USING DNA METABARCODING TO CHARACTERIZE THE DIETS OF INVASIVE WILD PIGS (*SUS SCROFA*) IN THE SOUTHEASTERN UNITED STATES

by

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(Under the Direction of James C. Beasley)

ABSTRACT

Biological invasions are one of the leading causes of global biodiversity loss. Invasive wild pigs (*Sus scrofa*) are ecological generalists that threaten numerous taxa and ecosystems worldwide. Amphibians and islands are among groups and systems sensitive to impacts from wild pigs but with limited research in many regions across wild pig's range. In this thesis, I used DNA metabarcoding to characterize wild pig diets in SC, USA and GA barrier islands, USA, quantified differences between sexes (Chapter 2), islands (Chapter 3), and seasons, and identified taxa vulnerable to wild pig predation in the study region. My results revealed wild pigs in the southeastern US exhibit opportunistic, plastic diets that vary temporally and spatially, and that wild pigs primarily consume vertebrate species with fossorial habits likely encountered while rooting, such as amphibians. Collectively, my research informs management of wild pigs by highlighting potentially vulnerable taxa in the southeastern USA.

INDEX WORDS: Amphibians; Diet; Invasive species; Islands; Next-generation sequencing; Sus scrofa; Wild pigs

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Invasive species present a critical threat to global biodiversity and community function, second only to habitat loss and fragmentation (Allendorf & Lundquist, 2003; Gurevitch & Padilla, 2004). Invasive wild pigs (*Sus scrofa*; Linnaeus, 1758) are one of the most widespread and invasive mammal species, found on all continents except Antarctica, as well as many islands (Massei & Genov, 2004; Barrios-Garcia & Ballari, 2012). While many common names are used for wild pigs across their range (e.g., feral swine, wild boar, feral pigs, feral hogs), the term "wild pig" is used to broadly describe Eurasian wild boar outside of their native range, feral domestic pigs, and hybrids of wild boar and domestic pigs (Keiter, Mayer, & Beasley 2016; Smyser et al. 2020). Although the full extent of the impacts of wild pigs on a global scale remains unknown, wild pigs have been estimated to be a threat to hundreds of taxa globally and have already been a primary factor in the extinction of several species (Risch et al., 2021). A growing body of literature (Didero et al., 2023) seeks to describe and quantify the economic and ecological impacts of wild pigs, including to agriculture and natural resources (Bankovich et al., 2016; Engeman et al., 2016). Documented negative impacts of wild pigs are widespread and include crop damage, livestock and wildlife depredation, disease transmission to livestock and wildlife, and destruction of property and ecosystems (Bevins et al., 2014; McClure et al., 2018; R. Miller et al., 2017; Shwiff et al., 2018).

The success of wild pigs throughout their introduced range can largely be attributed to their adaptability and opportunistic, omnivorous food habits (Ballari & Barrios-García, 2014; Hegel et al., 2019). Wild pigs display diverse feeding behaviors including browsing, grazing, rooting, scavenging and predation (Loggins et al. 2002; DeVault, Rhodes, & Shivik 2003; Wilcox & van Vuren 2009). Although wild pig diets are dominated by plant material, they also consume fungi, invertebrates, fish, amphibians, reptiles, birds, and mammals (Wilcox & van Vuren, 2009; Ballari & Barrios-García, 2014; Hegel et al., 2019). Earthworms are commonly consumed and often the most frequent animal material detected (Challies, 1975; Baubet et al., 2003; Ballari & Barrios-García, 2014; Anderson et al., 2018). The extreme variation in food types consumed by wild pigs contributes to their adaptability, allowing them to alter their diets as needed across locations and seasons depending on food availability. Seasonal variability in wild pig diets has been described extensively across their range. For example, acorns are a prominent component of wild pig diets during the fall and winter of mast years (Loggins et al., 2002; Giménez-Anaya et al., 2008). Rooting for underground food items was also observed during winter, when above-ground vegetation was limited (Baron, 1982), while aboveground herbage increased in importance during the spring growing season (Wood & Roark, 1980). Jolley et al. (2010) detected green anoles (*Anolis carolinensis*) and eastern fence lizards (*Sceloporus undulatus*) in colder winter months when the reptiles likely sought warmth on the ground in leaf litter, making them vulnerable to wild pigs rooting during that time. Spatial variation in wild pig diets has also been observed on various scales. For example, while plants dominate the diets of wild pigs overall, wild pigs consume a higher proportion of animal matter and fungi compared to wild boar in their native range (Ballari & Barrios-García, 2014). There is also evidence that the proportion of animal matter consumed can vary with food availability across altitudes (Challies, 1975; Baubet et al., 2003). The extreme diet plasticity observed demonstrates the need for investigating wild pig diets both seasonally and spatially to determine their impacts on native flora and fauna. In particular, islands are among the systems with some of the highest potential risks from wild pigs, yet few studies have investigated diets of wild pigs on islands.

Islands are disproportionately vulnerable to impacts from introduced vertebrates such as wild pigs compared to mainland systems due to high levels of endemism, smaller population sizes, reduced genetic diversity, and lack of defenses against introduced herbivores and predators compared to mainland species (Bowen & van Vuren, 1997; Frankham, 1997; Blumstein & Daniel, 2005). An estimated 23% of herpetofauna, 22% of invertebrate, 12% of bird, and 10% of plant species endemic to islands are believed to be threatened by wild pigs based on the IUCN’s Red List of Threatened Species, but studies investigating these estimated impacts on specific islands reman limited (Risch et al., 2021). Destructive rooting by wild pigs, where wild pigs overturn soil while foraging for plants and invertebrates, is associated with various negative impacts globally (Bankovich et al., 2016; Oldfield & Evans, 2016a; Gray et al., 2020). Island systems can have varying responses to such disturbances, with plant communities taking from 6 months to over 18 years to recover depending on the species composition and environmental attributes of the island (Baron, 1982; Cole & Litton, 2014). In addition to vegetation impacts, wild pigs are known to predate nests of sea turtles, with nest mortality reaching up to nearly 100% in some cases (Engeman et al., 2016; Butler et al., 2020). Coastal bird species (eggs, chicks, and adults) are also considered at risk of predation by invasive wild pigs, with population declines in bird species such as the Lord Howe Island woodhen (*Gallirallus sylvestris*) (Miller & Mullette, 1985) and Galapagos rail (*Laterallus spilonotu*) (Coblentz & Baber, 1987; Donlan et al., 2007) attributed primarily to wild pigs. Documentation of impacts to invertebrates on islands beyond general estimates based on co-occurrence of invertebrates and wild pigs is limited. On a barrier island off the coast of Mississippi, USA, wild pigs foraged for coastal invertebrates on the beaches during summer months, although the extent or potential impacts to these species was not reported (Baron, 1982). Although estimates of the number of plant and wildlife species threatened by wild pigs on islands are substantial, studies on wild pig diets and the associated impacts of wild pig foraging on islands are greatly lacking.

In addition to islands species, Risch et al. (2021) described herpetofauna, particularly amphibians, as the taxa proportionally most threatened by wild pigs in mainland Australia, the U.S., and Europe based on the International Union for the Conservation of Nature's (IUCN) Red List of Threatened Species. Herpetofauna are vulnerable to wild pigs due to both indirect impacts from loss of habitat via rooting (Means & Travis, 2007; Maerz et al., 2015; Rossell et al., 2016) and direct predation (Jolley et al., 2010). Amphibians concentrate in wetlands during the breeding season and typically remain within approximately one kilometer of breeding habitat the rest of the year (Rittenhouse & Semlitsch, 2007; Marshall et al., 2023). Wild pigs select extensively for wetlands and other habitats in proximity to water (Clontz et al., 2021). This results in substantial overlap in use of wetlands between wild pigs and amphibians, facilitating opportunistic consumption of amphibians and destruction of important amphibian habitat via rooting. Given current global declines in many amphibian populations (Stuart et al., 2004; Pimm et al., 2014), there is an urgent need for a greater understanding of the extent of wild pig impacts to amphibians.

The southeastern United States has a well-established population of wild pigs (Lewis et al., 2019) which were first introduced to Florida by Spanish explorers in the early 16th century (Mayer et al., 2020). Since then, wild pig populations have expanded across much of North America (McClure et al., 2015; Aschim & Brook, 2019). The southeastern USA has been identified as a region of biodiversity conservation concern (Olson & Dinerstein, 2002), accounting for over half of herpetofauna species diversity in Canada and the U.S., as well as many mammal and bird species in both inland and barrier island systems (Brown, 1997; Jensen et al., 2008; Potter et al., 2015). Therefore, the southeastern USA provides an important study area to investigate potential impacts of invasive wild pigs to sensitive taxa and islands.

Additionally, most wild pig diet studies to date have relied on traditional methods of dietary assessment, consisting of manual examination of stomach contents, and typically reported percent of volume and/or percent frequency of occurrence for each identified food type (e.g., Everitt & Alaniz, 1980; Giménez-Anaya et al., 2008; Jolley et al., 2010). However, omnivorous diets present a unique challenge for visually examining stomach contents (De Barba et al., 2014; Robeson et al., 2018). Food types have differential rates of digestion, with difficult-to-digest plant materials taking much longer than materials such as egg shells and soft tissues, suggesting that consumption of some animal groups such as amphibians could be underestimated (Fournier-Chambrillon et al., 1995; De Barba et al., 2014; Oja et al., 2017). Molecular methods such as DNA metabarcoding can provide a more sensitive and comprehensive analysis of diet from scat samples or stomach contents, and are increasingly being used to characterize wildlife diets (Kohn & Wayne, 1997). Primers targeting the CO1 gene for animals and the trnL or rbcL genes for plants have allowed studies to successfully investigate carnivore (Rodgers & Janečka, 2012; Shao et al., 2019), herbivore (Kress & Erickson, 2007; Kartzinel et al., 2015; Nakahama et al., 2021), and omnivore diets (De Barba et al., 2014; Ducotterd et al., 2021). To take advantage of this methodology, Robeson et al. (2018) developed a protocol and primers to utilize metabarcoding for wild pig diet analysis from fecal samples. Beyond this study, use of metabarcoding to assess wild pig diet is limited to one study in Florida (Anderson et al. 2018) and three studies for wild boar in their native range (Estonia, southern India, and Japan) (Oja et al. 2017; Saito et al. 2020; ter Schure et al. 2021). While both traditional and molecular methods can provide useful information, further examination of wild pig diets with molecular methods is needed, particularly to ensure vertebrate consumption is not underestimated.

The goal of this thesis is to characterize the diets of invasive wild pigs in the southeastern USA, with a focus on using DNA metabarcoding to provide insight into the potential impacts of wild pig foraging on vertebrates. In Chapter 2, I document the vegetation and vertebrate communities consumed by wild pigs in South Carolina throughout the year and quantify differences in the diet composition of wild pigs across seasons and between males and females. I accomplished this by collecting fecal samples from 222 adult wild pigs from June 2017 through September 2018 on the Savannah River Site in Aiken, South Carolina, an area with a high concentration of isolated wetlands, and using DNA metabarcoding to detect and identify the prey items with primers targeting the chloroplast trnL (UAA) intron for plants (Taberlet et al., 2007) and the 12S mitochondrial rDNA gene for vertebrates (Valentini et al., 2016). In Chapter 3, I investigate the diets of wild pigs on three barrier islands in Georgia to quantify differences in diet composition between islands and among seasons. To accomplish this goal, I collected fecal material from 171 adult wild pigs that were culled May 2022 – July 2023 as part of ongoing management programs on Blackbeard Island, Sapelo Island, and Cumberland Island. To identify food items in the samples, I used DNA metabarcoding with the same primers and primers targeting the mitochondrial cytochrome c oxidase subunit I (COI) gene for invertebrates (Zeale et al., 2011). This thesis collectively aims to provide empirical molecular evidence regarding the food habits of an invasive mammal in the southeastern USA and determine potential impacts to vulnerable island systems and vertebrate taxa.

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CHAPTER 2

DNA METABARCODING REVEALS CONSUMPTION OF DIVERSE COMMUNITY OF AMPHIBIANS BY INVASIVE WILD PIGS (*SUS SCROFA*) IN THE SOUTHEASTERN UNITED STATES

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Abstract

Invasive wild pigs (*Sus scrofa*) are one of the most widespread, destructive vertebrate species globally. Their success can largely be attributed to their generalist diets, which are dominated by plant material but also include diverse animal taxa. Wild pigs are demonstrated nest predators of ground-nesting birds and reptiles, and likely pose a threat to amphibians given their extensive overlap in wetland use. DNA metabarcoding of fecal samples from 222 adult wild pigs culled monthly from 2017 - 2018 revealed a diverse diet dominated by plant material, with 166 plant genera from 56 families and 18 vertebrate species identified. Diet composition varied seasonally with availability for plants and was consistent between sexes. Amphibians were the most frequent vertebrate group consumed and represented the majority of vertebrate species detected, suggesting amphibians are potentially vulnerable to predation by wild pigs in our study region. Mammal, reptile, and bird species were also detected in pig diets, but infrequently. Our results highlight the need for research on the impacts of wild pigs on amphibians to better inform management and conservation of imperiled species.

Introduction

Invasive species present a significant threat to global biodiversity and community function that is second only to threats from habitat loss and fragmentation (Barrios-Garcia & Ballari, 2012). Invasive wild pigs (*Sus scrofa*), which include Eurasian wild boar outside of their native range, feral domestic pigs, and their hybrids (Keiter et al., 2016; Smyser et al., 2020), are one of the most widespread and prolific invasive vertebrates globally, occurring on all continents except Antarctica as well as many islands (Massei & Genov, 2004; Barrios-Garcia & Ballari, 2012). Although the full extent of wild pig impacts on a global scale remains unknown, wild pigs pose a significant threat to hundreds of taxa and have been a primary factor in the extinction of several species (Risch et al., 2021). In the United States (U.S.), wild pigs are responsible for a wide range of negative impacts including damage to crops, livestock depredation, disease transmission, destruction of property and ecosystems, and depredation of wildlife (Bevins et al., 2014; R. Miller et al., 2017; McClure et al., 2018; Shwiff et al., 2018). Their distributions in the US have been estimated to overlap with over 85% of imperiled species that could be directly impacted by wild pigs through habitat destruction or predation (McClure et al., 2018). A growing body of literature (Didero et al., 2023) seeks to describe and quantify their economic impact, including to agriculture and natural resources (Bankovich et al., 2016; Engeman et al., 2016).

Wild pigs are dietary and habitat generalists that display a wide array of feeding behaviors, including browsing, grazing, rooting, scavenging, and predation (Loggins et al., 2002; Wilcox, 2015; Turner et al., 2017). In particular, rooting, where pigs overturn soil in search of food, has been associated with negative impacts to both plants and animals (Barrios-Garcia & Ballari, 2012; Bankovich et al., 2016). Disturbances from rooting reduce populations and overall diversity of native plant species and allow for the establishment of exotic plants (Bankovich et al., 2016; Oldfield & Evans, 2016b). Rooting by wild pigs was also found to disrupt vital montane seep habitat for salamanders, resulting in decreased salamander abundance (Rossell et al., 2016) and has been implicated in the declines of Southern Dusky Salamanders (*Desmognathus auriculatus*) (Means & Travis, 2007; Maerz et al., 2015). Garabedian et al. (Garabedian et al., 2023) found that white-tailed deer (*Odocoileus virginianus*) alter their fine scale movements and space use in response to presence of even low densities of invasive wild pigs, suggesting an attempt at reducing competition. A broad range of taxa are thus impacted by wild pigs, with these impacts reaching from individual to community levels of organization.

In addition to indirect disruptions caused by rooting behaviors of wild pigs and wild boar, their generalist diets allow them to consume a wide variety of taxa, creating direct impacts on species through predation (Baber & Coblentz, 1987; Fournier-Chambrillon et al., 1995; Anderson et al., 2018; Robeson et al., 2018). Plants make up the largest component of diets of both wild pigs and wild boar in their native range, constituting 62% – 100% by volume and occurring in nearly 100% of stomach samples (Baber & Coblentz, 1987; Fournier-Chambrillon et al., 1995; Ballari & Barrios-García, 2014; Anderson et al., 2018). Wild pigs have also been found to consume fungi, insects, mollusks, crustaceans, fish, amphibians, reptiles, birds, and mammals (Ballari & Barrios-García, 2014). Earthworms are commonly consumed and often the most frequent animal material detected (Challies, 1975; Baubet et al., 2003; Ballari & Barrios-García, 2014; Anderson et al., 2018). Among vertebrate groups consumed, small mammals such as California voles (*Microtus californicus*) and Botta's pocket gophers (*Thomomys bottae*) have been found to occur in wild pig diets at high frequencies, with evidence of targeted predation (Wilcox & van Vuren, 2009; Wilcox, 2015). While predation has been documented, Wilcox and van Vuren (2009) noted that the vertebrates consumed were primarily fossorial or semi-fossorial small mammals and thus could have been taken opportunistically.

Although studies are limited, amphibians, reptiles, and ground-nesting birds could be similarly at risk of predation by wild pigs and native wild boar (Challies, 1975; Jolley et al., 2010; Mori et al., 2021; Robeson et al., 2018; Wilcox & van Vuren, 2009). Within their native range, wild boar consume chicks and eggs of ground-nesting birds, making them a potential threat to the conservation of these species (Santilli & Senserini, 2016; Oja et al., 2017; Mori et al., 2021). Invasive wild pigs have also been found to impact native birds. For example, on a small Australian island, wild pigs were implicated in the decline of a flightless bird, the Lord Howe Island woodhen (*Gallirallus sylvestris*), which was able to expand its distribution on the island following removal of wild pigs (Miller & Mullette, 1985). While amphibians and reptiles have thus far been found to occur at low frequencies in wild pig diets, Risch et al. (2021) described herpetofauna as the taxa proportionally most threatened by wild pigs in Australia, the U.S., and Europe based on the International Union for the Conservation of Nature's (IUCN) Red List of Threatened Species. This could largely be due to the overlap in use of wetlands between wild pigs and amphibians. Amphibians are known to concentrate in wetlands during breeding season and typically remain within a kilometer of breeding habitat the rest of the year (Rittenhouse & Semlitsch, 2007), and wild pigs select for wetlands and other habitats in proximity to water (Clontz et al., 2021). The significant threats posed by invasive wild pigs creates an urgent need for understanding the extent of these impacts, including wild pig predation on native vertebrate species.

The generalist and omnivore diet of wild pigs and native wild boars allows them to alter their diets as needed across locations and seasons depending on availability (Genov, 1981;

Loggins et al., 2002; Giménez-Anaya et al., 2008; Robeson et al., 2018). For example, while plants dominate the diets of *Sus scrofa* overall, wild pigs consume a higher proportion of animal matter and fungi than wild boar in their native range (Ballari & Barrios-García, 2014). Seasonal variability in wild pig diets has primarily been attributed to plant availability. For example, acorns are a prominent component of the diets of both wild pigs and wild boar during fall and winter (Loggins et al., 2002; Giménez-Anaya et al., 2008). Rooting for underground food items was also observed during winter, when above-ground vegetation was limited (Baron, 1982), while herbage and foliage increased in importance during the spring growing season (Wood & Roark, 1980). Studies describing seasonal trends in the consumption of animals are more limited but provide further evidence of opportunistic feeding. For instance, wild boar consumed ducks more frequently during molting season when they were more vulnerable to predation (Giménez-Anaya et al., 2008). Jolley et al. (2010) detected green anoles (*Anolis carolinensis*) and eastern fence lizards (*Sceloporus undulatus*) in December and January, when the lizards and anoles were likely most available to wild pigs as they sought out warmth in leaf litter. Due to this extensive seasonal variability, it is important to conduct year-round investigations of wild pig diets to create a full picture of their potential impacts to native species.

Although studies are more limited, sex is another potential factor influencing food selection by wild pigs, as females have the added energetic cost of reproduction and caring for large litters of young, sometimes multiple times per year (Chinn et al., 2022). Most studies have not found evidence of differences in diet composition between males and females (Loggins et al., 2002; Adkins & Harveson, 2006; Wishart et al., 2015). However, Wilcox and Van Vuren (2009) found that female wild pigs appeared to consume higher frequencies of vertebrates during summer and fall compared to males, which corresponded to periods of reduced physical condition (measured by rump fat) in their sampled wild pigs. They suggested that females might increase consumption of protein-rich vertebrate species due to higher energetic costs of reproduction (Wilcox & van Vuren, 2009). These conflicting results suggest that more research is needed to investigate the potential effect of sex on wild pig food selection throughout the year.

Most wild pig and native wild boar diet studies to date have relied on visual examination of stomach contents (Everitt & Alaniz, 1980; Fournier-Chambrillon et al., 1995; Giménez-Anaya et al., 2008). However, omnivorous diets present a unique challenge for visually examining stomach contents, as food materials can have differential rates of digestion, with difficult-to-digest plant materials remaining easier to detect and identify than animal materials such as egg shells and soft tissues, which quickly degrade and can be underrepresented (De Barba et al., 2014; Oja et al., 2017; Robeson et al., 2018). Molecular methods such as DNA metabarcoding can provide a more sensitive and comprehensive analysis of diet from fecal samples (Kohn & Wayne, 1997), and are increasingly being used to characterize omnivore diets (De Barba et al., 2014). However, use of molecular methods to assess wild pig and wild boar diets remains limited (Oja et al., 2017; Anderson et al., 2018; Robeson et al., 2018; ter Schure et al., 2021). Due to the concern regarding predation of vertebrates, more diet studies using DNA metabarcoding are needed to ensure predation events of vertebrate taxa are not underestimated. In particular, the southeastern U.S. was identified as a region of biodiversity conservation concern (Olson & Dinerstein, 2002) and accounts for over half of herpetofauna species diversity in Canada and the U.S., as well as many mammal and bird species (Brown, 1997; Jensen et al., 2008; Potter et al., 2015). However, wild pig dietary studies within this region are limited (Wood & Roark, 1980; Anderson et al., 2018; Robeson et al., 2018), despite long-established wild pig populations (Lewis et al., 2019; Mayer et al., 2020).

The goal of this study was to use DNA metabarcoding to assess the potential impacts of invasive wild pig diet on native plant and vertebrate species in South Carolina, U.S. Our objectives were to (1) characterize the vegetation and vertebrate communities consumed by wild pigs in South Carolina, (2) quantify differences in the dietary beta diversity of wild pigs across seasons and between males and females, and (3) identify which taxa appear most susceptible to predation by wild pigs in this study region. We predicted that diet composition would vary seasonally with availability. For example, oak (*Quercus* spp.) would be consumed most frequently in fall and winter months when acorns were available in higher quantities. Additionally, we hypothesized that consumption of plant material would be the same between males and females, but that vertebrates would be consumed more by females than males. Finally, we hypothesized that more amphibians would be detected in wild pig diets compared to previous studies due to the high amphibian diversity and abundance in the region, the shared use of wetlands by amphibians and wild pigs, and our use of DNA metabarcoding as opposed to traditional dietary methods.

Results

Diet Overview

Of the 222 samples collected, two were removed prior to analysis as they did not meet the criteria of taxonomic resolution down to family level. The final data set used for statistical analysis represented approximately balanced sexes, with 116 female samples, 101 male samples, and three of unknown sex. Across all samples, the total number of reads was 3,137,261 for plant (trnL) results and 8,763 for vertebrates (12S rDNA), with a mean read count of 14,260 per sample for plant data and 39.8 per sample for vertebrate taxa. The mean number of plant families consumed by individual wild pigs was 8 (±3, SD) and mean number of vertebrate species detected per sample was 0.11 (±0.50, SD). Only 17 samples out of the original 222 contained vertebrate prey items and of these, 12 were female and 5 were male wild pigs.

Plants were consumed more frequently than vertebrates, occurring in 100% of samples (Supplementary Table S1). Across all samples, 166 plant genera belonging to 56 plant families were recorded. The most frequent plant families also had the highest RRA in the diet, although the rank order of some families was slightly different between the two metrics (Fig. 1). Poaceae (grasses), Fabaceae (legumes; e.g., *Apios* spp., *Desmodium* spp., *Trifolium* spp.), Fagaceae (hard-mast species; i.e., *Quercus* spp.), and Rosaceae (forbs, soft-mast species; e.g., *Potentilla* spp., *Rubus* spp., *Prunus* spp.) were both the most frequently occurring and most abundant plant families, in descending order.

Vertebrates were identified to 18 species belonging to four classes (Amphibia, Reptilia, Mammalia, and Aves) (Table 1). Amphibians were the most frequent vertebrate group detected, occurring in 71% of samples with vertebrate DNA. Amphibians were also the most diverse group of vertebrates consumed, representing 12 of the 18 species detected. We detected more amphibian species in sampled wild pigs than other US studies, including studies in the southeastern region (Wood & Roark, 1980; Loggins et al., 2002; Jolley et al., 2010; Anderson et al., 2018). Barking tree frogs (*Hyla gratiosa*) were the most frequently detected vertebrate, occurring in 4 samples. Mammal, reptile, and bird species were detected infrequently, with eastern red bat (*Lasiurus borealis*) occurring most frequently in 3 samples. American crow (*Corvus brachyrhynchos*), wild turkey (*Meleagris gallopavo*), ruby-crowned kinglet (*Regulus calendula*) were the 3 bird species detected. Two mammal species, short-tailed shrew (*Blarina brevicauda*) and eastern red bat, and 1 reptile species, broadhead skink (*Plestiodon laticeps*), were also detected (Table1). We observed vertebrates in the diet primarily during late autumn through early spring, with only one sample containing vertebrates between May and August (Fig. 2). Amphibians, the dominant vertebrate taxon sequenced, drove this trend, with 88.2% of amphibian detections occurring December – April. Of the 17 amphibian detections found, 44.2% occurred during the amphibian breeding seasons (Fig. 3) (Jensen et al., 2008).

Beta Diversity

For plant abundance data, the NMDS randomization test converged on a stress value of 0.19, indicating that individual dissimilarities between plant compositions were effectively captured with 3 dimensions. The ANOSIM for plant beta diversity revealed a significant effect of month ($p < 0.001$, $R = 0.21$), with fall and winter months clustered distinctly from spring and summer months (Fig. 4). In contrast, sex did not appear to influence plant beta diversity in the diet as neither sex, nor the interaction of month and sex significantly affected plant beta diversity (Fig. 4).

All pairwise comparisons of plant beta diversity among months conducted with a PERMANOVA were significant (FDR adjusted $p < 0.05$) with the exception of mid-winter months (January x February) and late spring through early fall months (June x May, July x May, June x July, and June x September). Diet composition thus varied between most months but did demonstrate some level of seasonality with some months within seasons having similar compositions.

The Indicator Species Analysis revealed 16 indicator plant families out of 56 families detected (Supplementary Fig. S1). Poaceae and Fabaceae were the most frequently occurring plant families and were selected as indicator families throughout most of the year. Fagaceae (hard-mast) was found to be an indicator family for fall months, as well as July (Fig. 5). Juglandaceae (e.g., *Carya* spp.), also hard-masting species, was also an important dietary component during fall months. Pinaceae (pines) was a significant component of the diet in early fall and spring. In spring and summer months, soft-mast producing plants (Rosaceae) as well as shrubs, edicts, and vines (Amaryllidaceae, Arecaceae, Commelinaceae, Polygonaceae, Salicaceae, Smilacaceae, Violaceae, Vitaceae, and Zygophyllaceae) were identified as indicator families in the diet composition.

The trends of plant dietary composition identified by the SIMPER were similar to those revealed by the Indicator Species Analysis. Fabaceae, Fagaceae, Rosaceae, Vitaceae, and Pinaceae were identified as the plant families contributing to the most dissimilarity between months, followed by Polygonaceae, Juglandaceae, Arecacaea, Poaceae, Smilacaceae, and Typhaceae, respectively (Fig. 6). The discrepancy in relative ranking of Poaceae in the SIMPER as compared to the Indicator Species Analysis reflects the key difference in interpretation of the two analyses. Poaceae frequently occurred across many months (ranking high in the Indicator Species Analysis) and therefore was consistent throughout the year, reducing its contribution to seasonal variation in dietary composition and ranking relatively lower in the SIMPER compared to other families that occurred at high frequencies/abundances in only a few months.

Compositional Data Analysis for Plant Data

To account for the compositional nature of our data, we transformed the plant data using a centered log-ratio transformation (CLR) and conducted an ANOSIM analysis with this transformed data. Using CLR transformed data did not appear to have differing results from the ANOSIM with raw abundance data. Month significantly influenced the beta diversity of consumed plants ($p = 0.001$, $R = 0.08$). Sex and the interaction of sex and month were not significant.

Discussion

Wild pigs are one of the most invasive species globally, and present a threat to countless species worldwide (McClure et al., 2018; Risch et al., 2021). Examining their diets can provide insight into taxa that might be vulnerable to wild pig consumption and inform management and conservation decisions. Using DNA metabarcoding of wild pig fecal samples collected across a two-year period, our study revealed a highly diverse diet, with 166 plant genera from 56 families and 18 vertebrate species identified. Plants dominated the diet in both abundance and frequency of occurrence, and diet composition varied seasonally but not between sexes. Although vertebrates were consumed at lower frequencies compared to plant matter, we detected vertebrates spanning a relatively wide breadth of taxa, including vertebrate groups with fossorial, semi-fossorial, and terrestrial habits thought to be vulnerable to wild pig predation (amphibians, reptiles, small mammals, and ground-nesting birds). In particular, amphibians appear to be among the vertebrate classes more susceptible to predation by wild pigs within our study region, occurring most frequently and representing the majority of the species detected. Given current global declines in many amphibian populations (Stuart et al., 2004; Pimm et al., 2014) and extensive overlap in habitat use surrounding wetlands by both wild pigs and amphibians (Semlitsch & Bodie, 2003; Engeman et al., 2007; Rossell et al., 2016; Jones et al., 2018), our results highlight the potential vulnerability of amphibian populations to predation by wild pigs. Thus, this underlines the need for focused management of invasive pigs in localized habitats containing populations of imperiled species.

Consumption of plant material changed throughout the year with availability, as demonstrated in previous studies of wild pig and native wild boar diets (Ballari & Barrios-García, 2014). However, DNA metabarcoding allowed for identification of a greater taxonomic breadth to a finer resolution than many traditional wild pig diet studies (Wood & Roark, 1980; Herrero et al., 2004; Fournier-Chambrillon et al., 1995). Grasses were the most common and abundant vegetation consumed throughout the year, which is consistent with other studies in the region (Wood & Roark, 1980; Anderson et al., 2018). Edicts (e.g., Fabaceae) were also observed consistently across seasons. As predicted, fall and early winter months were characterized by hard masting species, with consumption of oaks and hickory peaking in October but continuing through March (Fournier-Chambrillon et al., 1995; Loggins et al., 2002; Anderson et al., 2018). Pines also were dominant in the diet during fall months, although this could have been incidental consumption of pine needles during rooting within pine stands. As hard mast availability dwindled in late winter and early spring, pines remained dominant in the diet along with ferns and wetland species such as *Sparganium* spp. and *Typha* spp., although pine detected during spring could in part be pollen. Corresponding to increased plant growth in spring and summer, wild pig diets increased in herbaceous vegetation including woody vine species (e.g., *Smilax* spp.) and soft-masting species (e.g., Rosaceae). We also observed a notable increase in consumption of oaks in July, likely comprising oak seedlings for which wild pigs are known consumers (Gómez & Hódar, 2008). This summer spike in consumption of oak indicates that targeted temporal and spatial removal of wild pigs might be needed in areas where oak recruitment is of management concern.

Compared to plants, DNA metabarcoding performed better for vertebrate taxa, allowing us to identify all detected vertebrates to species level. Although vertebrates were consumed less frequently than plants, wild pigs consumed species belonging to all four vertebrate groups of interest (amphibians, reptiles, small mammals, and ground nesting birds). It is important to note that absence of earthworms and other invertebrates in this study was due to our decision to focus on vertebrates with a vertebrate-specific primer, not necessarily lack of consumption by our sampled wild pigs. Amphibians were the primary class of vertebrates detected, comprising 12 of the 18 vertebrate species. This represents the most amphibian species identified in wild pig diets in the US to date, with prior studies detecting 0 – 5 amphibian species (Wood & Roark, 1980; Loggins et al., 2002; Jolley et al., 2010; Anderson et al., 2018). It’s possible that other studies using traditional methods might have underestimated amphibian presence due to rapid digestion. Anderson et al. (Anderson et al., 2018) used metabarcoding to examine wild pig diets in Florida and detected only 5 amphibian species but used a different 12S primer set that was not designed for *Bactrachia* amplification. Of the 12 amphibian species detected in our study, only one salamander, the southern two-lined salamander (*Eurycea cirrigera*) was found. The low numbers of salamander detections were surprising considering the fossorial habits and high abundance of salamanders in our study region. (Kinkead et al., 2007; Jensen et al., 2008). For example, we expected to detect Ambystoma spp. in the diets of wild pigs in our study area as these salamanders are semi-fossorial and common within and around wetlands. An *in silico* analyses performed by Jonah Ventures, LLC for our primer set revealed that the Batr01 primer set reference database is biased against some groups of salamanders including Ambystomatidae, suggesting that more salamanders could have been consumed by wild pigs in our study than shown here.

Consumption of amphibians appeared to demonstrate a seasonal trend, with detections clustered between late fall and early spring. Our amphibian detections were both during and outside the known breeding windows of these species, indicating that amphibians can be consumed in habitats used outside the breeding season (Jensen et al., 2008). DNA metabarcoding does not enable us to determine whether the amphibians detected in pig diets were eggs, larvae, or adults, or if individuals were deceased prior to consumption so we are limited in our current understanding of when and which habitats amphibians are most vulnerable. Given the rooting habits of pigs, we expect they are most likely consuming adults or juveniles around and within wetlands. Mortality to adults and juveniles could have significant impacts on amphibian populations than would scavenging of remnant tadpoles in drying wetlands (Biek et al., 2002).

Despite concerns regarding wild pig and wild boar predation on ground-nesting birds and reptiles (Challies, 1975; Miller & Mullette, 1985; Engeman et al., 2016; Oja et al., 2017), we found limited evidence of this occurring among the individuals sampled in our study. Wild turkey (*Meleagris gallopavo*) was a species of interest in our study area as a ground-nesting game bird, but was only detected in a single occurrence, and the timing of this detection in January (outside of nesting season) suggests this was likely a scavenged adult and unrelated to nesting behavior. Furthermore, only one reptile, the broadhead skink (*Plestiodon laticeps*) was detected in a single sample in April. While other studies in the U.S. have seen higher occurrences of small mammals in wild pig diets with over one third of samples containing small mammals (Wilcox & van Vuren, 2009; Anderson et al., 2018), the short-tailed shrew (*Blarina brevicauda*) was the only small mammal detected in our study and occurred in only one sample. These findings suggest that in South Carolina, amphibians appear to be among the more vulnerable wild pig prey groups in contrast to other vertebrate taxa that have been documented in the diet at higher levels elsewhere (Giménez-Anaya et al., 2008; Wilcox & van Vuren, 2009; Engeman et al., 2019). However, given the dominance of plants, more in-depth studies focused within periods of peak vulnerability of ground nesting birds or other concerned taxa (e.g., nesting seasons) are needed to fully capture the potential extent of impacts of wild pig predation.

When using molecular methods, primer biases can influence the breadth and depth of species detected (Pawluczyk et al., 2015; Deagle et al., 2019a). While our specific 12S primer (Valentini et al., 2016) biases could have potentially led to underrepresentation of bird, mammal, and reptile taxa detection in our study, Kluever et al. (2022) detected multiple local bird and mammal species in the diets of coyotes using the same primer set used in our research. This suggests that primer biases likely played a minimal role in our infrequent detections of birds and mammals and that the species we detected among these groups are likely representative of the actual species consumed by the sampled individuals. However, an *in silico* analysis by Jonah Ventures, LLC determined a bias of our Batr01 primer set against reptiles in addition to salamanders, suggesting that wild pigs could have consumed more reptile species than we were able to identify. Given the presence of small fossorial snakes and lizards on the SRS (Gibbons & Semlitsch, 1991), reptiles were likely underrepresented in our study.

Surprisingly, eastern red bats were detected more frequently than birds, reptiles, and other mammals. This is the first known documentation of wild pig consumption of a bat species. Eastern red bats are arboreal and select winter roosts in midstory to understory locations and occasionally in the leaf litter when temperatures are 0-10℃, potentially explaining the detection in January (Rivera-Giboyeaux, 2018), as an individual could have been accessible to a wild pig during torpor. However, temperatures did not drop below that threshold during our study period for the September detections (Rivera-Giboyeaux, 2017, 2018), which could have reflected scavenging of carcasses, predation of recently volant juveniles, or coprophagy of bat guano as DNA metabarcoding does not allow us to differentiate these forms of consumption from predation (Robeson et al., 2018).

While RRA can be tentatively interpreted as a semi-quantitative estimate of dietary importance, it is not a reliable predictor of number of individuals of each species consumed and thus we cannot determine how many individual vertebrates were consumed in each sample (Deagle et al., 2019a). As opportunistic foragers, wild pigs have been documented to consume large quantities of a single food item within a short period, with one stomach containing as many as 49 eastern spadefoot toads (Jolley et al., 2010). Therefore, our results are likely a conservative estimate of the number of individual vertebrates actually consumed. Furthermore, we sampled trapped wild pigs that consumed corn at bait sites for several days prior to capture while conditioning to the trap site. As trapped wild pigs thus had some level of a supplemented diet of easily accessible corn, they could have been consuming less vertebrates than wild pigs that were not being trapped and therefore were not provided with any level of diet supplementation, and the extent of vertebrates in wild pig diets may have been underestimated in our study compared to other scenarios.

Our findings suggest that wild pigs have the potential to pose an important predation risk to amphibian populations. As wild pigs prefer wetland habitat and forage within the leaf litter and upper soil layers (Gray, Roloff, Montgomery, et al., 2020; Clontz et al., 2021), they are likely to encounter amphibians frequently, particularly in regions of the world with high amphibian diversity, such as the southeastern U.S. (Jensen et al., 2008). Wild pigs are notorious for destroying critical wetland habitats through rooting while foraging (Engeman et al., 2007; Rossell et al., 2016; Keiter & Beasley, 2017; Jones et al., 2018), which may further exacerbate their impacts to vulnerable amphibian communities. As primarily opportunistic feeders, wild pigs could consume high volumes of amphibians in a short time period (Jolley et al., 2010). If they happen to encounter an amphibian breeding event while foraging, this could be detrimental to localized amphibian populations through the additive effect of direct predation and indirect habitat loss, particularly for those species that are already imperiled. While no threatened or endangered species were detected among our samples, this was not surprising due to the inherent low availability of rare species on the landscape. When combined with short duration over which dietary studies reflect consumed food items before they pass through the digestive system and the relatively low frequency that vertebrates were consumed (<8%) in this study, it is not unlikely that rare species would go undetected. However, we detected fossorial or semi-fossorial species that spend time in leaf litter and near wetlands which are life history traits similar to several species of concern in the southeastern U.S. such as gopher frogs (*Rana capito*) and reticulated flatwoods salamander (*Ambystoma bishopi*). Coupled with previous research on vertebrate species frequently detected in wild pig diets (Wilcox & van Vuren, 2009; Jolley et al., 2010), this suggests that species with these life history characteristics have the potential to be vulnerable to depredation by wild pigs in areas where their ranges overlap. Furthermore, our results provide further evidence that wild pig food habits can pose potential threats to imperiled wetland habitats and oak sapling recruitment, and thus would benefit from management of wild pig populations.

Collectively, our findings highlight the need for further research into the extent to which wild pigs may pose a threat to amphibian populations globally, both directly from predation but also indirectly through habitat modification during rooting. Additional spatio-temporal studies using molecular approaches across larger regions within biodiversity hotspots are needed to determine the extent that amphibians, bats, and imperiled species are being consumed across their range. Finally, more extensive targeted sampling in areas with species of concern should be conducted and more common species with similar life history traits could be utilized as a proxy for rare species to provide insight into how to approach management of wild pigs to best reduce the effects of wild pigs on taxa that are most vulnerable to their impacts.

Methods

Study area

This study was conducted at the U.S. Department of Energy's Savannah River Site (SRS) in west-central South Carolina. The SRS is located in the sandhills and the upper-coastal plain ecoregions of South Carolina, and dominated by upland pine forest, bottomland hardwood forest, and riparian habitats (White and Gaines 2000). Upland pine habitats (~50% of site) are comprised primarily of loblolly pine (*Pinus taeda*), longleaf pine (*P. palustris*), and slash pine (*P. elliottii*). Bottomland hardwoods (~25% of site) include *Taxodium* spp., *Liquidambar* spp., *Quercus* spp., and *Nyssa* spp. Upland hardwood forest, including *Carya* spp., *Acer* spp., *Quercus* spp., and shrubby/herbaceous habitat cover an additional 18% of the site (Clontz et al., 2022). The site hosts a high diversity of vertebrate species, with close to 100 herpetofauna species (GibbonsAuthor to whom correspondence should be addressed. et al., 1997) as well as many birds (McCallum et al., 2000), and mammals (Cothran et al., 1991). While the SRS has a perimeter fence to restrict public access to the site, wild pigs are able to move freely across the boundary, and have been present in the area since before the establishment of the SRS (Mayer & Beasley, 2018). Despite control efforts, wild pigs are abundant and widely distributed across the landscape (Keiter et al., 2017).

Data Collection

We sampled both male and female wild pigs between June 2017 and September 2018 that were live-trapped and culled as part of ongoing wild pig management on the SRS and individuals that were live captured and released for other research purposes. Sampled wild pigs received some level of dietary supplementation as traps were baited with corn. Fresh fecal samples were obtained in the field from culled wild pigs during necropsy or while under anesthesia from the distal colon/rectum. We sampled individuals evenly across the primary habitat types of the SRS and during all months throughout the study years, with approximately balanced sampling efforts across months and sexes. We selected wild pigs >1 year of age and larger than 25 kg to ensure they were large enough to consume vertebrate prey items and to be independently foraging. No wild pigs were euthanized specifically for this research. This study was approved by the University of Georgia Institutional Animal Care and Use Committee. All experimental protocols were conducted in accordance with the Institutional Animal Care and Use Committee under University of Georgia protocols A2015 05-004, A2015 12-017, and A2018 06-024. All methods were performed in accordance with the ARRIVE guidelines.

Samples were placed on ice in the field until they were transferred to an ultra-low temperature freezer (approximately -70 C) within a few hours. Samples were later shipped on dry ice to Jonah Ventures, LLC (Boulder, Colorado, USA) for molecular analysis of food items.

Laboratory analyses and data curation

All laboratory analyses were conducted by Jonah Ventures laboratory. To amplify plant taxa, we used a primer set targeting a section of the chloroplast trnL (UAA) intron – g (5’-GGGCAATCCTGAGCCAA-3’) and h (5’-CCATTGAGTCTCTGCACCTATC-3’ (Taberlet et al., 2007). To amplify vertebrate taxa, we utilized the Batr01 primer set, which targets the 12S mitochondrial rDNA gene -F (5’-ACACCGCCCGTCACCCT-3’) and R (5’-GTAYACTTACCATGTTACGACTT-3’) (Valentini et al., 2016) focused on the taxonomic group Batrachia but amplifies other vertebrate groups as well. We selected a vertebrate primer set to exclude invertebrates to prevent the anticipated high volumes of earthworms from masking the targeted but typically less frequent vertebrate taxa that were more central to our research question. Our methods used for DNA isolation and processing of sequences were similar to those described by Robeson et al. (Robeson et al., 2018) for trnL analyses with an updated form of the UNOISE (v3) pipeline to generate Operational Taxonomic Unit (OTU) sequences as Exact Sequence Variants (ESVs) (Callahan et al., 2017). Sequences were downloaded from GenBank and top hits with alignment query coverages of at least 90% and identities greater or equal to 85% were selected using NCBI BLAST. This was followed by a custom processing pipeline created by Jonah Ventures. We discarded sequences that could not be identified to the family level or were considered possible contaminants.

Statistical analysis

All analyses were conducted using R v 4.1.1 (R Core Team, 2021). Because distinct primers were used for plant and vertebrate data, they were examined separately. All analyses were performed on read count (abundance) data at the family level of taxonomic resolution unless otherwise stated. We also calculated both percent Frequency of Occurrence (%FOO) and Relative Read Abundance (RRA) (Deagle et al., 2019a) for use in visualizations and semi-quantification of the diet. %FOO is considered a more conservative approach to interpreting diet data, but it can lead to overestimation of low abundance food items, since as an occurrence metric all food items are given the same weight. RRA, or relative abundance, eliminates this concern but can be influenced by recovery biases, and is thus not always an accurate representation of the relative abundance of the food that was actually consumed (Deagle et al., 2019a). To account for these concerns and for generalizability, we provided both. We calculated %FOO for each food item by dividing the number of samples containing that food by the total number of samples, multiplied by 100. RRA was calculated by dividing the read count of each food item by the total number of reads for that marker and expressed as a percent.

For beta diversity, or diet composition, our data violated assumptions of normality, and we thus conducted non-metric multidimensional scaling (NMDS) to visualize trends between months and sexes. A three-dimensional solution from the lowest stress was used to run a randomization test with 1,000 permutations. We then utilized a non-parametric Analysis of Similarities (ANOSIM) with Bray-Curtis distance with 9,999 permutations to determine the effects of sex and month on beta diversity of wild pig diets. For pairwise comparisons of plant dietary beta diversity between months we conducted a PERMANOVA with Bray-Curtis distance with 9,999 permutations and False Discovery Rate (FDR) corrected p-values. The NMDS, ANOSIM, and PERMANOVA were conducted using the vegan package in R (Oksanen et al., 2022). Alpha diversity was also calculated and descriptions of these methods and results are provided in the Supplementary Information.

To further explore trends of plant beta diversity, we conducted a Similarity Percentages (SIMPER) analysis with 999 permutations using the vegan package in R (Oksanen et al., 2022), and identified the plant families contributing greater or equal to 50% of the dissimilarity between months. Additionally, we conducted an Indicator Species Analysis using the indicspecies package in R to further examine effects of month on beta diversity (Cáceres et al., 2022). In this analysis, an Indicator Value index is assigned to examine the relationship between each species (or taxon) within a community and the site group (or month). Permutations are used to identify statistically significant taxa that are most representative of the community at the given location or time, based on abundance and occurrence (Dufrene & Legendre, 1997). We used this analysis to identify the significant indicator species (or taxa) for each month. Taxa with higher Indicator Values are more representative of the community during that sampled month, providing a method to quantify the seasonal trends identified by the ANOSIM.

For analyses on vertebrates consumed, only 17 samples contained vertebrates and thus our analyses had limited power. We have therefore only included descriptive results for vertebrate data. To investigate potential drivers behind trends observed in timing of amphibian detections in the diet, we used estimated breeding seasons of the detected amphibians provided by Jenson et al. (2008).

Finally, due to rising concerns of the how the inherent compositional nature of data generated by high-throughput sequencing (HTS) might impact analyses and interpretation of metabarcoding data (Gloor & Reid, 2016; Gloor et al., 2017), we conducted compositional data analyses on plant data to ensure our results obtained from traditional statistical methods that ignore the compositional nature of HTS data was not impacting our results (Gloor & Reid, 2016; Quinn et al., 2019). Compositional data analyses entail performing ratio transformations to the raw abundance (read count) data and using alternate distance metrics to accommodate for the compositional nature of the data. We conducted the NMDS and ANOSIM again as described above, but replacing raw read count data with centered-log ratio (CLR) transformed data and Bray-Curtis distance with Aitchison distance, the Euclidean distance between CLR data (Gloor & Reid, 2016; Gloor et al., 2017).

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Tables and Figures

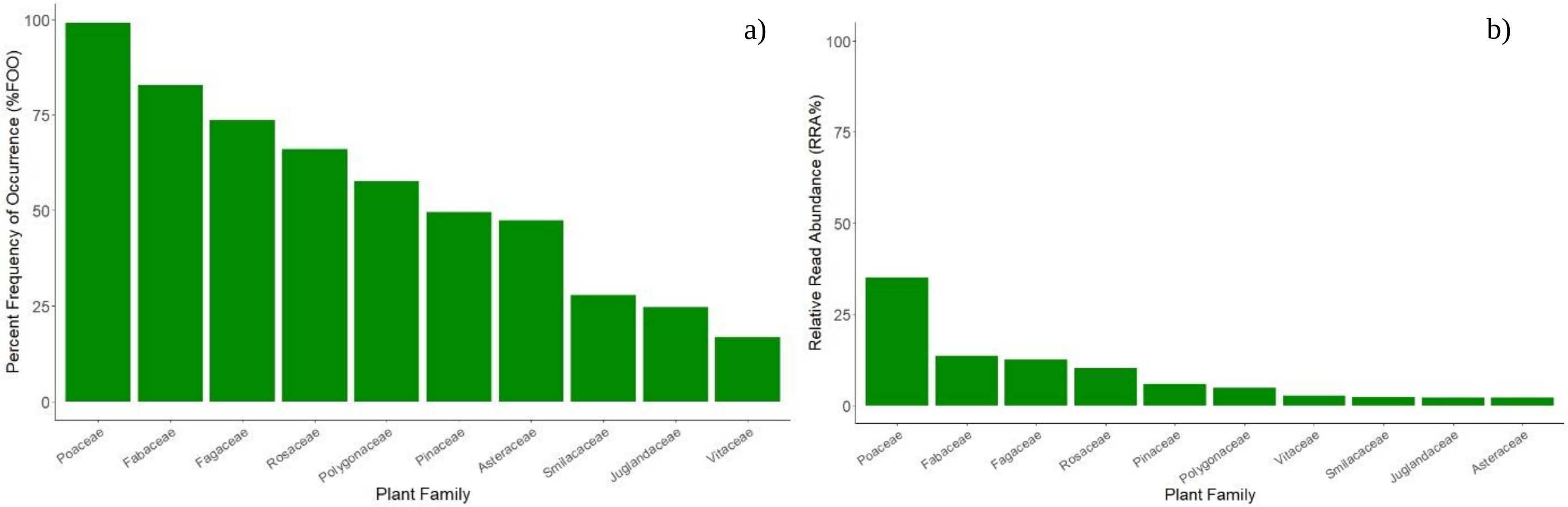

Fig. 2.1 a) Percent frequency of occurrence (%FOO; number of samples containing each food taxa divided by total number of samples and expressed as a percentage) of the 10 plant (trnL) families occurring most frequently throughout the year overall in the diet of wild pigs (*Sus scrofa*) in South Carolina, U.S. June 2017 - September 2018; and **b)** Relative Read Abundance (RRA%; total number of reads of each plant family divided by total number of reads and expressed as a percentage) of the top 10 plant (trnL) families most abundant throughout the year overall in the diet of our sampled wild pigs.

Table 2.1 Percent frequency of occurrence (%FOO; number of samples containing each food taxa divided by total number of samples [n = 220] and expressed as a percentage) and Relative Read Abundance (RRA%; total number of reads of each vertebrate species divided by total number of vertebrate reads and expressed as a percentage) of vertebrate species (12S mitochondrial rDNA) detected in the diet of wild pigs (*Sus scrofa*) in South Carolina, U.S. in June 2017 - September 2018; “n” represents number of wild pig samples containing that vertebrate species.

Class	**Species**	**Common name**	**n**	**FOO %**	**RRA %**
Amphibia	*Hyla chrysoscelis*	Cope's gray treefrog	1	0.45	0.43
	Hyla femoralis	Pine woods treefrog	1	0.45	1.77
	Hyla gratiosus	Barking treefrog	4	1.82	5.48
	Hyla squirellus	Squirrel treefrog	1	0.45	1.88
	Pseudacris feriarum	Upland chorus frog	1	0.45	0.24
	Pseudacris nigrita	Southern chorus frog	1	0.45	0.33
	Pseudacris ornata	Ornate chorus frog	1	0.45	1.31
	Rana catesbeiana	American bullfrog	2	0.91	21.92
	Rana clamitans	Green frog	2	0.91	23.54
	Rana sphenocephala	Southern leopard frog	1	0.45	0.14
	Scaphiopus holbrookii	Eastern spadefoot toad	1	0.45	15.70
	Eurycea cirrigera	Southern two-lined salamander	1	0.45	2.12
Reptilia	*Plestiodon laticeps*	Broadhead skink	1	0.45	1.81
Aves	*Corvus brachyrhynchos*	American crow	1	0.45	13.57
	Meleagris gallopavo	Wild turkey	1	0.45	3.74
	Regulus calendula	Ruby-crowned kinglet	1	0.45	3.75
Mammalia	*Blarina brevicauda*	Short-tailed shrew	1	0.45	0.47
	Lasiurus borealis	Eastern red bat	3	1.36	1.78

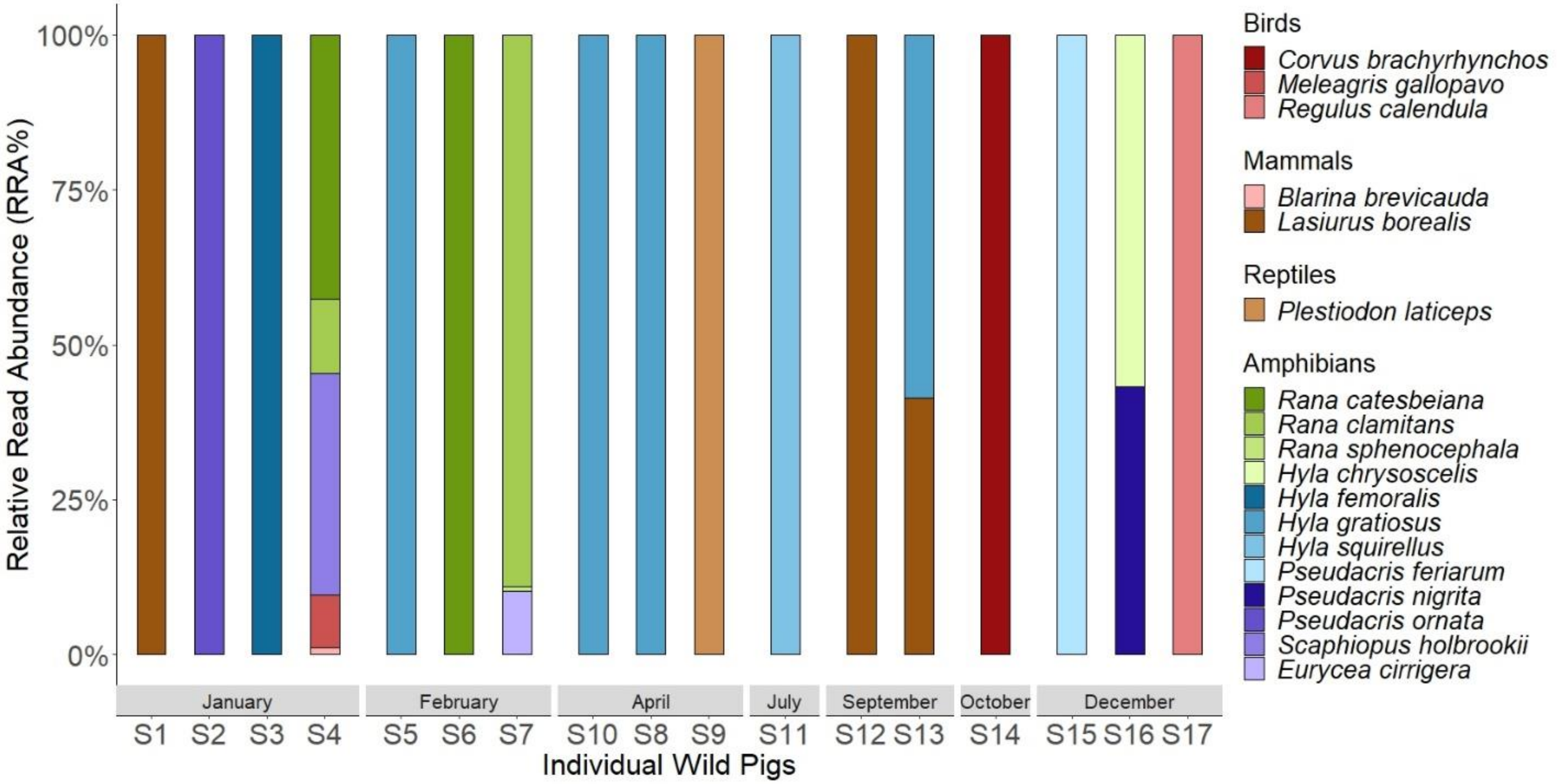

Fig. 2.2 Relative Read Abundance (RRA%; number of reads of each vertebrate species divided by total number of vertebrate reads per sample) of vertebrate species (12S mitochondrial rDNA) detected in each wild pig (*Sus scrofa*) diet sample per month in South Carolina, U.S. in June 2017 - September 2018.

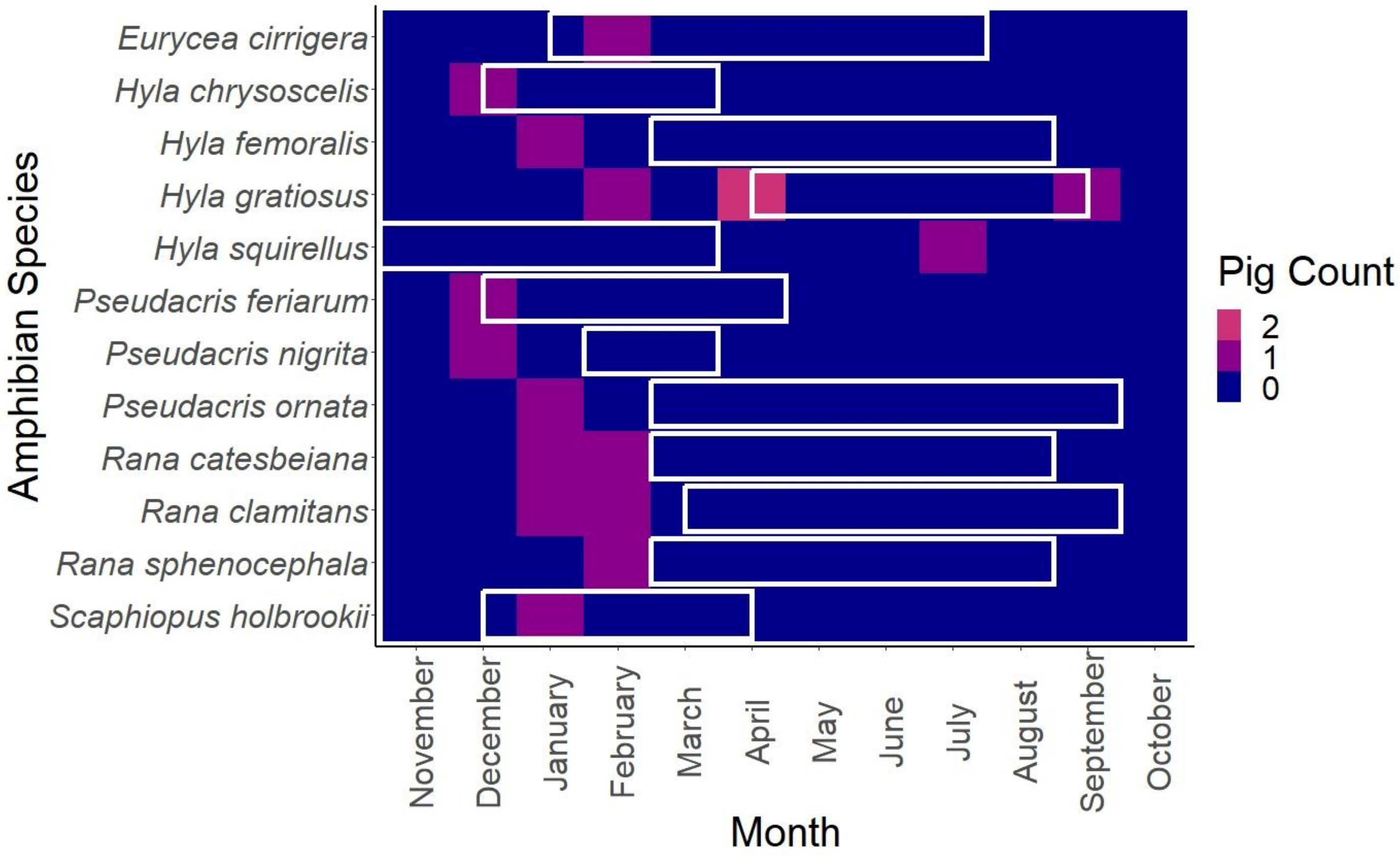

Fig. 2.3 Number of wild pig (*Sus scrofa*) diet samples collected in South Carolina, U.S. in June 2017 - September 2018 containing each detected amphibian species (12S mitochondrial rDNA) per month. White boxes indicate approximate breeding season of each amphibian species.

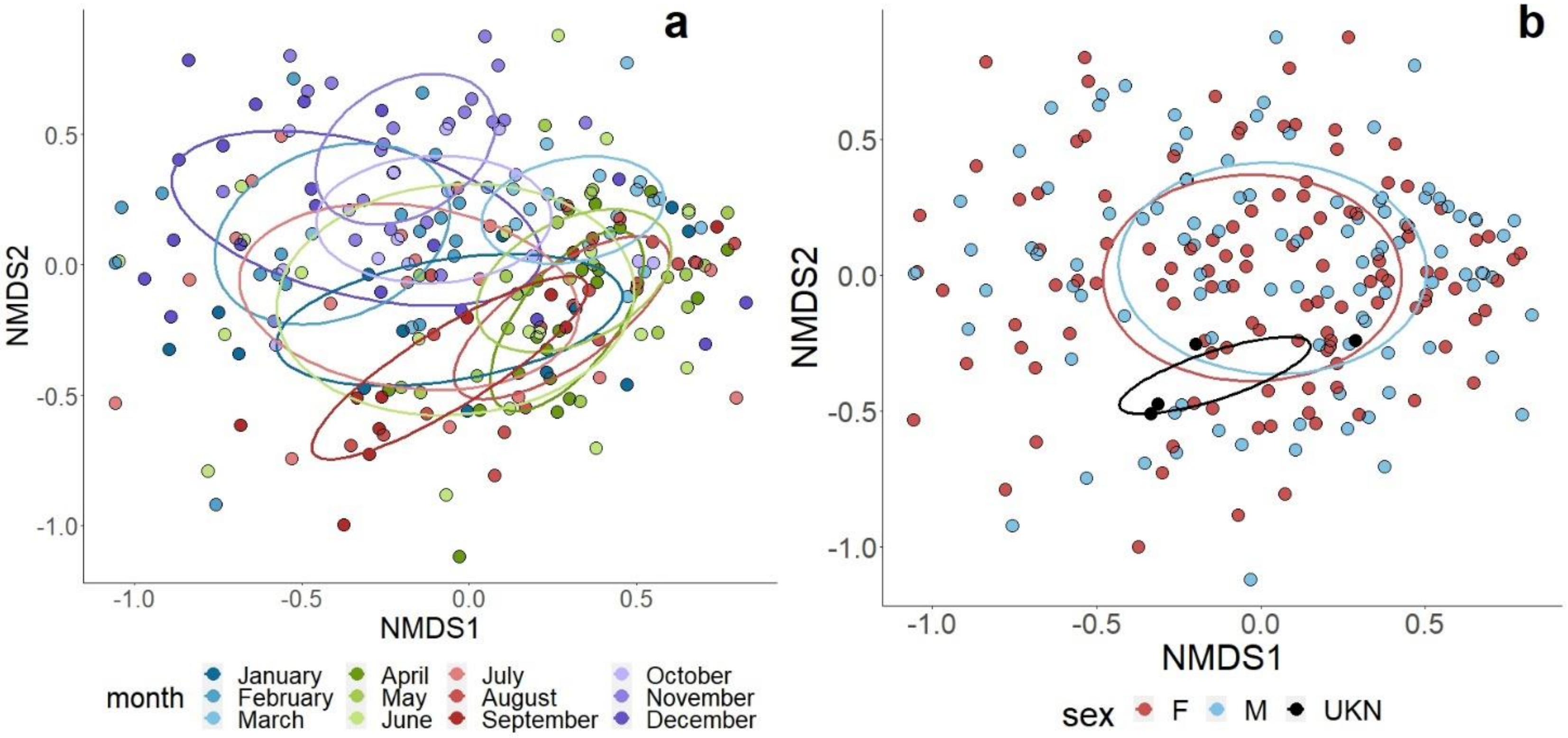

Fig. 2.4 a) Non-metric multidimensional scaling plot for plant families (trnL) detected in the diet of wild pigs (*Sus scrofa*) in South Carolina, U.S. in June 2017 - September 2018 by month; and **b)** non-metric multidimensional scaling plot for plant families (trnL) detected in the diet of wild pigs (*Sus scrofa*) in South Carolina, U.S. in June 2017 - September 2018 by sex.

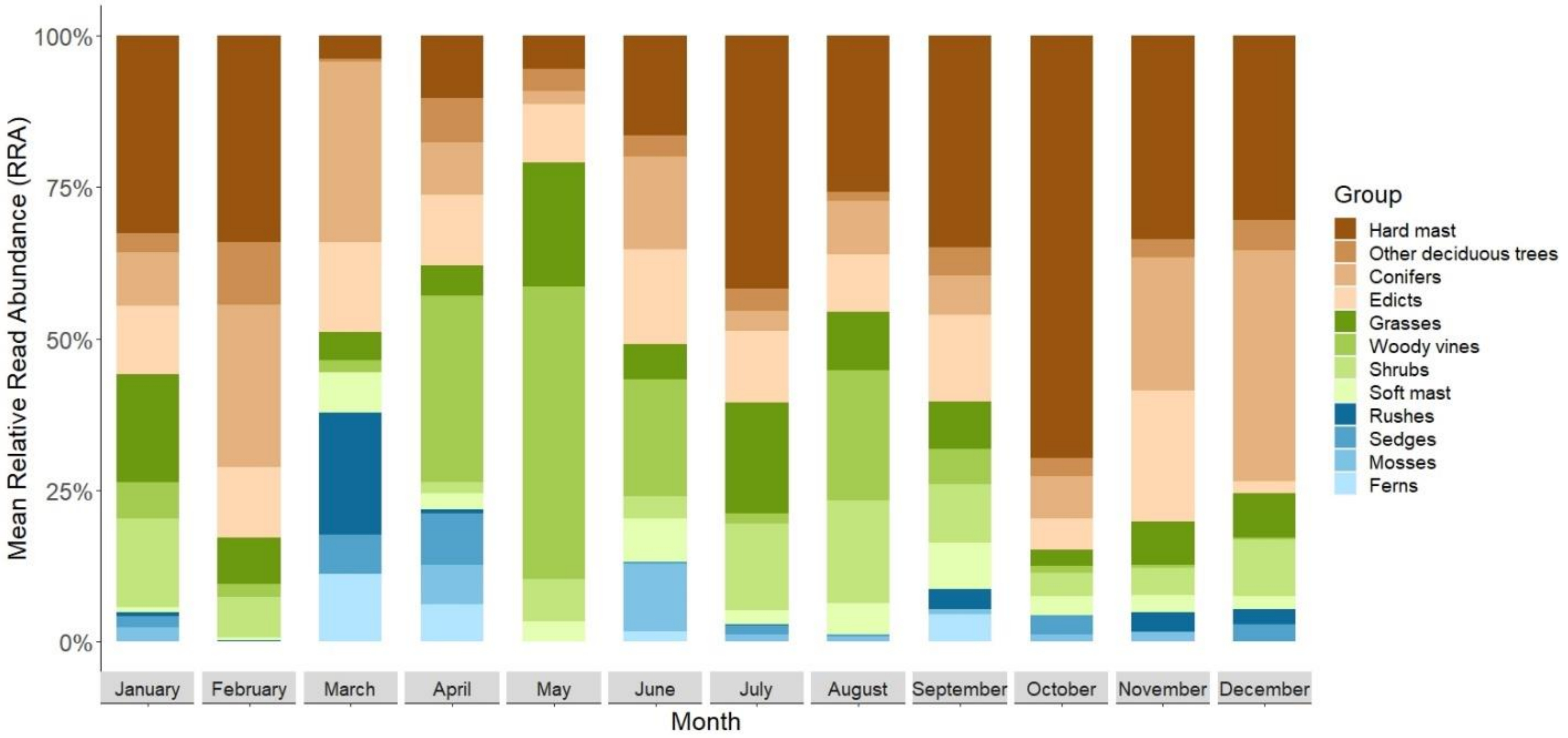

Fig. 2.5 Mean Relative Read Abundance (RRA%; %; mean number of reads of each plant family per month divided by total number of reads per month and expressed as a percentage) of plant functional groups (trnL) detected in the diet of wild pigs (*Sus scrofa*) in the South Carolina, U.S. per month for samples collected in June 2017 - September 2018. Assignment of plant genera to groups utilized in Fig. 5 is outlined in Supplementary Table 2.2.

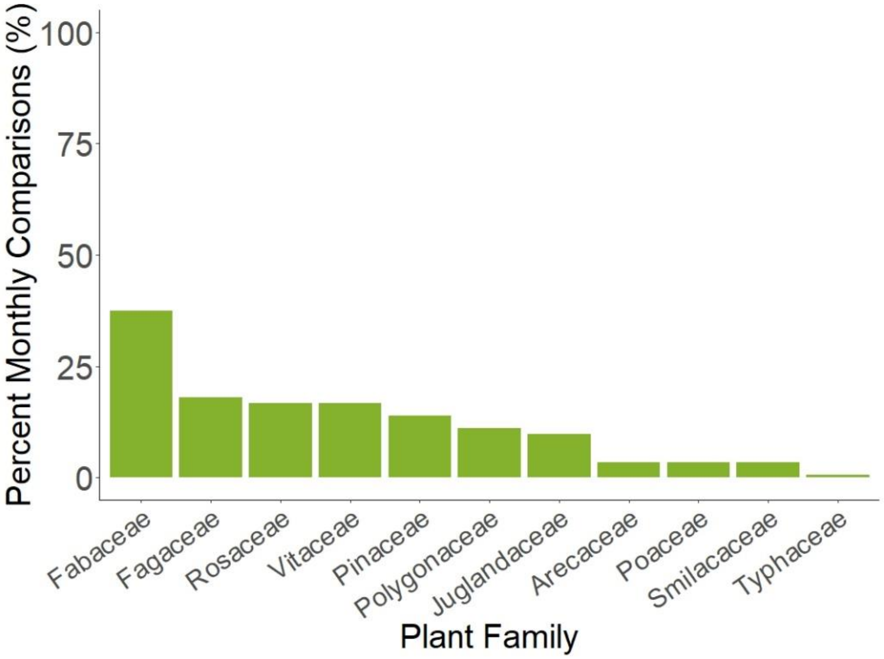

Fig. 2.6 Percent of monthly pairwise comparisons of plant families (trnL) contributing to ≥ 50% of monthly variation in diet composition of wild pigs (*Sus scrofa*) in the South Carolina, U.S. per month for samples collected in June 2017 - September 2018 derived from the SIMPER.

CHAPTER 3

INVASIVE WILD PIG (SUS SCROFA) DIETS ON BARRIER ISLANDS OF THE SOUTHEASTERN UNITED STATES

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Abstract

Biological invasions are one of the leading causes of reductions in global biodiversity. Islands are particularly sensitive to invasions, which often result in cascading impacts throughout island communities. Wild pigs (*Sus scrofa*) are globally invasive and pose threats to numerous taxa and ecosystems, particularly for islands where they have contributed to declines of many endemic species. However, the impacts of wild pig diets on the flora and fauna remain understudied in many island systems. We used DNA metabarcoding of wild pig fecal samples to quantify the seasonal diet composition of wild pigs on three barrier islands in the southeastern USA. Wild pigs exhibited a diverse diet dominated by plants but also including marine and terrestrial animals. The diet composition of plants varied seasonally and between islands. Consumption of invertebrates also changed seasonally, with a shift to coastal invertebrates, particularly crabs, in spring and summer. Vertebrates were found in <10% of samples, but spanned broad taxa including amphibians, fish, mammals, and reptiles. Species consumed by wild pigs indicate wild pigs use a variety of habitats within barrier islands for foraging, including forests, saltmarshes, and beaches. The observed shift to beach foraging during sea turtle nesting season suggests wild pigs have potential to hinder nesting success on islands without established management programs. These findings provide insight into the diverse food habits of wild pigs on barrier islands and highlight the need for removal of wild pigs from sensitive island ecosystems due to their potential impacts to native plant and animal communities.

Introduction

Biological invasions are a leading cause of global biodiversity loss, second only to habitat loss and fragmentation (Barrios-Garcia & Ballari, 2012; Bellard et al., 2016). Islands are biodiversity hotspots despite only encompassing ~5.5% of global terrestrial surface area (Kier et al., 2009; Kreft et al., 2008; Tershy et al., 2015). With high levels of endemism, islands are particularly vulnerable to invasions, driven by characteristics common among island species compared to mainland species including smaller population sizes, reduced genetic diversity, and lack of defenses against introduced herbivores and predators (Bowen & van Vuren, 1997; Frankham, 1997; Blumstein & Daniel, 2005; Fernández-Palacios et al., 2021). Indeed, greater than half of global extinctions have occurred on islands, with invasive species listed as the primary contributors (Tershy et al., 2015; Bellard et al., 2016). In particular, invasive mammals present the greatest impacts to insular species, primarily through predation (Szabo et al., 2012; Bellard et al., 2016; Doherty et al., 2016).

Wild pigs (*Sus scrofa*: non-native Eurasian wild boar, feral pigs, and their hybrids (Keiter et al., 2016; Smyser et al., 2020)) are among the most widespread and successful invasive mammals, with a global distribution across all continents except Antarctica, including many islands in the Pacific and Americas (Massei & Genov, 2004; Barrios-Garcia & Ballari, 2012). Their destructive rooting behavior and generalist, omnivorous food habits result in detrimental impacts across their range, including destruction of property and ecosystems, crop damages, and depredation of both livestock and wildlife (Bevins et al., 2014; McClure et al., 2018; Shwiff et al., 2018; Anderson et al., 2019). Wild pigs’ food habits are broad with many strategies exhibited including browsing, grazing, rooting, scavenging, and predation (Loggins et al., 2002; Wilcox & van Vuren, 2009; Turner et al., 2017). Across their range, wild pigs exhibit highly diverse diets dominated by plant material but also consisting of fungi, earthworms, insect larva, mollusks, crustations, fish, herpetofauna, birds, and mammals (Ballari & Barrios-García, 2014). Their generalist diets are highly variable and change spatially and temporally as a result of local food availability (Baron, 1982; Giménez-Anaya et al., 2008; Robeson et al., 2018). Hard mast, particularly acorns (*Quercus* spp.), is a preferred food of wild pigs and occurs in wild pig diets in high volumes and frequencies during fall and winter months when available, with a shift to herbaceous vegetation with green-up in spring (Loggins et al., 2002; Chapter 2 of this thesis). During a low mast year when acorns were not available, Baron (1982) observed higher instances of rooting of underground plant parts in a coastal ecosystem during the winter when other food items were scarce, which deceased in the spring and summer with the availability of herbaceous plants. Baron (1982) also noted that animal material appeared to increase in wild pig diets during summer months when dead fish and coastal invertebrates were available on beaches. Other instances of apparent opportunistic consumption of animals based on availability have also been recorded. For example, herpetofauna were consumed more frequently than other vertebrate groups in South Carolina, USA, where there is an abundance of amphibian and reptile species (Chapter 2 of this thesis). Conversely, wild pigs in California, USA consumed higher numbers of small mammals than other vertebrate taxa, including herpetofauna (Wilcox & van Vuren, 2009). The extreme plasticity of wild pig diets relative to locally available food resources necessitates thorough investigations of their food habits in ecosystems across their range, particularly sensitive areas such as islands, to better understand their impacts to native species.

The destructive rooting behavior, or displacement of soil while foraging, by wild pigs has documented negative effects to plant communities. Rooting by wild pigs is associated with reductions in vegetative cover and native plant species richness (Bankovich et al., 2016; Oldfield

& Evans, 2016a; Gray, Roloff, Kramer, et al., 2020). This is concerning for islands where approximately 10% of island endemic plants are considered threatened by wild pigs (Risch et al., 2021). However, wild pig consumption and rooting of plants remains relatively understudied in many island systems. On islands, the recovery period of vegetative communities appears to differ by location, likely due to habitat differences. For example, on the big island of Hawaii, USA, common forest plants recovered within 6.5 years whereas rare plants took longer than 18 years to recover after removal of wild pigs (Cole & Litton, 2014). In contrast, despite extensive rooting of habitats on a small sand barrier island in Mississippi, USA, vegetative cover was restored to original percent cover within only 6 months, perhaps because it is a system adapted to frequent environmental disturbances such as hurricanes (Baron, 1982). However, in some cases the recovery of plant biomass is replacement by invasive species (Bankovich et al., 2016), which devalues agricultural lands. Given this apparent site-specificity, further examinations are needed to better understand wild pig impacts to plant communities on islands throughout their range.

Wild pigs also impact native fauna primarily directly through predation. Wild pigs have been identified as a threat to hundreds of herpetofauna, birds, invertebrates, and mammals worldwide. On islands specifically, an estimated 23% of herpetofauna, 22% of invertebrate, and 12% of bird species endemic to islands are threatened by wild pigs based on the IUCN's Red List of Threatened Species, but studies of these impacts on specific islands still reman limited (Risch et al., 2021). Wild pigs are notorious for nest predation of sea turtles, with varying levels of nest mortality. On 12 barrier islands in Georgia, USA, wild pigs contributed to 19.63% of loggerhead sea turtle (*Caretta caretta*) egg losses (Butler et al., 2020). Studies on islands in Florida (Engeman et al., 2016) and South Carolina (Engeman et al., 2019), USA found that wild pig nest predation of sea turtles ranged from almost zero to nearly all nests depending on the year, likely due to intensity of wild pig removal efforts and whether remaining wild pigs had learned to exploit sea turtle nests. Coastal bird species (eggs, chicks, and adults) are also considered at risk of predation by invasive wild pigs and wild boar in their native range, although documentation remains limited. In a coastal Mediterranean wetland, birds (both adults and nests) were the primary vertebrate group consumed, with ducks occurring at high frequencies along with common moorhen (*Gallinula chloropus*) and western swamphen (*Porphyrio porphyrio*) (Giménez-Anaya et al., 2008). Wild pigs also have been found to contribute to population declines in bird species such as the Lord Howe Island woodhen (*Gallirallus sylvestris*) (Miller & Mullette, 1985) and Galapagos rail (*Laterallus spilonotu*) (Coblentz & Baber, 1987; Donlan et al., 2007).

Although wild pigs are known to consume high frequencies of invertebrates (Ballari & Barrios-García, 2014) and invertebrates are among the top groups thought to be threatened by wild pigs on islands (Risch et al., 2021), empirical evidence of impacts to invertebrates in the literature is almost non-existent. Wild pigs in mainland Alabama, USA were found to consume freshwater mussels at low frequencies but in concentrated areas, potentially impacting spatial distribution and community structure of mussel populations (van Ee et al., 2020), and suggesting that bivalves on islands could be similarly impacted. On a barrier island of Mississippi, USA, wild pigs foraged for coastal invertebrates on the beaches during summer months, although the extent and potential impacts to coastal invertebrates were not reported (Baron, 1982). Although estimates of insular animals threatened by wild pigs are substantial, studies on wild pig diets and impacts to species beyond marine turtles remain extremely limited on islands.

Despite the long-established, high density populations of wild pigs in the southeastern USA (Lewis et al., 2019; Mayer et al., 2020), the food habits of wild pigs on the numerous barrier islands of the region are understudied (Baron, 1982; Butler et al., 2020). Therefore, the goal of this study was to investigate the diets of wild pigs on three barrier islands off the coast of Georgia, USA using the molecular method of DNA metabarcoding which has been shown to be an efficient and effective method to determine the omnivorous diets of wild pigs (Anderson et al., 2018; Robeson et al., 2018). We tested the hypotheses that 1) wild pig dietary composition would change seasonally relative to the availability of food items; and 2) wild pig diet composition would not differ among islands due to the similar habitats and relative proximity of the islands. We predicted that wild pigs would primarily utilize inland habitats in autumn and winter (e.g., acorns [*Quercas* spp.] would dominate the diet), with a shift to herbaceous plants and increased foraging activity on dunes and beaches during spring and summer (Baron, 1982).

Methods

Study area

This study was conducted on three barrier islands off the coast of Georgia, USA (Fig. 1) with ongoing wild pig population control efforts. Blackbeard Island and Sapelo Island are adjacent islands separated by Blackbeard Creek. Cumberland Island is located approximately 52 km southeast of Sapelo Island. The islands are all characterized by pine and oak maritime forest, maritime scrub-shrub, salt marsh, dune, and beach habitats (Edwards et al., 2013; U.S. National Park Service, 2023). The maritime forests are dominated by oak (e.g., live oak [*Quercus virginiana*]), slash pine (*Pinus elliottii*), and loblolly pine (*Pinus taeda*) with a mid-story including a diversity of species such as American beautyberry (*Callicarpa americana*), Florida grape (*Vitis cinerea*), American holly (*Ilex opaca*), and yaupon holly (*Ilex vomitoria*). Maritime scrub-shrub habitats occur primarily on the protected inland sides of the dunes and include species such as wax myrtle (*Morella cerifera*), purple sandgrass (*Triplasis purpurea*), croton (*Croton* spp.), saw palmetto (*Serenoa repens*), silkgrass (*Pityopsis graminifolia var. tenuifolia*), spurge nettle

(*Cnidoscolus stimulosus*), and prickly pear (*Opuntia drummondii*). Salt marshes are characterized by smooth cordgrass (*Spartina alterniflora*), black needlerush (*Juncus roemerianus*), sea lavender (*Limonium carolinianum*), seaside oxeye (*Borrichia frutescens*), saltgrass (*Distichlis spicata*), and saltmarsh asters (*Symphyotrichum* spp.). Dunes have lower abundance of plant species than other habitats but include species such as sea oats (*Uniola paniculata*), saltwort or Russian thistle (*Kali* spp.), seashore dropseed (*Sporabolus virginicus*), salt hay (*Spartina patens*), and seabeach grass (*Panicum amarum*) (Edwards et al., 2013). The islands provide critical habitat for hundreds of birds, as well as herpetofauna and mammals, including species of conservation concern such as loggerhead sea turtles (*Caretta caretta*), seaside sparrows (*Ammodramus maritimus*), eastern diamondback rattlesnakes (*Crotalus adamanteus*), Wilson's plovers (*Charadrius wilsonia*), red knots (*Calidris canutus rufa*), and piping plovers (*Charadrius melodus*). The salt marshes, dunes, and beaches also host many coastal invertebrates such as crabs, ghost shrimp (*Palaemon paludosus*), and bivalves (Pearce, 2010).

Data Collection

We sampled 171 adult wild pigs that were culled as part of ongoing management programs across the three islands. Sampling occurred from May 2022 – July 2023 for Blackbeard Island and Sapelo Island (n = 121 samples) and February – April 2023 for Cumberland Island (n = 50 samples). We obtained samples from individuals estimated to be over 1 year old via tooth eruption and weighing > 34 kg to ensure the wild pigs sampled had a fully independent diet without supplementation of milk and were large enough to consume all available prey items. The majority of sampled wild pigs were baited with corn and live trapped prior to euthanization, and thus diets of these individuals were supplemented with corn to some degree. However, a similar study conducted in South Carolina utilized samples from wild pigs baited with corn and still observed a highly diverse diet following similar trends observed in other studies, suggesting trapped wild pigs still forage extensively in the environment despite diet supplementation with bait (Chapter 2 of this thesis). We collected fresh fecal samples from the colon/rectum during necropsy and stored them in an ultra-cold freezer until we shipped samples on dry ice to Jonah Ventures, LLC (Boulder, Colorado, USA) for next-generation sequencing. No wild pigs were culled specifically for the purpose of this study. All collection protocols were conducted in accordance with the University of Georgia Institutional Animal Care and Use protocol A2022 02-017.

Laboratory analyses and data curation

Jonah Ventures, LLC conducted all laboratory analyses (DNA extraction and amplification), sequencing, and initial bioinformatics following the general experimental protocol represented in Supplementary Fig. 1. We selected three primers targeting plants, invertebrates, and vertebrates in the diet. For plant taxa, we utilized a primer set targeting the chloroplast trnL (UAA) intron (forward 5‘CGAAATCGGTAGACGCTACG 3’and reverse 3‘CCATTGAGTCTCTGCACCTATC’ 5) (Taberlet et al., 2007). To amplify invertebrate taxa, we used ZBJ-ArtF1c and ArtR2c primers targeting a section of the mitochondrial cytochrome c oxidase subunit I (COI) gene (forward 5’ AGATATTGGAACWTTATATTTTATTTTTGG 3’ and reverse 5’ WACTAATCAATTWCCAAATCCTCC 3’) (Zeale et al., 2011). For vertebrate taxa, we utilized the batR01 primer set targeting the 12S mitochondrial rDNA gene (forward 5’ACACCGCCCGTCACCCT 3’ and reverse 5’ GTAYACTTACCATGTTACGACTT 3’) (Valentini et al., 2016), which was developed for aquatic vertebrates but amplifies other vertebrate taxa as well (Kluever et al., 2022). We utilized established protocols similar to those outlined in

Robeson et al. (Robeson et al., 2018) with an updated use Exact Sequence Variants (ESV) in place of Operational Taxonomic Units (OTU) (Callahan et al., 2017). We assigned taxa using a custom-built pipeline using both GenBank and Jonah Ventures voucher specimens, accepting assignments with > 90% agreement within 1% of the top hits. We discarded contaminant ESVs (taxa not occurring in the study region or with a relative abundance <0.01% of reads) (Deagle et al., 2019b) and ESVs that were not identified to the family level unless the ESVs were unique taxa not represented at a higher taxonomic level.

Statistical analysis

We conducted statistical analyses separately for each marker, with a taxonomic resolution of family level for plants and invertebrates and species level for vertebrates. We conducted all analyses using R v 4.1.1 (R Core Team, 2021). For diet summaries and visualizations, we calculated percent frequency of occurrence (%FOO) and percent relative read abundance (%RRA) for each food taxon. We calculated %FOO by dividing the number of samples containing that food item by the total number of samples, expressed as a percent, and %RRA by dividing the read count of each food item by the total number of reads for that primer set and expressed as a percent.

To examine diet composition (beta diversity) of plant and invertebrate families in the diet, we conducted separate three-dimensional non-metric multidimensional scaling (NMDS) analyses with 1,000 permutations to visualize trends in variation between islands and seasons. We then conducted a non-parametric Analysis of Similarities (ANOSIM) with Bray-Curtis distance with 9,999 permutations to quantify differences in dietary beta diversity between islands and seasons. We designated biologically relevant seasons for this study as follows: winter (December – February), spring (March – May), summer (June – August), and autumn (September – November)

(Chapter 2 of this thesis). If differences in beta diversity existed between islands, we conducted all downstream analyses of seasonality separately for the sites that differed. Because we only collected Cumberland and Sapelo Island samples during three months (February – April 2023), we performed tests of island differences in diet composition only for data collected in those months. If diet composition did not differ between islands, we pooled all samples containing that diet group (plants, invertebrates, or vertebrates) regardless of location for analyses of seasonality. When the ANOSIM revealed a significant impact of season we conducted a permutational multivariate ANOVA (PERMANOVA) with Bray-Curtis distance with 9,999 permutations and False Discovery Rate (FDR) corrected p-values to analyze pairwise comparisons. We used the vegan package in R (Oksanen et al., 2022) to conduct the NMDS, and ANOSIM, and PERMANOVA.

Upon identifying seasonal variation in diet composition, we investigated which prey taxa were driving the differences using a Similarity Percentages (SIMPER) analysis and Indicator Species Analysis. For the SIMPER, we used the vegan package in R (Oksanen et al., 2022) with 9,999 permutations to identify prey taxa that contributed to ≥ 50% dissimilarity in diet composition between seasons. We conducted the Indicator Species Analysis (Dufrene & Legendre, 1997) to identify prey taxa that contributed significantly to diet composition for each season using the indicspecies package in R (Cáceres et al., 2022).

Due to the low number of wild pig diet samples containing vertebrates (n = 12), our sample size was too small to conduct robust statistical analyses for that group. Therefore, we provided only a diet summary and descriptive results for the vertebrate component of the diet.

Results

Diet Overview

Wild pigs on barrier islands in Georgia, USA exhibited a diverse, omnivorous diet, with plants occurring in 168 samples (98.2%), invertebrates in 101 samples (59.1%), and vertebrates in 12 samples (7.0%). Plants (trnL) were identified to the family level for analyses, with 39 families detected. The total read count for plants was 2,949,035 with a mean read count of 17,553.78 per sample (± 6,784.46 S.D.). Analyses for invertebrates (ArthCO1) were also conducted at the family level, with 65 invertebrate families detected. Invertebrates had a total read count of 404,723 with a mean of 3,880.04 reads per sample (± 5,256.80 S.D.). Vertebrates (BatR01) had the highest level of taxonomic resolution with 9 species detected. The vertebrate total read count was 6,285 with a mean of 36.75 (± 202.52 S.D.) per sample.

Plant (trnL) Beta Diversity

Effects of Location

After sequence filtering and removal of contaminants, plants were detected in 168 samples. The NMDS (stress = 0.17) and ANOSIM ($p = 0.0001$, $R = 0.2257$) revealed site differences in plant diet composition, and the PERMANOVA identified Cumberland Island as significantly different from the other two islands (FDR adjusted $p = 0.002$), whereas Blackbeard and Sapelo Islands did not differ from each other. The SIMPER test identified seven plant families that were driving these differences with >50% dissimilarity between Cumberland and the other two islands: Fagaceae (oaks: *Quercus* spp.), Arecaceae (palms), Typhaceae (bulrushes: *Typha* spp.), Pinaceae (pines: *Pinus* spp.), Polygonaceae (buckwheats: *Eriogonum* spp. and *Rumex* spp.), Urticaceae (nettles: *Parietaria* spp.), and Pontederiaceae (pickerel-weeds: *Pontederia* spp.) (Fig. 2, Fig. 3, Fig. 4, and Supplementary Tables S1 and S2).

Effects of Season

Given the differences in plant composition between Cumberland Island and the other two islands, Cumberland Island (n=50) was separated from Blackbeard Island and Sapelo Island for samples with plants present (n=118) for all further plant analyses. As Cumberland Island samples were only collected during three months (February – April 2023), no seasonal analyses were conducted at this location.

The NMDS with three dimensions for plants on Blackbeard and Sapelo Islands converged on a stress value of 0.16, demonstrating that plant composition dissimilarities were effectively captured within three dimensions. The NMDS plot (Fig. 5) displays apparent clustering between seasons, suggesting seasonal differences in plant dietary beta diversity. The ANOSIM revealed a significant difference in plant dietary composition among seasons ($p = 0.0002$, $R = 0.13$) and the PERMANOVA for pairwise comparisons indicated that all seasons were different from one another ($p \leq 0.01$).

The SIMPER test for seasonal variation in plant dietary beta diversity identified seven plant families contributing to ≥50% of dissimilarity. The families leading dissimilarity in plant composition for winter and spring were Chenopodiaceae (goosefoot family: *Atriplex* spp., *Dysphania* spp., *Salicornia* spp.), Urticaceae, Fagaceae, and Typhaceae, respectively. For winter and summer, Verbenaceae (verbena family), Chenopodiaceae, Typhaceae, and Fagaceae contributed to the dissimilarity. Typhaceae and Arecaceae drove dissimilarity between winter and autumn. For spring and summer, the plant families contributing to dissimilarity were Verbenaceae, Urticaceae, Chenopodiaceae, and Fagacea. Spring and autumn dissimilarity was led by Pontederiaceae, Fagaceae, and Arecaceae. Finally, the dissimilarity between summer and autumn was driven by Fagaceae and Arecaceae.

The Indicator Species Analysis for seasonality of plant beta diversity identified five plant families that dominated the dietary composition for specific seasons. For winter, Typhaceae ($p = 0.0001$) and Hypericaceae (St. John's-worts; $p = 0.01$) were indicator families. In the spring, Oxalidaceae (wood sorrels) was the indicator family ($p = 0.03$). Fagaceae was the indicator family for summer ($p = 0.003$). Lastly, for autumn, the indicator family was Arecaceae ($p = 0.0001$).

Invertebrate (ArthCO1) Beta Diversity

Effects of Location

Out of the 171 wild pig diet samples, 101 contained invertebrates. No differences in wild pig consumption of invertebrates were found between islands (ANOSIM: $p = 0.09$, $R = 0.03$). Therefore, we pooled data across islands for seasonal analyses of invertebrates.

Effects of Season

The NMDS with three dimensions converged on a stress value of 0.0001, indicating invertebrate diet composition did have dissimilarity between seasons, but some samples within seasons had diet compositions so similar that they clustered almost identically (Fig. 6). This is exemplified in Fig. 6 where despite 101 points (one per sample), only 11 are visible because they have the same NMDS scores due to similar compositions and thus are stacked in the plot, giving the appearance of a smaller sample size. Therefore, variation in relative abundance of invertebrate families consumed between samples was low within each season, but composition of invertebrate families varied significantly between seasons (ANOSIM: $p = 0.0001$, $R = 0.17$). Pairwise comparisons from the PERMANOVA analysis demonstrated differences in invertebrate dietary beta diversity for all seasonal comparisons except winter and autumn (Table 1).

The SIMPER test of seasonality for composition of invertebrates in wild pig diets identified 11 invertebrate families that contributed to ≥50% dissimilarity between seasons (Table 2, Fig. 7). These families belonged to class Insecta (insects) and Malacostraca (crustaceans). Malacostraca families (Panopeidae and Ocypodidae) contributed dissimilarity only for winter – spring and summer – autumn comparisons (Fig. 7). The Indicator Species Analysis identified three invertebrate families that made significant contributions to invertebrate diet composition, all for autumn: Noctuidae (p = 0.01), Mycetophilidae (p = 0.05), and Dicyrtomidae (p = 0.05). The families identified in these two analyses as driving seasonal diet differences were also among the 10 families most frequent and abundant for invertebrates overall (Fig. 8).

Vertebrate Beta Diversity

Due to the low number of samples containing vertebrates (n = 12), no statistical analyses were conducted. Of the 12 samples with vertebrate detections, five were on Cumberland Island and seven were on Blackbeard Island. All vertebrate detections occurred in winter and spring between February and June (Fig. 9). Vertebrate species consisted of four amphibians (n = 4 samples), 1 reptile (n = 3 samples), two small mammals (n = 3 samples), and two fish (n = 2 samples) (Table 3). Green anoles (*Anolis carolinensis*) were detected most frequently (n=3), which were detected on both Cumberland and Blackbeard Islands. Cottontail rabbits (*Sylvilagus* sp.) were detected twice on Blackbeard Island in February and May. All other species occurred in only one sample each.

Discussion

Islands contribute greatly to global biodiversity and are disproportionally vulnerable to invasive species such as wild pigs. However, despite their widespread distribution across many island systems globally and demonstrated impact to island biodiversity, studies of wild pig food habits on islands remain limited. Using DNA metabarcoding of wild pig fecal samples from three barrier islands in Georgia, USA, our results revealed a diverse diet with 39 plant families, 65 invertebrate families, and 9 vertebrate species detected. Composition of plants and invertebrates in the diet of sampled individuals varied seasonally, and composition of plants in the diet differed between some islands, showcasing the opportunistic feeding habits of wild pigs based on local availability. In addition to insect larva, coastal invertebrates, particularly crabs, were a prominent component in wild pig diets during the spring and summer. This coincides with peak nesting activity of endangered sea turtles, which nest extensively on coastal islands, suggesting wild pigs are actively foraging in beach habitats during periods of peak sea turtle vulnerability. Ongoing turtle nest caging programs on the sampled islands appeared to be highly effective, as no sea turtles were detected in our study and no sea turtle nests on Blackbeard Island were reported to have wild pig predation events. Vertebrates occurred infrequently in wild pig diets but included diverse taxa: amphibians, reptiles, fish, and small mammals, with herpetofauna accounting for most vertebrate species and detections. These results indicate that wild pigs on barrier islands in the southeastern US exploit resources from all available habitats with the potential to inflict widespread impacts to sensitive flora and fauna.

Consistent with the diets of wild pigs across their range, wild pigs sampled in this study exhibited a diet dominated by plants, which occurred in ~98% of samples (Ballari & Barrios-García, 2014). Despite similar habitats across all islands, plant beta diversity for Cumberland

Island, located approximately 52 km southeast of the other two islands, was substantially different from Sapelo and Blackbeard Islands. Across all three sampled islands wild pigs consumed the same families of plants, but at different frequencies and abundances. Oaks, palms, and bulrushes were among the taxa contributing greatest to these differences. This likely reflects differences in relative availability of plants on the islands, demonstrating the opportunistic nature of wild pig foraging behavior. For example, Cumberland Island experienced a high mast year during the study period (Dough Hoffman, pers. comm., 2023), with many acorns available through April, while Blackbeard and Sapelo Islands experienced a low mast year with considerably fewer acorns produced during the study period (Patrick Helm, pers. Comm., 2023). On Cumberland Island, where acorns were highly available, oak was detected frequently and at high abundances through spring. Conversely, on Blackbeard and Sapelo, where acorns were less available, oak was detected infrequently and represented a small proportion of the diet from autumn through spring. This site specificity is further evidenced when comparing this study to other wild pig DNA metabarcoding studies in the southeastern USA, all using the trnL marker. Wild pigs in this study on Georgia barrier islands consumed 39 plant families compared to the 56 families consumed in mainland South Carolina (Chapter 2 of this thesis) and 66 families identified in mainland Florida (Anderson et al., 2018), likely reflecting lower species diversity of plants on these islands compared to the mainland. While many of the most common and abundant plant families detected across these three studies were the same, their comparative abundance and frequency of occurrence in the diet differed among sites (Anderson et al., 2018; Chapter 2).

As predicted, wild pig consumption of plants also varied seasonally with availability as is the case across their range (Ballari & Barrios-García, 2014). On Blackbeard and Sapelo Islands, where a low mast year resulted in limited availability of acorns, the winter diet of wild pigs was dominated by forbs and grasses (Poaceae). Grasses remained relatively abundant and common in the diet throughout the year, as seen in other studies in the southeastern USA (Anderson et al., 2018; Chapter 2). Spring and summer trends were similar to those found in Chapter 2 of this thesis in mainland areas of the same region: cattails, rushes, and sedges were most abundant in the diet from winter through mid-spring, when both soft masting species (Ebenaceae, Ericaceae, Rosaceae) and deciduous trees/shrubs including oak increased in the diet. Oak remained frequent and abundant in the summer along with forbs and grasses. In autumn, palms dominated plant composition in the diet with a notable drop-off in oak. This differs from autumn plant composition elsewhere in the region, where oak (acorns) dominated the diet (Chapter 2), again likely due to the limited availability of acorns on Blackbeard and Sapelo Islands during this study.

Invertebrates were also an important component of wild pig diets in our study, occurring in approximately 59% of samples. Wild pigs consumed a broad diversity of invertebrates, with 65 families detected representing insects, arachnids, springtails, centipedes, snails, native earthworms, crabs, bivalves, jellyfish, and anemones. Diet composition of invertebrates did not differ among islands but varied considerably among seasons. Ghost and fiddler crabs (Ocypodidae), marsh and shore crabs (Sesarmidae), and mud crabs (Panopeidae) constituted the largest portion of invertebrates consumed and were detected primarily in spring and summer when they were readily available on beaches (both live and carcasses) (Patrick Helm, personal communication). While wild pigs are efficient scavengers (Turner et al., 2017), we observed evidence that wild pigs were actively predating invertebrates on beaches, with substantial holes from rooting during spring and summer months (Fig. 10). Although studies are limited, wild pigs on a barrier island in Mississippi, USA (Baron, 1982) exhibited similar use of crabs in the summer, suggesting wild pigs on barrier islands in the Southeastern USA likely extensively forage on beaches during summer. Insects were common throughout the year but represented the largest proportion of the diet in autumn, along with springtails. Flying invertebrates, including the most frequent and abundant insects in the diet (e.g., woodlice, March flies, and owlet moths) were likely consumed as eggs or larva in the soil or on vegetation. Autumn was also when native earthworms (Sparganophilidae) were detected, collectively indicating wild pigs shifted rooting and foraging activity into forested areas during autumn. Notably absent from the diet were invasive earthworms, which are common in North America and typically an important component of wild pig diets (Anderson et al., 2018; Baubet et al., 2003). The lack of invasive earthworms reflects either a low abundance of invasive earthworms on the sampled islands or gaps in the reference database for our ArthCO1 primer that did not allow taxon assignment of earthworms. Winter consumption of invertebrates consisted primarily of springtails (Collembola), which are active year-round, and woodlice, which are inactive but available to wild pigs rooting under leaf litter/debris in winter.

Vertebrates were detected infrequently (approximately 7% of samples) but included many vertebrate groups (amphibians, reptiles, fish, and small mammals) with the exception of birds. Herpetofauna were the most frequent and abundant vertebrate species consumed, occurring in 58.3% of vertebrate samples and representing 55.6% of all vertebrate species detected. This is consistent with the results of Chapter 2 of this thesis in mainland areas of South Carolina using the same primer, and provides further evidence that among vertebrates herpetofauna, and particularly amphibians, are vulnerable to predation by wild pigs. However, fewer amphibian species were detected in this study (4 species) than in Chapter 2 (12 species).

This discrepancy in number of amphibian species occurring in wild pig diets is likely due to differences in environmental availability between the mainland and barrier islands. Specifically, the barrier islands sampled in our study have fewer freshwater wetlands to support amphibian populations than mainland areas of the southeastern US, and consequently have reduced diversity and abundance of amphibians compared to mainland areas in the region (Laerm et al., 2000). Fish were detected infrequently, and given the species of fish detected, they were likely scavenged carcasses that washed up on the beach, as found by Baron (1982) on a Mississippi barrier island. Wild pigs have also been documented to consume species of freshwater fish in mainland areas of the southeast, although it is unknown if the detections were scavenging or predation events (Anderson et al., 2018). Notably absent from wild pig diets were sea turtles, given the extensive evidence of sea turtle nest predation on other islands, and our observation of rooting on beaching for crabs during nesting season (Engeman et al., 2019; Butler et al., 2020). However, this result was anticipated due to intensive wild pig control efforts as well as predator exclusion netting over turtle nests were implemented during our survey period. In silico analyses conducted by Jonah Ventures, LLC revealed that this lack of sea turtle detections was likely not due to gaps in the references database, but was not able to rule out false negatives due to potential primer biases of BatR01. However, no sea turtle nest predation events by wild pigs were recorded on Blackbeard Island during the two nesting seasons in our study period. Therefore, as our sampling efforts on the other islands were limited during sea turtle nesting season, it is unlikely that our lack of loggerhead sea turtle detections was due to false negatives in this study. However, given the high levels of observed beach foraging activity by wild pigs for crabs and other marine invertebrates during the summer when sea turtles and shorebirds are nesting on beaches, our data suggest there is great potential for wild pigs to encounter nests in the absence of monitoring and control programs. Thus, conservation plans for sites with threatened or endangered coastal species might benefit from the removal or exclusion of wild pigs during spring and summer months in the southeastern US.

Conclusions

Overall, these results demonstrate that wild pigs on barrier islands in the southeastern USA exhibit an opportunistic, omnivorous diet that follows similar broad trends in other studies. Diet composition of wild pigs varied seasonally and by location according to availability, and even fine-scale differences between sites with the same overall habitats were reflected in the composition of wild pig diets. This study is the largest known sampling effort of wild pig diets on southeastern USA barrier islands and provides a thorough description of the extreme diversity of species consumed in the region. Wild pigs appeared to forage across all habitat types on the islands, making ample use of the coastal invertebrates available seasonally on the beaches in addition to species in the forest, marsh, and dune habitats. While sensitive sea turtles and shorebirds were not detected in this study, the extensive foraging activity by wild pigs on beaches during nesting season indicates that wild pigs could pose significant threats to these species in coastal areas where wild pig management programs are not in place. As wild pigs are abundant and widely distributed across many islands in the region and around the world, these findings highlight the need for removal of wild pigs from sensitive island ecosystems due to their potential direct impacts to native plant and animal communities and destructive rooting of sensitive habitats.

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Tables and Figures

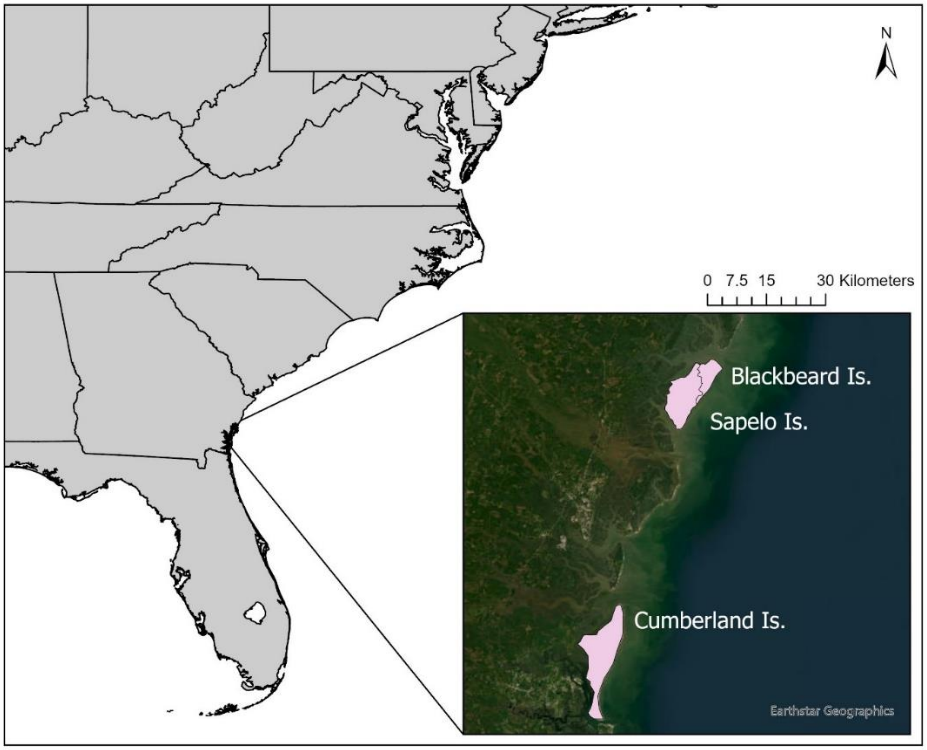

Fig. 3.1. Sampling locations for wild pig (*Sus scrofa*) diets on three barrier islands in Georgia, USA from May 2022 through July 2023.

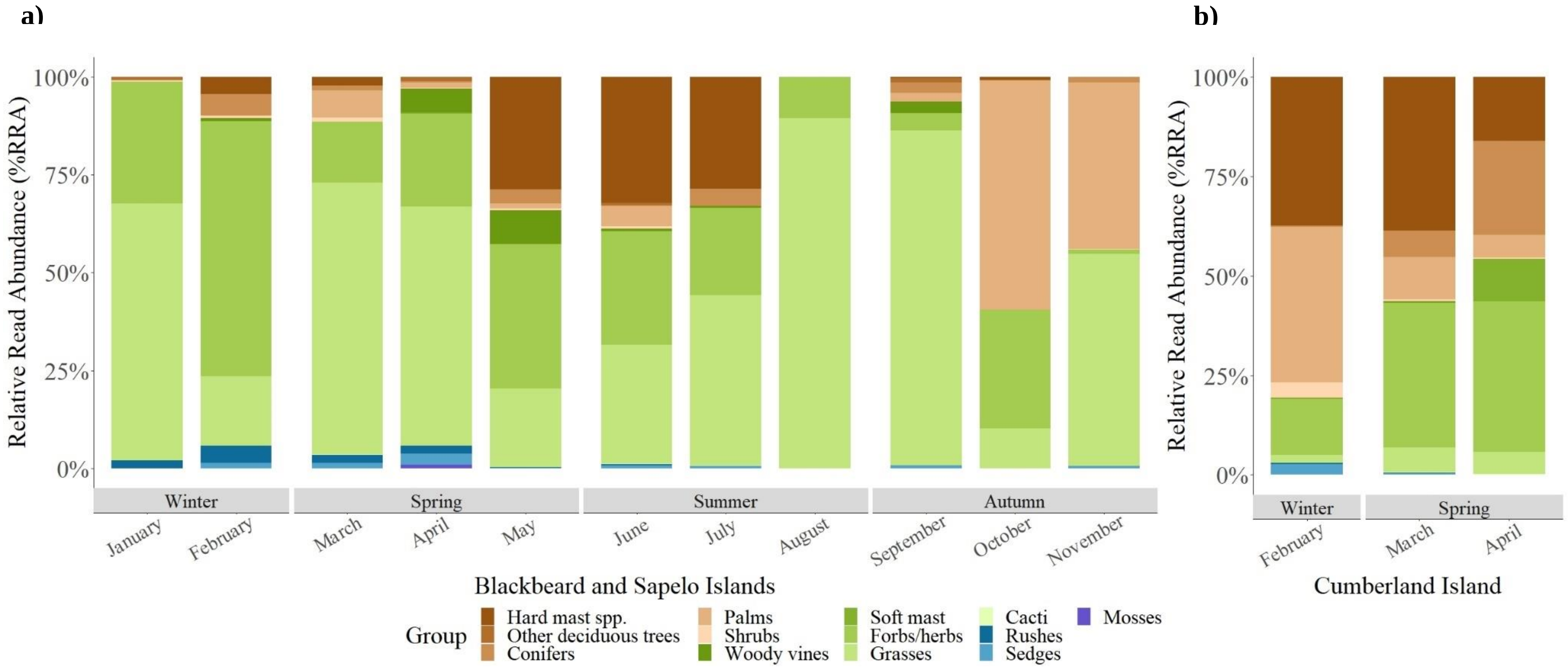

Fig. 3.2. Percent relative read abundance (%RRA; the read count of each plant group divided by the total number of reads for the trnL marker and expressed as a percent) of plant groups consumed by wild pigs throughout the year on **a)** Blackbeard and Sapelo Island, Georgia, USA from May 2022 – July 2023 and **b)** Cumberland Island, Georgia, USA from February – April 2023. Classifications of plant families into the groups used in Fig. 2 are provided in Supplementary Table 3.3.

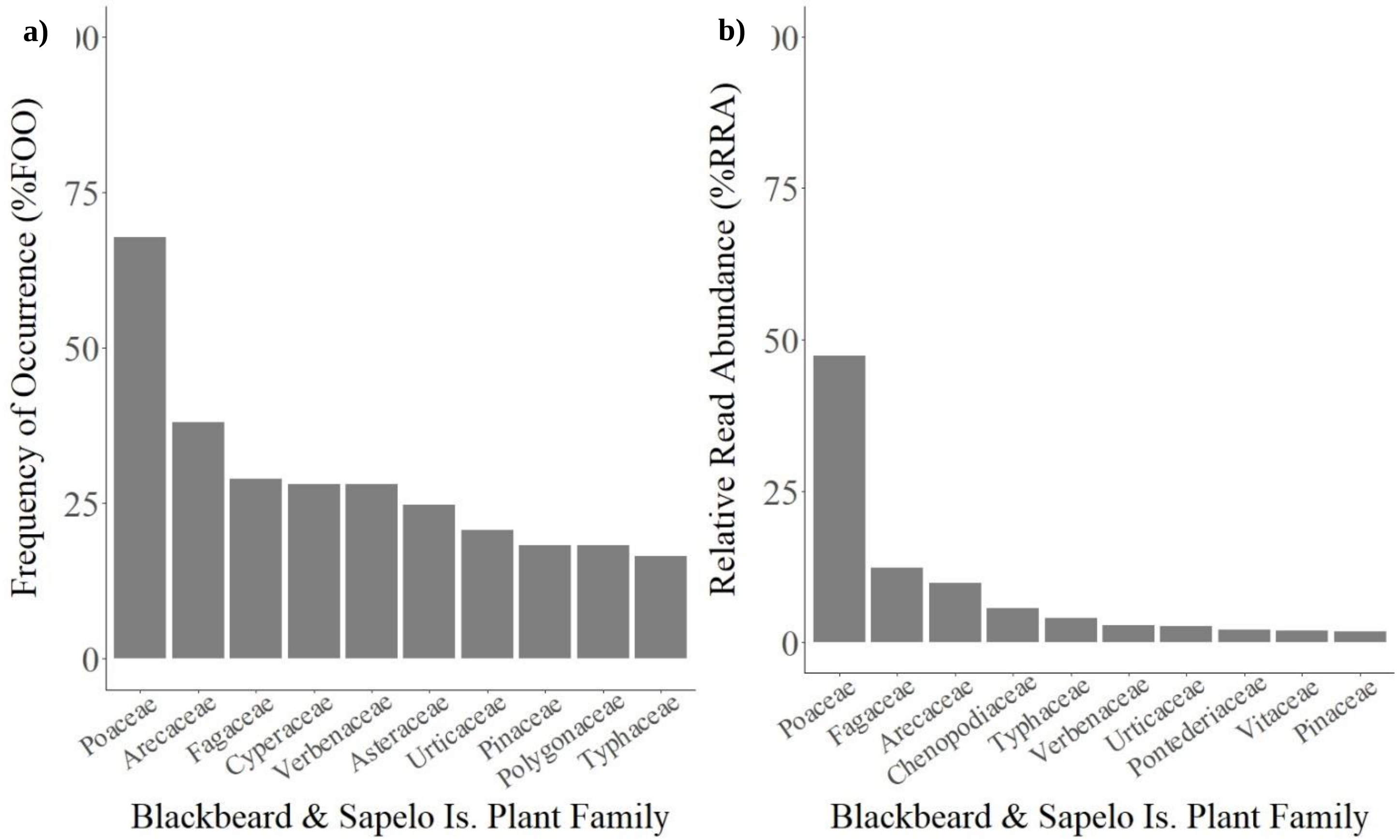

Fig. 3.3 a) Top 10 plant families for percent frequency of occurrence (%FOO; number of samples containing each plant family divided by the total number of samples on Blackbeard and Sapelo Islands [n=121]) in the diets of wild pigs (*Sus scrofa*) on Blackbeard and Sapelo Islands, Georgia, USA from May 2022 – July 2023; and **b)** Top 10 plant families for percent relative read abundance (%RRA; the read count of each plant family divided by the total number of reads for the trnL marker and expressed as a percent) in the diets of wild pigs (*Sus scrofa*) on Blackbeard and Sapelo Islands, Georgia, USA from May 2022 – July 2023.

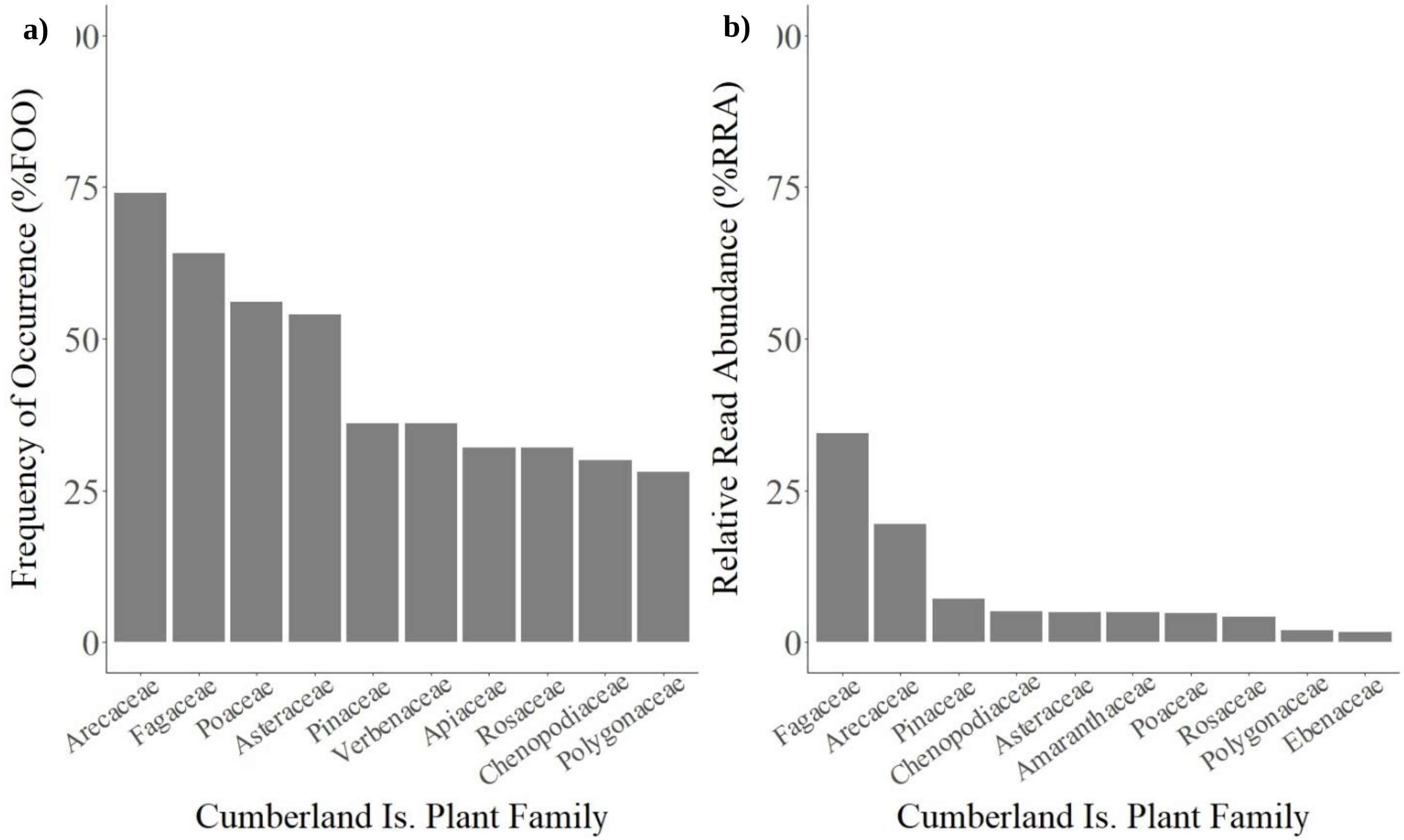

Fig. 3.4 a) Top 10 plant families for percent frequency of occurrence (%FOO; number of samples containing each plant family divided by the total number of plant samples on Cumberland Island [n=50]) in the diets of wild pigs (*Sus scrofa*) on Cumberland Island, Georgia, USA February – April 2023; and **b)** Top 10 plant families for percent relative read abundance (%RRA; the read count of each plant family divided by the total number of reads for the trnL marker and expressed as a percent) in the diets of wild pigs (*Sus scrofa*) on Cumberland Island, Georgia, USA February – April 2023.

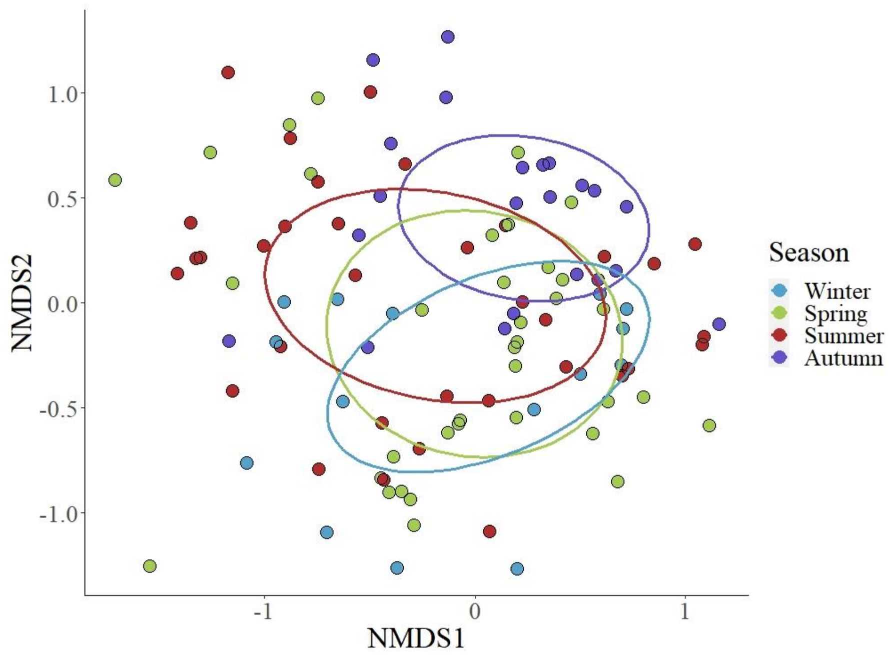

Fig. 3.5. Non-metric multidimensional scaling (NMDS) plot for plant families (trnL) detected in the diets of wild pigs (*Sus scrofa*) on Blackbeard and Sapelo Islands, Georgia, USA from May 2022 – July 2023.

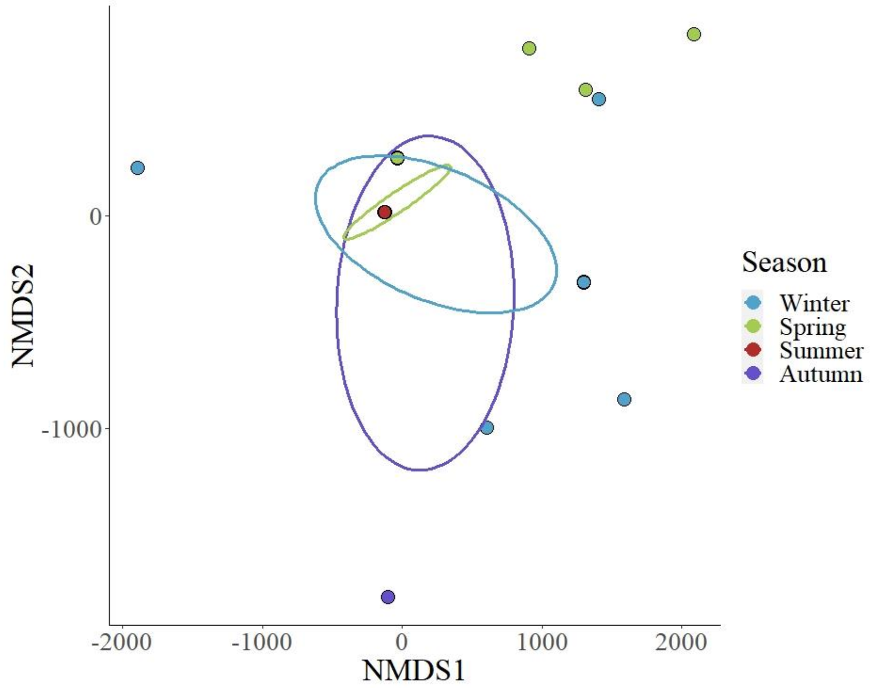

Fig. 3.6. Non-metric multidimensional scaling (NMDS) plot for invertebrate families (ArthCO1) detected in the diets of wild pigs (*Sus scrofa*) on three barrier islands, Georgia, USA from May 2022 – July 2023.

Table 3.1. Results of the FDR corrected PERMANOVA for pairwise comparisons of invertebrate family beta diversity between seasons in the diets of wild pigs (*Sus scrofa*) on three barrier islands of Georgia, USA from May 2022 – July 2023.

Season 1	**Season 2**	**FDR corrected p-value**
Winter	Spring	0.004
Winter	Summer	0.007
Winter	Autumn	0.354
Spring	Summer	0.044
Spring	Autumn	0.047
Summer	Autumn	0.047

Table 3.2. Results of SIMPER test for invertebrate families contributing to ≥ 50% dissimilarity between seasons in the diets of wild pigs (*Sus scrofa*) on three barrier islands of Georgia, USA from May 2022 – July 2023.

Season 1	**Season 2**	**Family**	**Common family name**
Winter	Spring	Cecidomyiidae	Gall midges
		Ceratopogonidae	Biting midges
		Gryllidae	True crickets
		Bibionidae	March flies
		Panopeidae	Mud crabs
Winter	Summer	Sciaridae	Dark-winged fungus gnats
		Armadillidiidae	Woodlice/pill bugs
		Gryllidae	True crickets
		Ceratopogonidae	Biting midges
Spring	Summer	Ceratopogonidae	Biting midges
		Hydropsychidae	Net-spinning caddisflies
		Armadillidiidae	Woodlice/pill bugs
		Bibionidae	March flies
Spring	Autumn	Bibionidae	March flies
		Noctuidae	Owlet moths

Summer	Autumn	Dicyrtomidae	Globular springtails
		Mycetophilidae	Fungus gnats
		Armadillidiidae	Woodlice/pill bugs
		Ceratopogonidae	Biting midges
		Ocypodidae	Ghost and fiddler crabs
		Noctuidae	Owlet moths

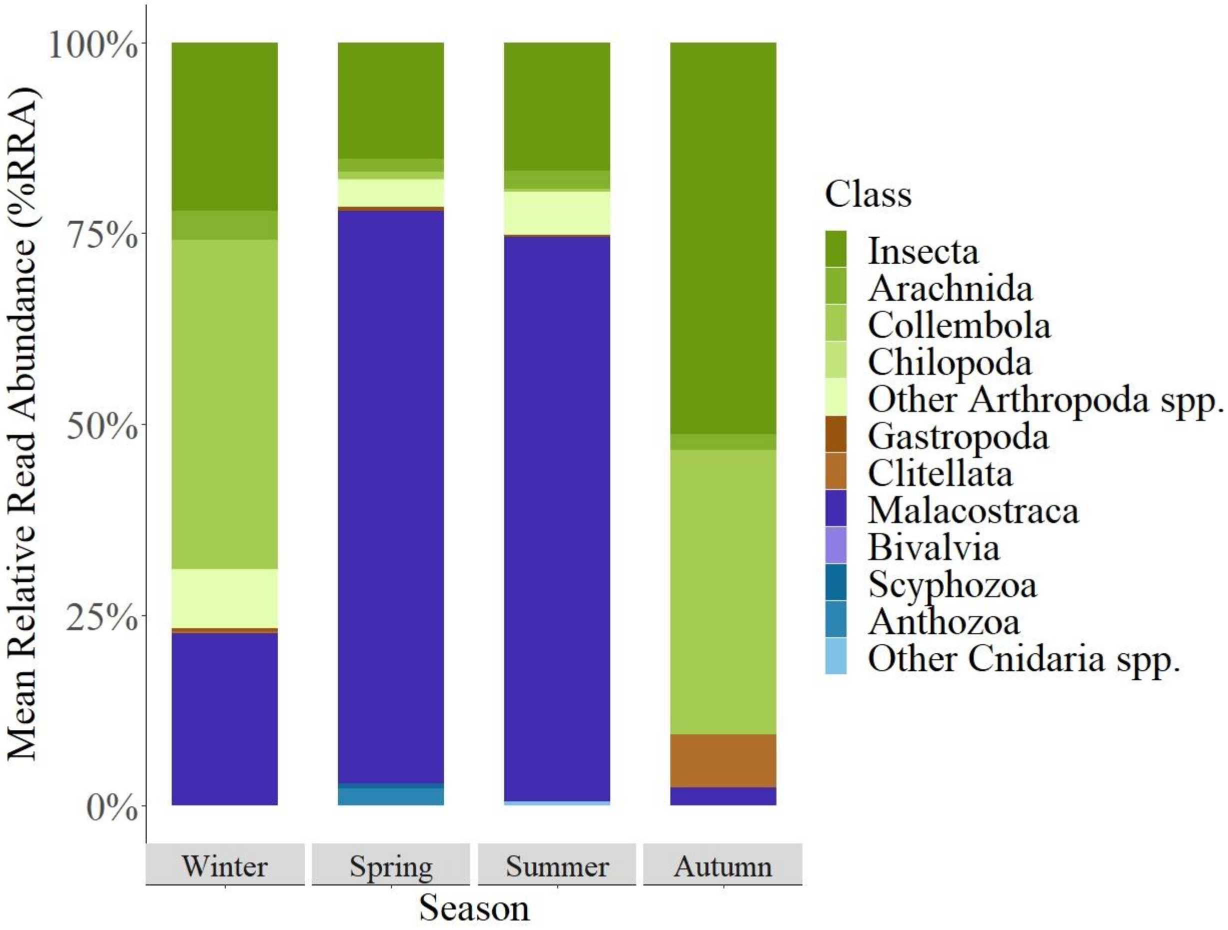

Fig. 3.7. Mean percent relative read abundance (mean %RRA; the mean read count of each invertebrate group divided by the total number of reads for that season for the ArthCO1 marker and expressed as a percent) of invertebrate classes consumed by wild pigs throughout the year on three barrier islands, Georgia, USA from May 2022 – July 2023.

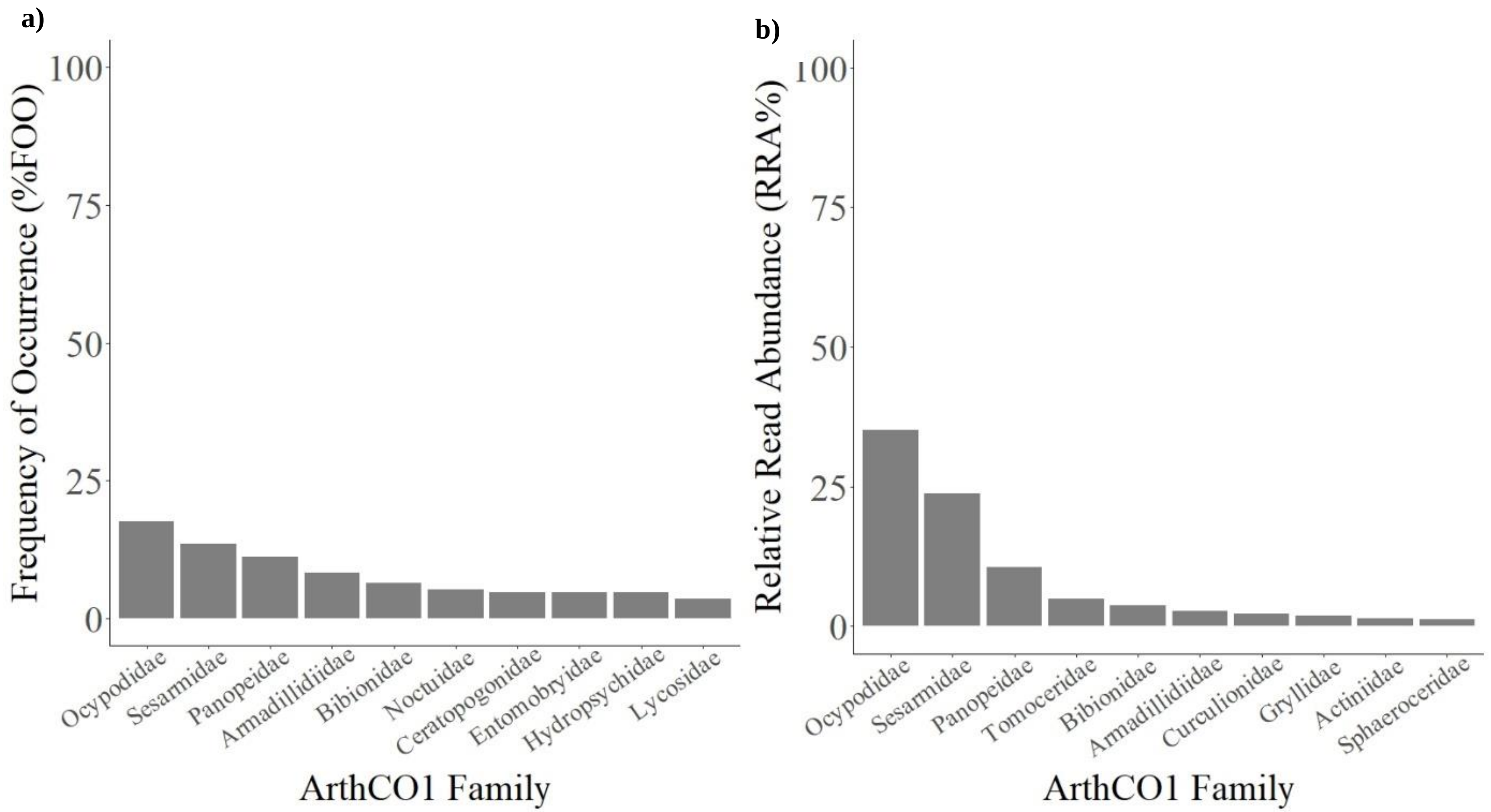

Fig. 3.8 a) Top 10 invertebrate families for percent frequency of occurrence (%FOO; number of samples containing each invertebrate family divided by the total number of samples [n=171]) in the diets of wild pigs (*Sus scrofa*) on three barrier islands, Georgia, USA from May 2022 – July 2023; and **b)** Top 10 invertebrate families for percent relative read abundance (%RRA; the read count of each invertebrate family divided by the total number of reads for the trnL marker and expressed as a percent) in the diets of wild pigs (*Sus scrofa*) on three barrier islands, Georgia, USA from May 2022 – July 2023.

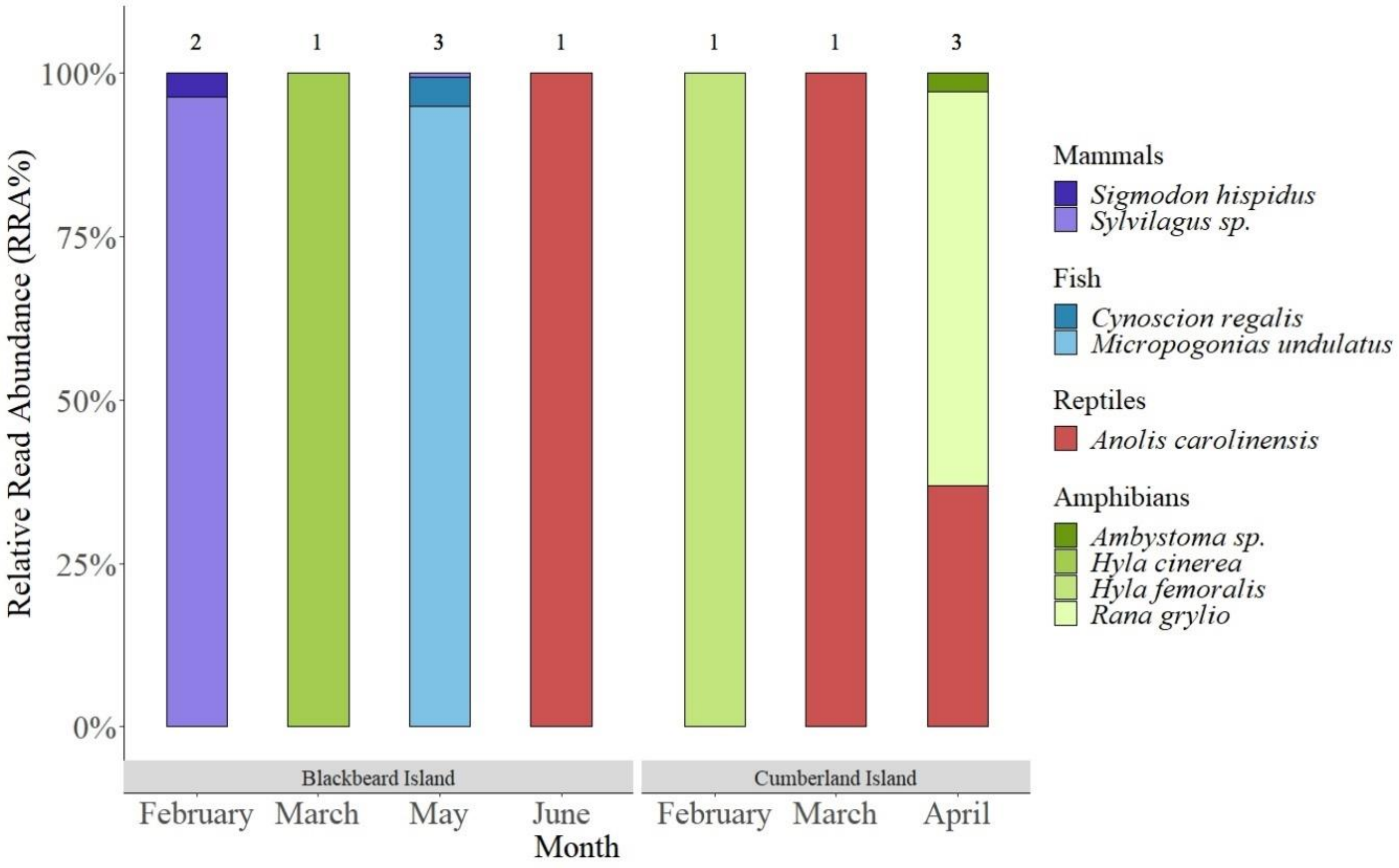

Fig. 3.9. Percent relative read abundance (%RRA; the read count of each vertebrate species divided by the total number of reads for the BatR01 marker and expressed as a percent) of vertebrate species consumed by wild pigs throughout the year on Blackbeard and Sapelo Island, Georgia, USA from May 2022 – July 2023 and Cumberland Island, Georgia, USA from February – April 2023. Numbers over each bar represent number of samples containing vertebrates per month.

Table 3.3. Percent frequency of occurrence (%FOO; number of vertebrate diet samples containing each vertebrate species [n] divided by total number of diet samples [N] expressed as a percent) and percent relative read abundance (%RRA; number of reads of each vertebrate species detected divided by total number vertebrate reads from the BatR01 marker) in the diet of wild pig (*Sus scrofa*) on three barrier islands of Georgia, USA from May 2022 through July 2023.

Class	**Species**	**Common name**	**n**	**N**	**FOO %**	**RRA %**
Amphibia	*Ambystoma talpoideum.*	Mole salamander	1	171	0.585	0.36
	Hyla cinerea	Green treefrog	1	171	0.585	23.500
	Hyla femoralis	Pine woods treefrog	1	171	0.585	8.369
	Rana grylio	Pig frog	1	171	0.585	7.701
Reptilia	*Anolis carolinensis*	Green anole	3	171	1.754	36.420
Mammalia	*Sigmodo hispidus*	Hispid cotton rat	1	171	0.585	0.175
	Sylvilagus sp.	Cottontail rabbit genus	2	171	1.170	4.789
Actinopterygii	*Micropogonias undulatus*	Atlantic croaker	1	171	0.585	17.836
	Cynoscion regalis	Weakfish	1	171	0.585	0843

Fig. 10. Examples of wild pig rooting damage to maritime forest and beach habitats on Blackbeard Island during the study period.

CHAPTER 4

CONCLUSIONS

Invasive wild pigs (Sus scrofa) are one of the most widely distributed invasive species, and pose a significant threat to a myriad of taxa and ecosystems worldwide. As invasive wild pig populations continue to expand globally, there is growing concern regarding their potential impacts to sensitive plant and animal communities and habitats through destructive foraging (i.e., rooting) and generalist, opportunistic diet. Wild pigs are known to depredate island birds, reptiles (e.g., sea turtles), and small mammals, and have the potential to similarly threaten amphibians due to mutual use of wetland habitats by wild pigs and amphibians. While previous studies have demonstrated wild pig consumption of amphibians, most invasive wild pig diet studies to date have used manual observational methods and thus may have underestimated consumption of these and other soft-bodied species without feathers or hair. Furthermore, given the demonstrated temporal and spatial plasticity of wild pig diets, studies investigating the diets of wild pigs across all seasons and in locations with high potential vulnerability to wild pigs are needed for a comprehensive understanding of the impacts of wild pigs to sensitive species and ecosystems. In this thesis, I addressed these gaps in knowledge using DNA metabarcoding to conduct some of the most extensive molecular dietary studies of wild pigs to date. Here, I present findings on the influence of season, sex, and location on the dietary composition of wild pigs in the southeast USA and highlight taxa with the potential to be most impacted by the food habits of wild pigs in the study region.

In Chapter 2, I found that wild pigs in western South Carolina broadly exhibit a similar opportunistic diet to wild pigs across their range. Wild pigs had a diverse diet dominated by plant material (100% of samples), with 166 plant genera from 56 families and 18 vertebrate species identified. Diet composition varied seasonally with availability for plants and was consistent between sexes. Plant groups consumed by wild pigs followed similar broad patterns to diets of wild pigs in other studies in the region. Specifically, autumn and winter diets were characterized by acorns and evergreen species such as pines, and shifted to increased herbaceous plants in the spring and summer when environmental availability increased. Grasses were prominent in the diet throughout the year. Oaks also appeared to be an important component in wild pig diets, both in the form of acorns in colder months as well as small saplings that are accessible to wild pigs in the summer. This exploitation of acorns and young oaks by wild pigs suggests management of wild pig populations is important in locations with concerns about oak recruitment rates.

Similar to other dietary studies, vertebrates were detected in wild pig diets in South Carolina at lower frequencies than plants, but all terrestrial vertebrate classes were represented. Amphibians, in particular, were the most frequent vertebrate group consumed and represented the majority of vertebrate species detected. This research provides evidence that amphibians may be more vulnerable to predation by wild pigs than previously estimated, which has concerning implications given current global declines of amphibian populations and overlap in space use between wild pigs and amphibians. Therefore, my results emphasize the need for focused management of invasive pigs in habitats containing populations of imperiled amphibian species.

In Chapter 3, I found that plants once again dominated the diets of wild pigs (98% of samples) on barrier islands in Georgia, USA, with invertebrates also commonly consumed and vertebrates detected infrequently. I detected 39 plant families as well as 65 invertebrate families and 9 vertebrate species in the diets of sampled wild pigs across the sampled islands. Although wild pigs still exhibited a highly diverse diet, wild pigs on the barrier islands consumed fewer plant species than found in Chapter 2 of this thesis as well as other mainland dietary studies conducted in the region, likely reflecting reduced plant diversity on the sampled islands compared to the mainland. The relative proportion of plant families in the diet differed between study sites, with wild pigs on Cumberland Island exhibiting different composition than wild pigs on Blackbeard and Sapelo. This site-specificity was likely due to local availability, as Cumberland Island is located ~ 52 km of the other two islands and could thus have experienced environmental conditions different enough to alter availability. Season also influenced diet composition of wild pigs for both plants and invertebrates, with wild pigs shifting from inland species in the autumn and winter to increased foraging of coastal species, particularly crabs, in spring and summer. With limited acorn availability, autumn and winter on Blackbeard and Sapelo Island were dominated by palms, grasses, and forbs. Grasses and forbs remained common in the diet throughout the year, but decreased in relative importance in the diet in spring and summer when other herbaceous plants became available. As seen in Chapter 2 of this thesis, oak was detected frequently in the summer months, suggesting oak saplings may be an important food resource for wild pigs in addition to acorns. Invertebrates occurred less frequently than plants in the diet but demonstrated the highest diversity, with insects and crabs accounting for most invertebrates in the diet.

Vertebrates were detected infrequently in the diets of wild pigs among my island samples, but again included many taxa: amphibians, reptiles, small mammals, and fish. Despite extensive use of beaches, fish were detected infrequently and were likely scavenged based on the species consumed. Similar to my findings in Chapter 2, herpetofauna were detected more frequently and represented the most diversity among the vertebrates detected. The prominence of crabs in the diet as well as abundant observed rooting damage on beaches during sea turtle and shorebird nesting season suggest that wild pigs could greatly impact nesting success of sea turtles and shorebirds on beaches without dedicated management of wild pigs and protective caging of nests. My findings indicate that amphibians and reptiles, particularly sea turtles, are at risk of depredation by wild pigs on barrier islands in the southeastern USA, and highlight the importance of comprehensive management programs in areas with sensitive species.

Collectively, my findings show that although general features of wild pig diets (i.e., prominence of plant material compared to animal material) are upheld in the diets of wild pigs in the southeastern USA compared to other studies across their range, the relative contribution of individual food items in the diet can vastly differ spatially and temporally, likely due to differences in environmental availability. I observed an influence of both seasonality and locality both between mainland and island ecosystems, but also among islands, which underlines the importance of conducting explorations of wild pig diets across all seasons and regions where wild pigs are found in order to capture a complete image of global impacts. Excluding seasons from a study or assuming that affected taxa will be the same in different ecosystems could lead to incorrect or incomplete conclusions and management plans. Vertebrate species were consumed relatively infrequently but were highly diverse. Species exhibiting terrestrial, fossorial, or semi-fossorial habits accounted for the vast majority of prey species (80.8%), opportunistically consumed by rooting wild pigs. My results indicate that in the southeastern USA, amphibians may be the vertebrates most vulnerable to both direct and indirect impacts by wild pigs, and that wild pigs in coastal areas extensively forage on beaches during nesting seasons of sensitive shorebirds and marine turtles, putting these species at risk of nest depredation by wild pigs. These findings highlight that vulnerable herpetofauna and sensitive island ecosystems in the southeastern USA could greatly benefit from continuation and expansion of wild pig control programs.

APPENDIX

A. Supplementary Analysis 1: Alpha Diversity

Methods

To examine dietary alpha diversity, we calculated Shannon's Diversity Index using the "diversity" function in the vegan package in R (Oksanen et al., 2022). We tested the data for normality and then conducted an Analysis of Variance (ANOVA) to investigate the effects of month and sex on alpha diversity. This was followed by a Tukey's test using the agricolae package in R in the event that significance ($\alpha = 0.05$) was found (Mendiburu, 2021).

Results

Plant family data had a mean Shannon's diversity index score of H = 1.24. An ANOVA of Shannon's diversity index revealed a significant effect of month (F11,208 = 3.13, $p < 0.01$). Tukey's Honestly Significant Difference (HSD) Test for multiple comparisons for month found that the mean H score was significantly lower in May (H=1.02) than January (H=1.49; p = 0.02, 95% Credible Interval [C.I.] = 0.03-0.89) and February (H=1.44; p = 0.02, 95% C.I. = 0.03-0.80) but not between other months. Neither sex, nor the interaction of month and sex were significant.

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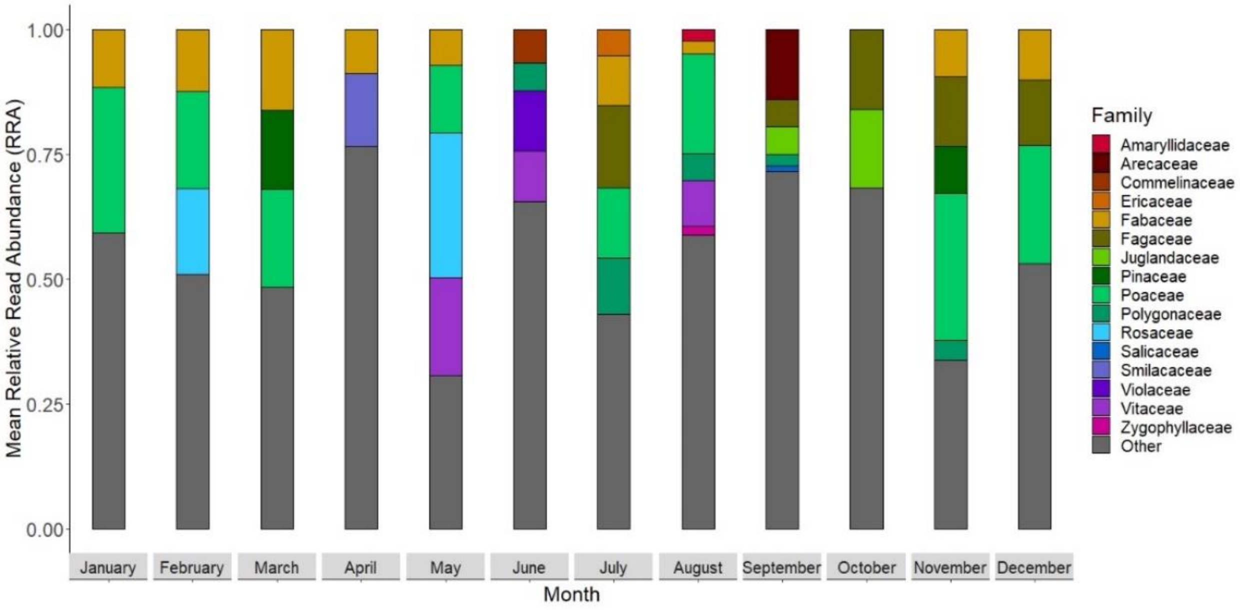


B. Supplementary Figure 2.1 Mean relative read abundance (RRA) per month of the 16 plant families identified in the Indicator Species Analysis for the diet of wild pigs (*Sus scrofa*) in South Carolina, U.S., June 2017 - September 2018. “Other” represents the RRA of the remaining 40 plant families consumed by wild pigs that did not contribute significantly to variation between months.

C. Supplementary Table 2.1 Number of diet samples containing each food taxa (n), percent frequency of occurrence (%FOO; n divided by total number of samples [220]) and mean relative read abundance (RRA%; number of reads of each food taxa divided by total number of trnL reads) of the plant families detected in the diet of wild pigs (*Sus scrofa*) in South Carolina, U.S.

Family	n	%FOO	%RRA
Poaceae	218	99.09	34.56
Fabaceae	182	82.73	12.68
Fagaceae	162	73.64	13.68
Rosaceae	145	65.91	9.72
Polygonaceae	127	57.73	4.36
Pinaceae	109	49.55	6.12
Asteraceae	104	47.27	1.91
Smilacaceae	61	27.73	1.81
Juglandaceae	54	24.55	3.02
Vitaceae	37	16.82	2.66
Solanaceae	36	16.36	0.18
Onagraceae	30	13.64	0.39
Cupressaceae	28	12.73	0.40
Salicaceae	27	12.27	0.32
Arecaceae	26	11.82	2.45
Rubiaceae	26	11.82	0.28
Altingiaceae	25	11.36	0.25
Typhaceae	23	10.45	1.22
Caryophyllaceae	22	10.00	0.14
Cyperaceae	22	10.00	0.26
Plantaginaceae	22	10.00	0.07
Chenopodiaceae	19	8.64	0.17
Malvaceae	16	7.27	0.12
Commelinaceae	15	6.82	0.24
Brassicaceae	14	6.36	0.03
Geraniaceae	14	6.36	0.03
Amaranthaceae	13	5.91	0.18
Violaceae	13	5.91	0.25
Oxalidaceae	12	5.45	0.05
Comandraceae	11	5.00	0.09
Juncaceae	11	5.00	0.22
Magnoliaceae	11	5.00	0.15
Ulmaceae	11	5.00	0.17
Anacardiaceae	9	4.09	0.08
Euphorbiaceae	9	4.09	0.04
Rhamnaceae	9	4.09	0.13
Grossulariaceae	8	3.64	0.04
Sapindaceae	8	3.64	0.08
Araceae	7	3.18	0.06
Convolvulaceae	7	3.18	0.07
Ericaceae	7	3.18	0.17
Cucurbitaceae	6	2.73	0.05
Ebenaceae	6	2.73	0.08

Amaryllidaceae	5	2.27	0.07
Aquifoliaceae	4	1.82	0.04
Ditrichaceae	4	1.82	0.06
Ephedraceae	4	1.82	0.06
Ophioglossaceae	4	1.82	0.09
Sphagnaceae	4	1.82	0.04
Zygophyllaceae	3	1.36	0.04
Berberidaceae	2	0.91	0.08
Cabombaceae	2	0.91	0.29
Cannabaceae	2	0.91	0.05
Polemoniaceae	2	0.91	0.07
Rutaceae	2	0.91	0.04
Nymphaeaceae	1	0.45	0.08

D. Supplementary Table 2.2 Breakdown of plant genera and associated family assigned to each plant group in Fig. 2.5 detected in the diet of wild pigs (*Sus scrofa*) in South Carolina, U.S.

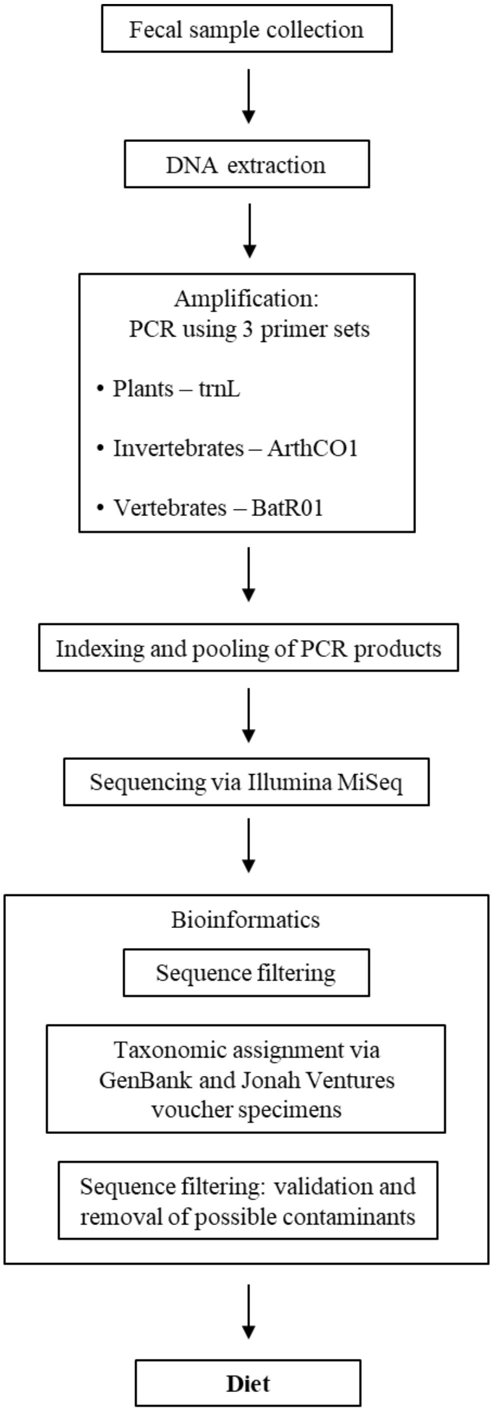
Plant Group	**Family**	**Genus**
Conifers	Cupressaceae	*Cunninghamia*
	Pinaceae	*Abies*
		Cedrus
		Pinus
		Pseudotsuga
Deciduous trees	Altingiaceae	*Liquidambar*
	Betulaceae	*Alnus*
		Betula
	Ebenaceae	*Diospyros*
	Fabaceae	*Robinia*
	Platanaceae	*Platanus*
	Rhamnaceae	*Alphitonia*
	Salicaceae	*Populus*
		Salix
	Sapindaceae	*Acer*
	Simaroubaceae	*Ailanthus*
	Ulmaceae	*Ulmus*
Ferns	Ophioglossaceae	*Botrychium*
		Ophioglossum
Edicts	Amaranthaceae	*Alternanthera*

	Amaranthus
	Bassia
	Chenopodium
	Oxybasis
Amaryllidaceae	*Allium*
Apocynaceae	*Apocynum*
Araceae	*Steudnera*
Araliaceae	*Hydrocotyle*
Asteraceae	*Bidens*
	Lactuca
	Taraxacum
	Tragopogon
Boraginaceae	*Phacelia*
Brassicaceae	*Alyssum*
	Brassica
Caryophyllaceae	*Cerastium*
	Silene
	Stellaria
Commelinaceae	*Commelina*
Cucurbitaceae	*Cucumis*
Euphorbiaceae	*Cnidoscolus*
	Euphorbia
Fabaceae	*Apios*
	Arachis
	Astragalus
	Dalea
	Desmanthus
	Desmodium
	Glycine
	Hedysarum
	Hoffmannseggia
	Kummerowia
	Lespedeza
	Lotus
	Lupinus
	Medicago
	Melilotus
	Onobrychis
	Pisum
	Rhynchosia
	Trifolium
	Vicia
Geraniaceae	*Geranium*
Hypericaceae	*Triadenum*
Lamiaceae	*Lamium*
Malvaceae	*Hibiscus*

		Sida
	Onagraceae	*Chamaenerion*
		Ludwigia
		Oenothera
	Oxalidaceae	*Oxalis*
	Passifloraceae	*Passiflora*
	Penstemon	*Penstemon*
	Plantaginaceae	*Linaria*
		Plantago
		Veronica
	Polemoniaceae	*Linanthus*
	Polygalaceae	*Polygala*
	Polygonaceae	*Eriogonum*
		Persicaria
		Polygonum
		Rumex
	Primulcaeae	*Douglasia*
	Rosaceae	*Geum*
		Potentilla
	Rubiaceae	*Galium*
		Richardia
		Spermacoce
	Santalaceae	*Comandra*
	Solanaceae	*Capsicum*
	Violaceae	*Viola*
Grasses	Poaceae	*Aegilops*
		Andropogon
		Aristida
		Bouteloua
		Bromus
		Chasmanthium
		Coleataenia
		Cynodon
		Digitaria
		Eriochloa
		Hesperostipa
		Hordeum
		Muhlenbergia
		Oryza
		Paspalum
		Phalaris
		Poa
		Pseudosasa
		Puccinellia
		Saccharum
		Sacciolepis

		Setaria
		Sporobolus
		Thinopyrum
		Triticum
Hard mast	Fagaceae	*Quercus*
	Juglandaceae	*Carya*
Mosses	Ditrichaceae	*Ceratodon*
	Funariaceae	*Funaria*
	Grimmiaceae	*Grimmia*
	Sphagnaceae	*Sphagnum*
Sedges	Cyperaceae	*Carex*
		Cyperus
		Eriophorum
		Fuirena
		Rhynchospora
Shrubs	Anacardiaceae	*Toxicodendron*
	Aquifoliaceae	*Ilex*
	Asteraceae	*Artemisia*
	Berberidaceae	*Berberis*
	Caprifoliaceae	*Symphoricarpos*
	Chloranthaceae	*Hedyosmum*
	Convolvulaceae	*Ipomoea*
	Cornaceae	*Cornus*
	Ephedraceae	*Ephedra*
	Fabaceae	*Acacia*
		Calliandra
		Prosopis
		Sesbania
	Hydrangeaceae	*Hydrangea*
	Lauraceae	*Persea*
	Magnoliaceae	*Magnolia*
	Moraceae	*Ficus*
	Myricaceae	*Morella*
	Rhamnaceae	*Ceanothus*
		Frangula
	Rosaceae	*Coleogyne*
	Zygophyllaceae	*Larrea*
Soft mast	Ericaceae	*Vaccinium*
	Grossulariaceae	*Ribes*
	Moraceae	*Morus*
	Rosaceae	*Prunus*
		Rubus

	Solanaceae	*Solanum*
	Ulmaceae	*Celtis*
Rushes	Juncaceae	*Juncus* *Luzula*
	Sparganiaceae	*Sparganium*
	Typhaceae	*Typha*
Woody vines	Aristolochiaceae	*Aristolochia*
	Fabaceae	*Wisteria*
	Smilacaceae	*Smilax*
	Vitaceae	*Parthenocissus* *Vitis*



E. Supplementary Figure 3.1 Flowchart detailing steps of the experimental protocal of DNA metabarcoding for diet compoistion of wild pig (*Sus scrofa*) on three barrier islands in Georgia, USA from May 2022 through July 2023.

F. Supplementary Table 3.2 Percent frequency of occurrence (%FOO; number of plant diet samples containing each plant family [n] divided by total number of diet samples [N] expressed as a percent) and percent relative read abundance (%RRA; number of reads of each plant family detected divided by total number plant reads from the trnL marker) in the diet of wild pig (*Sus scrofa*) on Blackbeard and Sapelo Islands in Georgia, USA from May 2022 through July 2023.

Family	***n***	***N***	***%FOO***	***%RRA***
Poaceae	82	121	67.769	47.374
Arecaceae	46	121	38.017	9.878
Fagaceae	35	121	28.926	12.388
Cyperaceae	34	121	28.099	0.792
Verbenaceae	34	121	28.099	2.779
Asteraceae	30	121	24.793	0.959
Urticaceae	25	121	20.661	2.710
Pinaceae	22	121	18.182	1.837
Polygonaceae	22	121	18.182	1.413
Typhaceae	20	121	16.529	3.966
Vitaceae	20	121	16.529	1.996
Juncaceae	17	121	14.050	0.798
Pontederiaceae	15	121	12.397	2.064
Chenopodiaceae	14	121	11.570	5.654
Fabaceae	13	121	10.744	1.490
Onagraceae	12	121	9.917	0.439
Rubiaceae	11	121	9.091	0.123
Rosaceae	10	121	8.264	1.118
Saururaceae	10	121	8.264	0.135
Myricaceae	9	121	7.438	0.227
Apiaceae	8	121	6.612	0.337
Oxalidaceae	8	121	6.612	0.103
Solanaceae	7	121	5.785	0.300
Hydrocharitaceae	6	121	4.959	0.062
Euphorbiaceae	5	121	4.132	0.128
Altingiaceae	4	121	2.339	0.165
Cactaceae	4	121	2.339	0.015
Commelinaceae	3	121	1.754	0.029
Ebenaceae	3	121	1.754	0.040
Hypericaceae	3	121	1.754	0.143
Primulaceae	3	121	1.754	0.026
Caryophyllaceae	2	121	1.170	0.047
Salicaceae	2	121	1.170	0.119
Adoxaceae	1	121	0.585	0.032
Bryopsida_c	1	121	0.585	0.053
Juglandaceae	1	121	0.585	0.048

Rhamnaceae	1	121	0.585	0.094
Rutaceae	1	121	0.585	0.034
Sapindaceae	1	121	0.585	0.083

G. Supplementary Table 3.2 Percent frequency of occurrence (%FOO; number of plant diet samples containing each plant family [n] divided by total number of diet samples [N] expressed as a percent) and percent relative read abundance (%RRA; number of reads of each plant family detected divided by total number plant reads from the trnL marker) in the diet of wild pig (*Sus scrofa*) on Cumberland Island in Georgia, USA from February through April 2023.

Family	***n***	***N***	***%FOO***	***%RRA***
Arecaceae	37	50	74	9.878
Fagaceae	32	50	64	12.388
Poaceae	28	50	56	47.374
Asteraceae	27	50	54	0.959
Pinaceae	18	50	36	1.837
Verbenaceae	18	50	36	2.779
Apiaceae	16	50	32	0.337
Rosaceae	16	50	32	1.118
Chenopodiaceae	15	50	30	5.654
Cyperaceae	14	50	28	0.792
Polygonaceae	14	50	28	1.413
Myricaceae	11	50	22	0.227
Rubiaceae	10	50	20	0.123
Juncaceae	8	50	16	0.798
Cactaceae	6	50	12	0.015
Fabaceae	6	50	12	1.49
Hydrocharitaceae	5	50	10	0.062
Pontederiaceae	5	50	10	2.064
Urticaceae	5	50	10	2.710
Euphorbiaceae	4	50	8	0.128
Onagraceae	4	50	8	0.439
Commelinaceae	3	50	6	0.029
Oxalidaceae	3	50	6	0.103
Vitaceae	3	50	6	1.996
Caryophyllaceae	1	50	2	0.047
Ebenaceae	1	50	2	0.040
Sapindaceae	1	50	2	0.083

H. Supplementary Table 3.3 Classification of plant groups for plant families and genera (if available) detected in the diets of wild pigs (Sus scrofa) on three barrier islands in Georgia, USA from May 2022 through July 2023.

Plant Group	**Family**	**Genus**
Cacti	Cactaceae	*Opuntia*
Conifers	Pinaceae	*Pinus*
Hard mast	Fagaceae	*Quercus*
	Juglandaceae	*Carya*
Other deciduous trees	Altingiaceae	*Liquidambar*
	Salicaceae	*Populus*
	Sapindaceae	*Acer*
Forbs/herbs	Acanthaceae	*Ruellia*
	Aizoaceae	*Sesuvium*
	Amaranthaceae	*Alternanthera*
	Apiaceae	*Centella*
	Araceae	*Wolffiella*
	Araliaceae	*Hydrocotyle*
	Asteraceae	*Lactuca*
		Liatris
		Parthenium
	Brassicaceae	
	Campanulaceae	*Wahlenbergia*
	Caryophyllaceae	
	Chenopodiaceae	*Atriplex*
		Dysphania
		Salicornia
	Commelinaceae	*Commelina*
		Tradescantia
	Convolvulaceae	*Ipomoea*
	Euphorbiaceae	*Cnidoscolus*
		Croton
		Euphorbia
	Fabaceae	*Apios*
		Centrosema
		Lespedeza
		Medicago
		Vicia
	Hydrocharitaceae	*Limnobium*
	Hypericaceae	*Hypericum*
		Hypoxis
	Lamiaceae	*Salvia*
		Teucrium
	Onagraceae	*Ludwigia*

		Oenothera
		Oxalis
	Plantaginaceae	*Bacopa*
		Plantago
	Plumbaginaceae	*Limonium*
	Polygonaceae	*Eriogonum*
		Rumex
	Pontederiaceae	*Pontederia*
	Primulaceae	*Lysimachia*
		Samolus
	Rosaceae	*Potentilla*
	Rubiaceae	*Galium*
		Pentodon
		Spermacoce
	Rutaceae	
	Saururaceae	*Saururus*
	Solanaceae	
	Typhaceae	*Typha*
	Urticaceae	*Parietaria*
	Verbenaceae	
	Violaceae	*Viola*
	Vitaceae	*Cissus*
Grasses	Poaceae	*Andropogon*
		Anthoxanthum
		Bromus
		Cenchrus
		Chasmanthium
		Cynodon
		Digitaria
		Distichlis
		Echinochloa
		Eustachys
		Leersia
		Paspalum
		Paspalum
		Setaria
		Spartina
		Stenotaphrum
		Uniola
Mosses	Class Bryopsida	
Palms	Arecaceae	
Rushes	Juncaceae	*Juncus*

Sedges	Cyperaceae	*Carex*
		Cyperus
		Eleocharis
		Fuirena
		Rhynchospora
Shrubs	Adoxaceae	*Sambucus*
	Aquifoliaceae	*Ilex*
	Cupressaceae	*Juniperus*
	Oxalidaceae	
	Lauraceae	*Persea*
	Myricaceae	*Morella*
	Rhamnaceae	*Rhamnus*
Soft mast	Ebenaceae	*Diospyros*
	Ericaceae	
	Rosaceae	*Prunus*
Woody vines	Smilacaceae	*Smilax*
	Vitaceae	*Parthenocissus*
		Vitis

I. Supplementary Table 3.4 Percent frequency of occurrence (%FOO; number of invertebrate diet samples containing each invertebrate family [n] divided by total number of diet samples [N] expressed as a percent) and percent relative read abundance (%RRA; number of reads of each invertebrate family detected divided by total number invertebrate reads from the ArthCO1 marker) in the diet of wild pig (*Sus scrofa*) on three barrier islands of Georgia, USA from May 2022 through July 2023.

Class	***Family***	***n***	***N***	***%FOO***	***%RRA***
Arachnida	Lycosidae	6	171	3.509	0.111
	Melicharidae	5	171	2.924	0.102
	Psoroptidae	4	171	2.339	0.067
	Tarsonemidae	4	171	2.339	0.715
	Eupodidae	3	171	1.754	0.586
	Eriophyidae	2	171	1.17	0.043
	Ixodidae	2	171	1.17	0.042
	Ctenizidae	1	171	0.585	0.312
	Haplozetidae	1	171	0.585	0.024
	Linyphiidae	1	171	0.585	0.464
	Scheloribatidae	1	171	0.585	0.033
Chilopoda	Cryptopidae	1	171	0.585	0.072
Collembola	Entomobryidae	8	171	4.678	0.666

	Tomoceridae	2	171	1.17	4.909
	Dicyrtomidae	1	171	0.585	0.137
	Isotomidae	1	171	0.585	0.218
	Sminthurididae	1	171	0.585	0.134
Insecta	Bibionidae	11	171	6.433	3.712
	Noctuidae	9	171	5.263	0.259
	Ceratopogonidae	8	171	4.678	0.951
	Hydropsychidae	8	171	4.678	0.731
	Carabidae	5	171	2.924	0.519
	Curculionidae	5	171	2.924	2.337
	Cecidomyiidae	4	171	2.339	0.235
	Chironomidae	4	171	2.339	0.203
	Sciaridae	4	171	2.339	0.379
	Tabanidae	4	171	2.339	0.219
	Aphididae	3	171	1.754	0.029
	Cynipidae	3	171	1.754	0.990
	Elateridae	3	171	1.754	0.256
	Tenebrionidae	3	171	1.754	0.227
	Calliphoridae	2	171	1.17	0.028
	Chrysomelidae	2	171	1.17	0.041
	Gryllidae	2	171	1.17	1.848
	Staphylinidae	2	171	1.17	0.014
	Anthocoridae	1	171	0.585	0.413
	Corduliidae	1	171	0.585	0.023
	Crambidae	1	171	0.585	0.026
	Culicidae	1	171	0.585	0.089
	Ephydridae	1	171	0.585	0.306
	Ichneumonidae	1	171	0.585	0.171
	Limoniidae	1	171	0.585	0.140
	Muscidae	1	171	0.585	0.018
	Mycetophilidae	1	171	0.585	0.145
	Mymaridae	1	171	0.585	0.039
	Nitidulidae	1	171	0.585	0.084
	Scirtidae	1	171	0.585	0.023
	Sepsidae	1	171	0.585	0.355
	Sphaeroceridae	1	171	0.585	1.326
	Tachinidae	1	171	0.585	0.154
	Tipulidae	1	171	0.585	0.040
	Trichogrammatidae	1	171	0.585	0.011
Clitellata	Sparganophilidae	1	171	0.585	0.061
Gastropoda	Succineidae	4	171	2.339	0.428
Bivalvia	Montacutidae	1	171	0.585	0.054
Malacostraca	Ocypodidae	30	171	17.544	35.187

	Sesarmidae	23	171	13.45	23.848
	Panopeidae	19	171	11.111	10.591
	Armadillidiidae	14	171	8.187	2.720
	Portunidae	2	171	1.17	0.088
	Asellidae	1	171	0.585	0.027
	Eriphiidae	1	171	0.585	0.062
	Ligiidae	1	171	0.585	0.025
Scyphozoa	Stomolophidae	1	171	0.585	0.459
Anthozoa	Actiniidae	1	171	0.585	1.476