within the environment (McClelland *et al.* 1997; Fry 2006). However, we did not observe such enrichment in the White Ibis individuals in this study.

Parasite infracommunity composition differed among bird species. As with stable isotopes, we noted significant differences between conspecifics from mainland and Keys wildlife centers. The three bird species shared a "common core" parasite community consisting of taxa such as *Ascocotyle* sp. and dilepidid cestodes that use fishes as intermediate hosts and are therefore commonly found in fish-eating birds (Scholz *et al.* 1997, 2011; Anderson *et al.* 2009; Shamsi *et al.* 2009; Drago and Lunaschi 2011). The dominance of these two parasite taxa indicates that fishes are an important component of south Florida wading bird diets, especially for Great Egret and Great Blue Heron. Similarly, several of the parasite taxa listed in Fig. 3 (and see Gumbleton *et al.* 2019) are associated with piscivorous diets (e.g., *Echinochasmus* sp. and *Apatemon* sp.). *Tetrameres* sp., *Arhythmorhynchus* sp., and *Ibirhnychus* sp. use invertebrates as intermediate hosts but often include fishes as auxiliary hosts; we suggest that fishes are the likeliest conduits for these taxa into south Florida wading birds. Not all of the parasites in this study are transmitted through fishes: some strigeids (aside from *Apatemon* sp.) are transmitted by amphibians or reptiles, and *Plagiorhynchus* sp. has a fully terrestrial life cycle using isopods as intermediate hosts. Although we do not have individual life histories or gut contents data for our birds, it seems likely that intraspecific differences in infracommunity species composition between mainland and Keys birds are largely being driven by ecological differences and the availability of intermediate hosts.

The key result of this study is the significant correlation between the stable isotope profile of individual birds with both infracommunity parasite species richness and composition. Analysis of stable isotope profiles and parasite communities converged towards similar conclusions: i.e., a predatory diet consisting largely of fish and regional differences in carbon sources/prey communities. Consilience between the two approaches validates them and highlights their potential usefulness in the study of dietary preferences and feeding ecology. That said, isotope and parasite data do not always agree with direct observations of gut contents (or each other). Isotopes and parasites only weakly predicted or contradicted gut content data in pumpkinseed (*Lepomis gibbosus*) sunfish: their parasite communities were strongly indicative of copepod consumption, but copepods were never observed among the gut contents; in addition, stable isotope models failed to retain gastropods, the second most abundant prey per the gut contents (Locke *et al.* 2013). Unlike the present study, Locke

et al. (2013) found that their isotope and parasite data did not converge. For example, isotope models proposed gastropods as the most important prey item in their fish, but the parasite community did not reflect this. Although they detected weak correlations in their multivariate analyses (i.e., fishes with similar gut content communities tended to have similar isotope profiles and parasite communities), these correlations became weaker still when they controlled for confounding factors such as spatial autocorrelation and host size (Locke *et al.* 2013).

These caveats aside, our data support the notion that combining the two approaches provides more insight on foraging ecology than either approach alone. Using the combined approach of stable isotope analysis and parasite identification makes it possible to better elucidate wading bird feeding ecology. In the present study, stable isotope analysis provided information on trophic interactions based on $\delta^{15}N$, allowing discrimination of piscivores from birds with more varied diets, while $\delta^{13}C$ detected broad differences in geographic foraging location; analysis of the parasite community provided insights into the likely trophic relationships of these species in their south Florida habitats. Stable isotope profiles cannot identify species-specific prey in the diet; and parasite identification does not provide in-depth information on foraging location and carbon sources. These two techniques are complementary and, used together, provide useful insights into feeding ecology and trophic interactions.

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