URBANIZATION OF THE WHITE IBIS (*EUDOCIMUS ALBUS*): REPRODUCTIVE PRODUCTIVITY AND NESTLING WEST NILE VIRUS DYNAMICS

by

JULIA SILVA SEIXAS

(Under the Direction of Sonia M. Hernandez)

ABSTRACT

Monitoring the reproductive productivity of urban colonies of wild birds is paramount to understand their adaptation to anthropogenic environments. The White Ibis (*Eudocimus albus*) is a wading bird iconic to the Everglades ecosystem that has become ubiquitous to the urban landscape in south Florida. Despite having specific breeding requirements, the ibis has already established a few urban colonies. This study aimed at comparing the productivity of an urban colony of ibises to those in the Everglades. Nestling mortalities were also investigated and revealed three ibis with West Nile virus disease in 2020. The urban colony showed high Mayfield nest success rates in 2020 (70.8%) and 2021 (76.4%). The results presented herein suggest that urban colonies are potentially suitable for ibis breeding however, continued studies on additional urban rookeries are needed.

INDEX WORDS: White Ibis; urbanization; reproductive productivity; nest success; West Nile virus

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DEDICATION

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CHAPTER 1

LITERATURE REVIEW

Reproductive Productivity

The Greater Everglades Ecosystem in Florida was the target of an intense water drainage process that influenced rapid urban and agricultural development in the early 20th century. This drastic landscape alteration resulted in a 70% deficit in annual water budget, 50% loss of territory and 90-95% decline in wading bird abundance in the Everglades (Perry 2004). Impacts to wading bird species were further exacerbated by compartmentalization of the central Everglades, which in addition to the reduced water budget, also resulted in an altered seasonal timing of water release. The gross dewatering of the estuary may have had the greatest impact on wading bird populations, since this zone was the location of highest productivity and the site of most of the large historical breeding colonies (Frederick et al. 2009).

The hydrology in the Everglades changes drastically throughout the year, producing distinct wet and dry seasons. The former occurs from May to October, when precipitation peaks and the populations of aquatic fauna are allowed to grow (Acosta and Perry 2001; Ruetz et al. 2005). Starting in November, the dry season is characterized by a decline in rainfall, causing the water levels to recede. The reduction in available habitat concentrates aquatic prey in ever shallower waters, where they can be more easily preyed upon by wading birds, particularly tactile feeders (Gawlik 2002; Trexler and Goss 2009). The timing of the breeding seasons of these birds is strongly correlated to periods where preferred prey is abundant (Gawlik 2002). Changes in hydrological conditions were described to dictate fluctuations in prey concentrations, which are considered to be one the primary drivers of nesting colony size and reproductive success of tactile feeders that depend on high concentrations of prey to forage and reproduce (Frederick and Odgen 2001; Crozier and Gawlik 2003a). The White Ibis (*Eudocimus albus*; hereafter "ibis") is one of the most abundant species in the Everglades and it readily responds to variations in availability of prey (Gawlik 2002; Semones 2003; Herring and Gawlik 2013; Heath et al. 2020). It was established as an indicator of the status of the Everglades restoration, as the species is well-studied, abundant, highly dependent on prey influenced by hydrological fluctuations (Crozier and Gawlik 2003a; Frederick et al. 2009). Ibises show extremely low nest-site fidelity and, due to its nomadic nature, they can easily explore novel foraging and breeding grounds (Frederick et al. 1996; Frederick and Ogden 1997). The flexibility of the ibis to adapt to a new breeding and non-breeding range combined with the severe habitat deterioration in south Florida possibly encouraged this species to explore resource-abundant anthropogenic environments.

The White Ibis

White Ibises are nomadic wading birds iconic to the Everglades ecosystem in Florida. They naturally forage in shallow coastal marshes, wetlands and mangrove swamps (Bildstein 1993; Boyle et al. 2014; Kushlan 1979). As probing tactile feeders, their diet consists of terrestrial insects and aquatic organisms such as crayfish, crabs, and fish (Bildstein 1993; Heath et al. 2020). They will select for ridges with < 19 cm of depth and sparse vegetation for foraging (Lantz et al. 2011; Binkley et al. 2019). In coastal areas, nestlings and juveniles feed mostly on freshwater prey due to their inability to tolerate heavy salt loads (Johnston and Bildstein 1990; Cocoves et al. 2021). During the breeding season, ibises consume primarily crayfish, while fish and terrestrial arthropods are targeted as secondary prey (Kushlan 1979; Bildstein 1993; Boyle et al. 2014).

Breeding site selection is still a poorly understood process within ibis population dynamics (Heath et al. 2020). This species exhibits a very low nest-site fidelity, which is possibly promoted by the unpredictable variations in weather and foraging conditions. Consequently, these birds will travel long distances during their search for the optimal breeding site (Bildstein 1993; Frederick et al. 1996). Such locations comprise freshwater or marine tree islands, where a multitude of wading birds nest (e.g., Great Egrets *Ardea alba*, Snowy Egrets *Egretta thula*, Little Blue Herons *Egretta caerulea*, Black-crowned Night Herons *Nycticorax nycticorax*, etc), and high densities of preferred prey are found within 60 km of the colony (Kushlan 1979; Boyle et al. 2014). The waters surrounding these islands are frequently inhabited by alligators, which confer protection against terrestrial predators (e.g., raccoons, snakes etc; Frederick and Collopy 1989a; Nell et al. 2016). The onset of breeding is variable. It usually occurs sometime between February and June in south Florida, during the wetland dry season (Frederick and Collopy 1989b). The nesting cycle lasts approximately 60 - 80 days, providing enough time in the Everglades for the young to fledge before foraging conditions deteriorate as a result of increased rainfall. Ibises are socially monogamous, and females will typically lay one egg per day on alternate days (Bildstein 1993). Although extra-pair copulations are common, they do not account for more than 6% of all fertilizations (Frederick 1987). Incubation lasts about 21 days. Clutch sizes typically range from two to four nestlings, however previous studies claim that often only one will fledge per nest as parents will prioritize the oldest when feeding. Yet, fledging rates may vary depending on the environmental conditions, resource availability, timing in the breeding season, poor experience in nesting birds, or a combination of the aforementioned factors (Kushlan 1977; Frederick and Collopy 1989b; Bildstein 1993; Semones 2003).

Nesting colonies are characteristically occupied by multiple species, where the white ibis is usually the most numerous (Frederick et al. 2009). Nesting pairs within a colony fluctuate annually from a few tens to hundreds of thousands of individuals, often changing radically in the same colony from year to year (Bildstein 1993; Frederick and Ogden 2001). In the Everglades, ibises commonly exhibit supranormal nesting events in years immediately following severe droughts, which has been previously correlated with an increase in crayfish availability (Frederick and Ogden 2001; Dorn and Cook 2015; Cocoves et al. 2021). Nest success and colony size depends heavily on the availability of preferred prey, such as crayfish (Frederick and Collopy 1989b; Cocoves et al. 2021). Crayfish densities fluctuate depending on weather conditions and are often found at high concentrations following rapid wetland drying rates. Consequently, ibises tend to show greater nesting success and numbers of nest initiations when water recession rates are faster. Abandonment is the most common source of nesting failure in the Everglades, sometimes accounting for over 90% of failures, and it usually occurs as a response to a decline in foraging conditions (Frederick and Collopy 1989b; Frederick et al. 1996; Heath et al. 2020). Failure can also result from predation, severe weather conditions (e.g., storms, tidal inundations), and increased aggression among nesting birds (Frederick and Collopy 1989a, 1989b; Bildstein 1993; Frederick et al. 1996; Heath et al. 2020). Ibises may re-nest if failure occurs early in the nesting stage (e.g., egg laying, incubation) or breeding season (Bildstein et al. 1992; Heath et al. 2020). They may also re-nest following a successful early-nesting attempt, however the likelihood and frequency at which this event occurs remains unclear (Heath et al. 2020). Although loss of nest content due to predation is usually fairly low, common wading bird nest predators include small mammals, black-crowned night herons, crows, owls, and snakes, including the invasive Burmese python (Frederick and Collopy 1989a; Orzechowski et al. 2019).

As a result of habitat deterioration, ibis breeding activity has declined by 87% across the Everglades between the early 1900s and the 1990s (Frederick and Ogden 2001; Crozier and Gawlik 2003a). However, they are still numerous and are categorized under *Least Concern* by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (BirdLife International 2016). The draining of the Everglades also caused a shift in nesting from mostly coastal ecotones to primarily freshwater inland areas. Coastal colonies may be temporally more stable and supply constant minimal recruitment (Kushlan 1977; Frederick et al. 2009). Large irruptive breeding events at coastal colonies were probably characteristic of the pre-drainage period and have recently been reported in 2018, probably in response to a period of high crayfish abundance (Cocoves et al. 2021). However, while natural habitats provide variable environmental conditions for breeding, anthropogenic environments may be a more reliable alternative since they offer more stable foraging conditions in the form of provisioned food (e.g., urban refuse, handouts from the public).

Urbanization of the White Ibis

As a response to the loss of habitat, a sector of the South Florida population has been increasingly found in urban parks, residential neighborhoods, zoos, landfills, and other artificial wetlands since the 1990s (Frederick and McGehee 1994; Hernandez et al. 2016). Yet, it is still in its early stages of adapting to anthropogenic environments. In urban areas, ibis aggregate in large flocks, come in close contact with a variety of species (including peridomestic and exotic waterfowl; Fig. 1.1), and consume poor quality food and water, often provisioned by people.

During their transition to living in urban environments, ibises have experienced shifts in behavior, movement, and diet, which may impact their health and ecology. For instance, GPS transmitters revealed alterations in the movement patterns of urban ibises when compared to those captured in natural areas: ibises with high levels of urban habitat use dispersed less, undertook shorter breeding seasons, and showed non-breeding season foraging site fidelity to urban areas (Kidd 2018). Stable isotope analyses have shown that ibises that had isotopic signatures similar to processed food used more urban habitat, had lower body condition, lower ectoparasite scores, assimilated more omnivorous food, and had lower diet diversity, when compared with ibises in natural areas. This suggests that urban ibises are heavily provisioned with anthropogenic food (mainly highly processed carbohydrates) that are not part of their natural diet. This appears to offer a trade-off by providing low-quality, but easily accessible, calories that may not support high mass but may increase time available for behaviors such as preening to clear ectoparasites (Murray et al. 2018). Until very recently, even highly urbanized ibises would still return to the Greater Everglades ecosystem to breed (Kidd 2018). One of the largest and oldest urban ibis rookeries documented is located on the Solid Waste Authority of West Palm Beach, which is adjacent to a landfill and bordered on the western side by Grassy Waters Preserve, a natural area. Urban colonies are defined herein as those located within human-dominated habitats and artificial wetlands. Additional urban rookeries have been identified in Palm Beach County, one of which is readily accessible to researchers (Rumbold et al. 2009; Welch 2016).

Although ibises will primarily target crayfish to feed their nestlings, changes in the hydrology of wetlands can promote switches in prey types when conditions are less than ideal (Kushlan 1979). They may fly long distances daily (approximately 30 – 60 km) in search for food and will forage in novel areas if necessary (Frederick et al. 2009). Ibises can also become more generalist if resources are scarce. In fact, urban refuse is already considered an opportunistic alternative (Dorn et al. 2011; Boyle et al. 2014). Resource abundant parks, zoos, and landfills may be particularly interesting to breeding ibises because they offer a continuous source of food that is readily available, thus reducing the dependence of ibises on hydrological cycles and the need to fly long distances to search for prey. However, increased consumption of this low-quality food could be detrimental to the nestlings growth and health, and other factors, like increased nest predation by urban-associated predators, may translate to important trade-offs (Heiss et al. 2009).

Given time, we predict ibises will learn to exploit urban environments throughout their annual cycle more extensively. For instance, the populations of Australian Ibises (*Threskiornis molucca*) in urban landscapes have been markedly increasing since the 1950s as a result of their successful adaptation to urban breeding and the continuous influx of natural ibises to anthropogenic landscapes (Smith et al. 2013). Currently considered a nuisance species, they will scavenge garbage containers and harass and steal food from people (Corben and Munro 2006). They are nomadic wading birds like the White Ibis, however some have become urban residents, where they will mostly occupy landfills (Martin et al. 2011; Martin et al. 2012). Australian Ibises breed in urban areas regularly, and these rookeries showed higher fledging rates than their natural counterparts (Corben and Munro 2016). Similarly, European White Storks (*Ciconia ciconia*) have also used urban areas for nesting, and they will consistently return to such locations to breed due to their high nest-site fidelity. Nesting in anthropogenic environments is also advantageous to this species, with nests built near dumping grounds showing a higher productivity (Massemin-Challet et al. 2006). Additionally, urban resident storks showed larger clutch sizes than their migrant conspecifics (Djerdali et al. 2016).

The reproductive success of urban White Ibises is currently unknown. In the case of Australian Ibises and European White Storks, their ability to breed in urban areas has allowed them to withstand large-scale habitat conversion but has also created human-wildlife conflicts. Thus, understanding the contribution of urban rookeries is paramount to monitor the increasing of White Ibises to anthropogenic environments and to predict the need for future management of urban populations.

West Nile Virus Dynamics

While anthropogenic environments may offer alternative resources to species with declining natural habitats, there are costs associated with this transition. In fact, shifts in wildlife behavior and health can increase exposure and susceptibility to pathogens (Becker et al. 2015). Factors such as stress, large aggregations, poor water and food quality, novel multi-specific interactions, and sedentarism provide opportunities for infection and disease development (Bradley and Altizer 2007). Urban white ibises are subject to distinct pathogen dynamics, potentially influenced by the consumption of contaminated food and water and the frequent and close contact with various species.

West Nile Virus

West Nile virus (WNV) is a mosquito-borne flavivirus (genus *Flavivirus*; family *Flaviviridae*) of global public health concern. It was first isolated from a febrile woman in Uganda in 1937 and has since rapidly spread (Smithburn et al. 1940). While the route of entry remains unclear, WNV was introduced in New York in 1999, causing outbreaks in humans, and exotic and wild birds (Lanciotti 1999). The strain isolated from the 1999 outbreaks (NY99) was most closely related to the strain circulating in Israel in 1998. The virus has since evolved, giving rise to novel strains within the US, and it is now found in the 48 contiguous states, in addition to Canada and Latin America, including South America.

West Nile virus is part of the Japanese encephalitis serocomplex, which includes the St. Louis encephalitis virus (SLEV), Murray Valley encephalitis virus, and Kunjin virus (Lanciotti 1999). Virions are spherical, 50 nm in diameter, and enveloped with an adherent lipid structure. The RNA is linear, single-stranded, plus sense, and approximately 11 kb long. Infection initially begins at the epidermal Langerhans cells (Samuel and Diamond 2006). The virus then infects macrophages in the lymph nodes and subsequently migrates to the spleen, kidneys, and spinal cord. The final target of WNV is the neurons in the brain. West Nile virus has a wide host range and is capable of infecting several taxa, including reptiles, birds, and mammals (Ip et al. 2014). Mammals are dead-end hosts; however, they can still develop severe clinical symptoms. In fact, 40% of cases in horses are fatal (Hall and Khromykh 2004). Most human infections are asymptomatic, however serious illness can still occur, particularly in the elderly (Centers for Disease Control and Prevention [CDC] 2018). Common symptoms reported in humans include fever, headaches, joint pains, vomiting, and diarrhea, but can also progress to serious neurologic symptoms such as convulsions, tremors, paralysis, and coma.

West Nile virus is primarily transmitted between birds and mosquitoes, however direct bird-to-bird transmissions may also occur (Banet-Noach et al. 2003). Infected birds develop a viremic period of 1-7 days, and usually die within 4-8 days (Komar et al. 2003). Clinical signs typically range from lethargy to torticollis, seizure, and ataxia. Individuals that survive develop detectable antibodies starting on day 4 post-infection that protect them against future infections (Styer et al. 2006; Brault et al. 2004). WNV-neutralizing antibodies can persist for up to 12 months in naturally infected rock pigeons (*Columba livia*) and fish crows (*Corvus ossifragus*), and over 4 years in raptors (Gibbs et al. 2005; Wilcox et al. 2007; Nemeth et al. 2008). The latter can also experience some level of protection upon subsequent exposures. Birds can also acquire immunity to WNV passively. Maternal antibodies have been detected across several avian species; however, their persistence and level of protection varies widely. While domestic chicken (*Gallus gallus domesticus*) chicks can remain protected against WNV infections for up to 42 days post-hatch, house sparrows (*Passer domesticus*) that inherit maternal antibodies are not protected beyond their first week of life (Nemeth and Bowen 2007; Nemeth et al. 2008).

West Nile virus has been detected in over 60 species of mosquitoes (CDC 2016a). However, *Culex* spp. appear to be primarily responsible for WNV transmission because they are competent vectors and are frequently associated with WNV foci (Turell et al. 2005; Molaei et al. 2006). There is some regional variation with respect to which *Culex* species will be important vectors, with *Cx. pipens* and *Cx. restuans* in northeast and north-central North America, *Cx. nigripalpus* and *Cx. erraticus* in southeastern US, and *Cx. tarsalis* in western North America (Blackmore et al. 2003; Reisen et al. 2004; Turell et al. 2005; Cupp et al. 2007). These largely generalist and ornithophilic mosquitoes now transmit WNV to over 300 avian species in the US, including captive, exotic, and free-living specimens (CDC 2016b).

Impacts on the North American Avifauna

Susceptibility to infection and disease varies greatly among birds, with raptors and some passerines such as members of the Corvidae family being highly susceptible to clinical disease and mortality. Population impacts are difficult to document in raptors and most studies rely on data from rehabilitation centers. Clinical signs and pathologic lesions are variable among raptor species, with great horned owls (*Bubo virginianus*) and hawks, particularly red-tailed hawks (*Buteo jamaicensis*), being generally more susceptible to severe disease, placing them at a higher risk of experiencing population declines due to their increased mortalities (Ellis et al. 2007; Saito et al. 2007; Nemeth et al. 2009). Although raptors are commonly reported with WNV infections, they are only moderately competent hosts. Banerjee et al. (2017) demonstrated a correlation between host competency, and body mass and phylogeny, which predicted that smaller passerines would develop higher viremias and thus potentially infect more mosquitoes than larger non-passerine species. Passerines are indeed excellent reservoirs and play an important role in WNV transmission and amplification in natural settings. Some of these birds are among the most competent hosts for this pathogen, including species of the families Corvidae, Icteridae, Fringillidae, and Passeridae (Kilpatrick et al. 2007). In fact, the American robin (*Turdus migratorius*) has been shown to account for as many as approximately 60% of WNV-infected mosquitoes across urban areas within Maryland and Washington, DC (Kilpatrick et al. 2006). Some passerine species are also highly susceptible to mortality, often resulting in declines or seasonal fluctuations of local populations after the arrival of the virus, including the American crow (*Corvus brachyrhynchos*), blue jay (*Cyanocitta cristata*), yellow-billed magpie (*Pica nuttalli*), American robin, eastern bluebird (*Sialia sialis*), tufted titmouse (*Baeolophus bicolor*), Carolina chickadee (*Poecile carolinensis*), black-capped chickadee (*P. atricapillus*), and house wren (*Troglodytes aedon*; LaDeau et al. 2007; Wheeler et al. 2009).

Kilpatrick and Wheeler (2019) argue that, with a few exceptions, WNV has generally caused low to moderate impacts on avian populations. In fact, some species were reported to have recovered to their pre-WNV introduction abundances. Factors that facilitate recovery are related to the spatial and temporal patterns characteristic of vector activity and subsequent disease transmission, which provide refugia to a number of susceptible species. Nonetheless, some corvids will still experience marked population declines due to their notorious susceptibility to WNV-associated mortality, reaching rates as high as 100% in American crows (Komar et al. 2003; Kilpatrick and Wheeler 2019). In fact, crow mortalities are used as early indicators to detect WNV activity (Eidson et al. 2001). Since then, the testing of dead wild birds for WNV infections and reporting of positive results have been encouraged extensively and are integral to the public health agenda.

West Nile Virus in Aquatic Birds

West Nile virus infections have not been extensively documented in wild aquatic birds. This is possibly a consequence of their general reduced susceptibility to disease and mortality, resulting in a lack of noticeable population declines (Reisen et al. 2009; Hofmeister et al. 2015). Additionally, access to rookeries is difficult, and mortalities are usually not investigated due to restrictions imposed by researchers such as time spent on the colony or lack of interest (Frederick et al. 1993; Kuiken et al. 1999). However, WNV has been reported in a number of free-living water birds and was responsible for a few outbreaks (Rocke et al. 2005; Johnson et al. 2010; Ip et al. 2014).

In 2013, a mortality event involving 15,000-20,000 eared grebes (*Podiceps nigricollis*) was reported in the Great Salt Lake, UT (Ip et al. 2014). All of the carcasses collected tested positive for WNV infection, suggesting the mortalities were a result of a WNV outbreak. WNV-infected bald eagles were also documented in the area during the same period. A multi-state outbreak was also reported in American white pelicans (*Pelecanus erythrorhynchos*) across seven north-central states in 2002-2003 where over 9,000 chick mortalities were attributed to WNV (Rocke et al. 2005). Nestling and juvenile pelicans are thought to be more susceptible to infection and disease than adults, which restricts population level impacts to reduced recruitment and limits potential outbreaks to the breeding season (Rocke et al. 2005; Johnson et al. 2010). Because of the high mortality rate of young pelicans and the association with WNV cases in humans within surrounding communities, surveillance of pelican colonies has been proposed as an additional indicator for WNV activity (Johnson et al. 2010).

Wading birds seem to show a limited role in the epidemiology of WNV. In general, WNV potentially circulates at a very low prevalence in wading bird colonies, and it is probably not a significant cause of mortality for these nestlings (Reisen et al. 2005; 2009). In fact, previous studies in California have shown that natural wading bird rookeries were not considered to be sources of virus amplification. However, infections in cattle egret (*Bubulcus ibis*), black-crowned night heron, snowy egret, and great egret nestlings were reported in the California colonies (Reisen et al. 2005; 2009). Additionally, black-crowned night herons are considered moderately competent hosts for the virus based on experimental infections, and neutralizing antibodies to WNV were detected in wild double-crested cormorant (*Phalacrocorax auritus*) chicks (Reisen et al. 2005). West Nile virus was reported in two species of ibises in the US, a free-living white-faced ibis (*Plegadis chihi*) and a captive scarlet ibis (*Eudocimus ruber*), yet details of their infections are lacking (CDC 2016b).

Spatial and Temporal Patterns

As a vector-borne disease, the spatial distribution of WNV is dependent on the biology of the vector, which is dependent on habitat type, and thus can be modeled to indicate areas suitable for WNV-infected mosquitoes. Optimal environmental conditions for potential WNV hotspots include gentle slopes (0-6%), low elevations (<85 m), temperatures around 25ºC, and ~70 mm of weekly rainfall (Ozdenerol et al. 2008). A study analyzing different habitat types and WNV prevalence in mosquitoes revealed that increased wetland cover was associated with a decline in WNV-infected mosquitoes (Ezenwa et al. 2007). Although support is lacking, an interesting explanation to this lies in the presence of a diverse bird community that is inherent of this habitat, which largely comprises poor hosts, thus potentially leading to lower WNV infection rates (Ezenwa et al. 2006; 2007). In contrast, anthropogenic habitats, particularly urban/suburban areas, are considered more suitable for WNV and have consequently been associated with higher mortality rates in avian populations (Bradley et al. 2008; Ozdenerol et al. 2008; LaDeau et al. 2011; Kilpatrick and Wheeler 2019). Within urban environments, residential areas have been positively correlated with an increase in infected mosquitos, and abundance of competent vectors and avian hosts, when compared to adjacent urban wetlands (Gibbs et al. 2006; Johnson et al. 2012). Mosquitoes in large urban wetlands (>100 ha) had the lowest infection rates, possibly due to their richer avian communities and lower abundance of competent hosts (Johnson et al. 2012). Additional factors, such as the socioeconomic status of different neighborhoods, may also play an important role in controlling mosquitoes and disease transmission. For instance, wealthier communities are generally better equipped to prevent mosquito proliferation and are more preoccupied with avoiding transmission than low-income areas (Koram et al. 1995; David et al. 2009; LaDeau et al. 2013). In fact, pupae of WNV vectors were more commonly found in neighborhoods categorized as below median income level in both Washington, DC and Baltimore, MD (LaDeau et al. 2013).

Transmission of WNV is also dependent on the seasonality associated with mosquito activity, which peaks during the summer and early fall in temperate regions (Apperson et al. 2004; Ozdenerol et al. 2008). Changes in temperature and/or precipitation can nonetheless alter these patterns and allow for WNV to circulate outside its normal temporal scale (LaDeau et al. 2011; Johnson et al. 2012; Ip et al. 2014).

West Nile Virus in Florida

West Nile virus was first detected in Florida in a crow in Jefferson County in July 2001 (Blackmore et al. 2003). The primary WNV vectors in the state are *Cx. nigripalpus* and *Cx. pipiens quinquefasciatus* (Blackmore et al. 2003; Rutledge et al. 2003). Both species are ornithophilic, but will also feed on mammals, reptiles, and amphibians (Day et al. 2015). Gravid *Cx. nigripalpus* females are generalists and will oviposit in a variety of freshly flooded habitats. *Cx. p. quinquefasciatus* are more commonly found in urban areas due to the females' preference for nutrient-rich semipermanent freshwater habitats. Despite the presence of competent vectors and opportunities for amplification, Florida has never experienced a major epidemic or epizootic event in birds (Day et al. 2015). A possible explanation for this involves a reduced abundance of competent host avian species critical for viral amplification, and the environmental factors influencing the abundance and competence of *Cx. nigripalpus* and *Cx. p. quinquefasciatus,* such as the lack of favorable environmental conditions.

Since the introduction of WNV, Florida has employed surveillance programs that include sentinel chickens, monitoring of mosquito pools, and the reporting of positive avian carcasses. They also developed a five-level response system to WNV activity (ranging from no activity to medical threat) that they use when communicating with the public. West Nile virus circulates regularly in Florida, with annual clinical human cases and mortalities averaging 23 and 1 respectively from 2001 to 2020 (CDC 2019a; 2019b; 2020). The virus is usually detected in hundreds of sentinel chickens and up to a few dozen mosquito pools every year, indicating that WNV is commonly found circulating across the state (Florida Department of Health [FDOH] 2021).

Rationale and Significance

This study aims at strengthening our understanding of the trade-offs imposed by urbanization on ibises, as they are becoming increasingly adapted to anthropogenic habitats. A higher productivity (i.e., clutch sizes and fledging rates) at urban colonies could represent a potential for ibises to increase their exploitation of anthropogenic resources throughout their annual cycle, which will allow them to subsequently become permanent urban residents. A similar progression was previously observed in the Australian white ibis and European white stork (Corben and Munro 2006; Massemin-Challet et al. 2006; Martin et al. 2012; Djerdali et al. 2016). The increased suitability of urban breeding sites could motivate ibises to form novel urban rookeries and establish large colonies. However, this process is limited by the ibises' specific requirements for breeding (i.e., tree islands surrounded by alligators), which are possibly some of the factors that could be slowing down the birds' adaptation to urban environments. Ultimately, the potential increase in ibises inhabiting anthropogenic habitats could culminate in the escalation of human-wildlife conflicts as observed with the Australian white ibis, where they rapidly learned to steal food from the public (Martin et al. 2012). Additional potential future sources of conflict relevant to urban ibis breeders are associated with the establishment of urban colonies at undesirable locations, such as near restaurants and airports, where they could become a hazard and produce a lot of noise, foul odor, and waste, as previously seen with ardeids in other US states (Telfair et al. 2000; Grant and Watson 1995; Hattori 2009). Information on the productivity of urban colonies can serve to monitor the adaptive process of ibises to urban environments, follow urban population trends, predict these conflicts, and inform management. Additionally, it can serve as an indicator of pathogens circulating in these populations, which can be relevant to public health such as WNV.

Nestling mortality investigations are often not conducted in colonial nesting birds, however they provide valuable information that contribute to understanding productivity. Therefore, we collected fresh ibis nestling carcasses from the urban and natural colonies and submitted them for diagnostic evaluation in hopes to identify any potential diseases that could have been impacting those populations. Because urban ibises we studied were nesting within a golf course community, mortality investigations from this site could be particularly useful to inform public health authorities about pathogens that could be circulating among human populations. One of the pathogens of interest is WNV because of its relevance to public health and endemic status in most avian populations. West Nile virus infections have not been extensively documented in aquatic birds, particularly nestlings. Therefore, documenting infections and mortalities on these individuals provides valuable information that increases our understanding of this pathogen.

This study was designed to measure the reproductive productivity of an ibis urban colony and compare the following metrics with natural rookeries in the Everglades: number of nesting pairs, nest success, clutch sizes, fledging rates, and nestling growth rates. The urban site selected was readily available to researchers and was located within a golf course community. We also collected ibis nestling carcasses from a mortality event that occurred during the breeding season of 2020 and they were diagnosed with WNV disease. In addition to documenting these mortalities, we also analyzed plasma samples collected from the urban nestlings that were handled that year for anti-WNV antibodies.

Study Limitations

Annual ibis colony size and reproductive success fluctuate greatly, from approximately 100 – 20,000 nesting pairs and 5 – 70%, respectively (Frederick et al. 2009; Heath et al. 2020). These variations depend heavily on breeding site location and the local hydrological conditions (Frederick and Odgen 2001; Crozier and Gawlik 2003a; Semones 2003). Because of this fluidity, the two years of data collection proposed in this study functioned primarily to indicate whether urban colonies can be equally or even more productive than rookeries in the Everglades. Continued analyses for longer periods and over multiple urban colonies will allow for more conclusive results on whether such habitats are overall more or less advantageous for ibis breeding. Another limitation inherent of this study design is the sample bias toward nests and chicks that are accessible. To reduce the influence of this bias, extension ladders that can reach the average height of the trees on the island were used.

This is a study that produced a high disturbance to the urban colony because it demanded more frequent and longer visits than the once weekly 1-hour limit established for monitoring natural rookeries (Frederick, personal communication). This could have led to abandonments and fewer nest initiations or exploitation by urban predators that take advantage of adults that are flushed from the nest by researchers. For instance, the urban colony was occupied and/or visited by boat-tailed grackles (*Quiscalus major*), black-crowned night herons, fish crows, blue jays, and raptors, which are known nest predators (Frederick and Collopy 1989a). In an attempt to minimize the influence of avian predators, particularly boat-tailed grackles, which were abundant at the site, food was placed on the shore, away from the colony, in an effort to distract the birds during visits by researchers. The birds' response to the food supplementation was recorded to assess the effectiveness of this strategy. Unfortunately, it unknown how much the disturbance caused by researchers could have affected the results, however, avian behavior in response to routine human disturbance in addition to the presence of researchers were noted and compared.

An unexpected setback that affected data collection in this study was the SARS-CoV-2 pandemic that led to the cancellation of visits to the Everglades for sampling purposes in 2020.

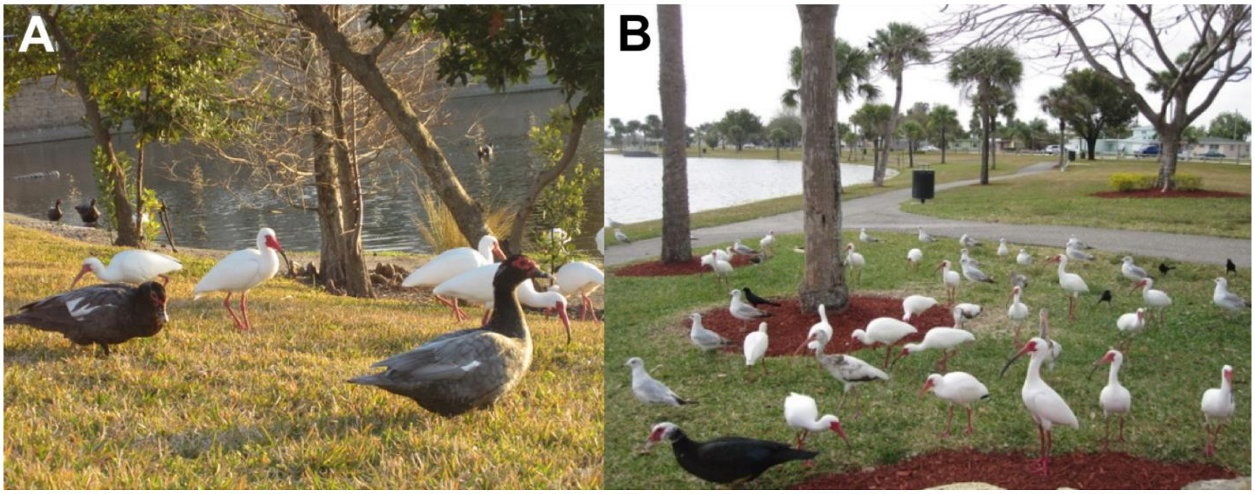

Figure 1.1. White Ibises (*Eudocimus albus*) waiting for handouts from people at urban parks come in close contact with peridomestic waterfowl (A) and other species and gather in large flocks all day (B).

CHAPTER 2

REPRODUCTIVE PRODUCTIVITY OF AN URBAN COLONY OF WHITE IBISES (*EUDOCIMUS ALBUS*)[1]

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[1] Seixas, J. S., Hernandez, S. M., Cooper, R. J., Frederick, P. C. To be submitted to *Waterbirds*.

ABSTRACT

Land-use changes such as urbanization and agriculture have caused many wildlife species to decline, yet some species benefit from novel resources offered by human-altered habitats. Such provisioning, often in the form of food, is widespread and can be intentional (e.g., bird feeders) or unintentional (e.g., landfills). The White Ibis (*Eudocimus albus*) is an increasingly urbanized wading bird in Florida that has been showing shifts in landscape use, diet, and movement patterns. Until very recently, most of the population returned to natural areas to nest but is now beginning to nest in urban areas. Ultimately, survival and reproductive success are the two key factors to determining the potential for urbanization to impart population-level impacts to urbanized species. We aimed to investigate the productivity of an urban colony of White Ibises in south Florida. A total of 64 and 67 nests were monitored until fledging via weekly nest checks in 2020 and 2021, respectively. Results were analyzed using the Mayfield survival method and compared with data collected in the Everglades during the same period. Additionally, a total of 36 and 41 nestlings were handled weekly from hatch until fledging to measure weight and standard morphometric measurements. Growth rates were compared with 39 chicks sampled at two colonies in the Everglades. The urban colony showed high nest success in 2020 (70.8%) and 2021 (76.4%) despite being subject to high levels of disturbance. We failed to detect differences in the growth rates and body condition of urban and natural nestlings, suggesting that urban chicks are provided with the necessary nutrients and calories to grow and fledge. The results presented herein suggest that urban environments are potentially suitable habitats for ibis breeding. Continued monitoring of additional urban rookeries is needed to more effectively evaluate whether urban environments are optimal alternatives to natural wetlands. Further studies analyzing nest predation, nestling diet, and the impact of anthropogenic food on

ibis productivity are also necessary to understand the factors promoting nest success in urban rookeries.

INTRODUCTION

The Urbanization of the White Ibis

The intensive wetland drainage in the Everglades between the 1930s and the 1990s resulted in a drastic reduction of habitat available for wading birds (Perry 2004). Tactile feeders such as the White Ibis (*Eudocimus albus*; hereafter "ibis") and Wood Storks (*Mycteria americana*) that depend on high concentrations of prey suffered severe population declines (Frederick et al. 2009). While historic breeding colonies used to support up to 100,000 – 200,000 and 14,000 – 20,000 breeding pairs of ibises and storks, they are now reduced to a maximum of 5,000 – 50,000 and 500 – 5,000 respectively (Frederick et al. 2009; Cook and Baranski 2019; Heath et al. 2020). As these two species are abundant, well-studied, highly dependent on prey influenced by wetland hydrology, and highly sensitive to slight environmental changes, they were both proposed as indicators of the status of the Everglades restoration (Frederick et al. 2009). The compartmentalization of the Everglades also promoted spatial changes in the locations chosen for ibis breeding. During the pre-drainage period, these birds used to show a preference for coastal ecotones. Now they heavily occupy the central Everglades, with these older freshwater/estuarine ecotone rookeries currently acting as buffers by providing constant minimal recruitment (Kushlan 1977; Frederick et al. 2009). Ibises are classified as nomads due to their low nest-site and foraging-site fidelity and unpredictable movement patterns (Melvin et al. 1999). These traits likely facilitated both the shift in nesting territories mentioned and their increasing colonization of anthropogenic territories in south Florida (Frederick and Ogden 1997).

Since the 1990s, ibises have become ubiquitous to the south Florida urban landscape, where they are routinely observed foraging in urban parks, zoos, landfills, and other artificial wetlands (Frederick and McGehee 1994; Hernandez et al. 2016). This dramatic change in habitat promoted shifts in behavior, movement, and diet in these birds, which impacted their health and ecology. For instance, GPS-tracked urban ibises were found to exhibit non-breeding season foraging site fidelity to urban areas, reduced dispersion, and shorter breeding seasons when compared to their monitored natural conspecifics (Kidd 2018). Stable isotope analyses of urban ibises indicated that the individuals with signatures similar to processed food offered by the public at urban parks exhibited lower body condition and ectoparasite scores, assimilated more omnivorous food, and had lower diet diversity, when compared with ibises inhabiting natural areas. This increased consumption of provisioned anthropogenic food seems to be offering these birds a trade-off by providing low-quality, but easily accessible calories that may not support a high body mass but allows more time for behaviors such as preening to clear ectoparasites (Murray et al. 2018).

Ibises have become well-established in urban areas during the non-breeding season, yet until the early 2000s, they would still return to the Greater Everglades ecosystem to breed (Kidd 2018). The limitation to breeding in anthropogenic environments is likely a consequence of this species’ strict breeding requirements. As colonial nesting wading birds, ibises usually breed in mixed species rookeries, and to avoid predation from terrestrial predators (e.g., raccoons, snakes), these colonies are formed on tree islands surrounded by alligators (Frederick and Collopy 1989a; Nell et al. 2016; Burtner and Frederick 2017). Despite this constraint, ibises have already established a few urban colonies in south Florida. Yet, it is unknown how successful urban nesters can be at reproducing in this alternative habitat. Ibis nest success and number of nesting pairs have both been repeatedly correlated with rapid water recession rates, which concentrates and allows access to crayfish, their preferred prey during the breeding season (Frederick and Collopy 1989b; Frederick and Odgen 2001; Crozier and Gawlik 2003a; Cocoves et al. 2021). In fact, irruptive breeding events have been registered at abnormally high crayfish abundances following severe droughts (Frederick and Odgen 2001; Cocoves et al. 2021). At reduced crayfish abundances, ibises usually resort to alternative food sources, resulting in lower reproductive productivities (Dorn et al. 2011; Boyle et al. 2014; Cocoves et al. 2021). Anthropogenic food, such as urban refuse, have already been established as one of such potential alternatives (Dorn et al. 2011; Boyle et al. 2014). Nevertheless, while provisioned food imparted negative impacts to natural ibis nesters through reduced recruitment, it has the potential to provide important benefits to the urban breeders, as below.

Breeding in Urban Environments

There are multiple factors that can motivate avian species to breed in urban environments, including availability of alternative habitat following natural habitat loss. However, food availability has been repeatedly demonstrated to be a key driver of urban nesting (Leston and Rodewald 2006; Chamberlain et al. 2009). The effects of urban food may not all be positive. Previous studies on passerines have shown that urbanization can result in reduced productivities (Chamberlain et al. 2009). This may present an ecological trap, since increased predation can be an issue within urban communities (Remes 2003; Robb et al. 2008). In fact, predation tends to be a problem to urban breeders, not as a result of the abundance of predators, but because of their increased predation rates (Chamberlain et al. 2009). Waterbirds are a group that has shown high tolerance to urbanization compared to other avian groups (Evans and Gawlik 2020).

Some species are already common breeders in urban landscapes, yet their reproductive productivity and the factors promoting their success are often poorly understood. Well-studied urban wading birds include the Australian Ibis (*Threskiornis molucca*) and the European White Stork (*Ciconia ciconia*). Some populations of these two species are well-adapted to urban environments, consume large amounts of anthropogenic food, and show increased reproductive productivity, specifically higher fledgling rates and larger clutch sizes, than their natural conspecifics (Corben and Munro 2006; Massemin-Challet et al. 2006; Djerdali et al. 2016). Furthermore, Wood Storks have already established urban residency, which was likely influenced by the abundance of anthropogenic food (Picardi et al. 2020). In fact, Evans and Gawlik (2020) found that, unlike natural Wood Stork colonies, urban rookeries were able to remain productive during years of poor hydrological conditions for foraging in natural wetlands by incorporating more anthropogenic food to their diets. Similarly, access to an abundant, reliable, and readily available source of food may be an advantage that ibises may experience from a diet rich in human-processed food, which has the potential to increase ibis productivity by allowing it to become less dependent on the extremely variable foraging conditions inherent to natural habitats. The suitability of urban habitats for avian reproduction seems to be highly dependent on the species, with some birds experiencing lower reproductive success due to increased predation and disturbance and others thriving in this new environment, likely as a result of the increased and continuous food supply. However, increased consumption of anthropogenic food may impart important trade-offs, such as a decrease in nestling growth rates (Heiss et al. 2009).

Urban colonies have not been extensively studied in waterbirds (Evans and Gawlik 2020). However, the trends demonstrated by Australian Ibises, European White Storks, and Wood Storks led us to believe that the White Ibis is also a successful urban breeder. The prospective of an increased reproductive output offered by urban colonies is key for the progression of the ibises' adaptation to urban environments. The ability to breed successfully in urban areas would enable this species to ultimately establish urban residency, which would confer them independency from natural environments. These productive urban colonies could subsequently become more numerous, allowing the urban ibis population to grow and expand more rapidly. An increasingly abundant urban population could be problematic. While public perceptions of ibises in south Florida are still positive, this could drastically change if this species becomes a nuisance. For instance, urban wading bird colonies have been repeatedly considered to be a source of human-wildlife conflicts (Telfair et al. 2000; Grant and Watson 1995; Hattori 2009). Examples of nuisance produced by these rookeries include noise, foul odor, and collision hazard to aircraft. With time, ibises could also eventually learn to harass and steal food from people, like the Australian Ibis (Corben and Munro 2006). However, the reproductive success of ibises in urban environments is unknown, and it is possible that this species is not highly productive in these environments or there may be additional factors related to its behavior and reproductive ecology that could be imposing a restriction on suitable anthropogenic sites for breeding. For example, ibises breeding in urban rookeries may still be relying on natural wetlands to forage. In fact, ibises may fly up to 60 km a day in search for foraging sites, allowing individuals breeding in urban colonies located near natural areas to exploit natural wetlands for food. Such specific requirements for breeding could limit the ibises' ability to expand in anthropogenic environments.

Based on the high reproductive productivities shown by urban populations of other similar wading bird species (e.g., Australian Ibis, European White Stork, and Wood Stork), we predicted that urban White Ibis colonies would also have high reproductive success rates. To address the potential for urban ibis colonies to become more common, an urban rookery in Palm Beach County was monitored and its reproductive productivity was compared to colonies in the Everglades. The growth rates of ibis nestlings were also measured to address some of the impacts of urban breeding to nestlings.

METHODS

Study Site

An urban colony located in West Palm Beach, FL, (26.825079, -80.149621) was selected for this study due to its accessibility to researchers. Urban colonies are defined herein as rookeries located within urban-dominated environments and artificial wetlands. The chosen colony sits on three adjacent islands (namely A, B, and C) ranging approximately 478-1312 m^2, surrounded by a golf course community, and located 35 m away from residences (Fig. 2.1). The vegetation consisted primarily of Sabal palms (*Sabal palmetto*), bald cypress (*Taxodium distichum*), willows (*Salix* spp.), Brazilian pepper (*Schinus terebinthifolia*), and grass. The site is known to attract hundreds of breeding water birds including White Ibises, Glossy Ibises (*Plegadis falcinellus*), Great Egrets (*Ardea alba*), Great Blue Herons (*Ardea herodias*), Snowy Egrets (*Egretta thula*), Little Blue Herons (*Egretta caerulea*), Tricolored Herons (*Egretta tricolor*), Black-crowned Night Herons (*Nycticorax nycticorax*), Anhingas (*Anhinga anhinga*), Double-crested Cormorants (*Phalacrocorax auritus*), Common Gallinules (*Gallinula galeata*), and Egyptian Geese (*Alopochen aegyptiaca*). White Ibises and Great Egrets are the two most abundant species nesting at this location, both with nesting pairs reaching between 100 – 200 annually.

Common predators or scavengers of nest contents that are found at this location include alligators, Boat-tailed Grackles (*Quiscalus major*; which also nest there), Fish Crows (*Corvus ossifragus*), Blue Jays (*Cyanocitta cristata*), rats, and raptors such as Red-shouldered Hawks (*Buteo lineatus*). The rookery suffers from continuous human disturbance ranging from loud chatter from golf players and nearby residents to landscaping activities that circulated daily around the golf course. Additionally, the islands are also littered with golf balls given its proximity to one of the golf holes.

Data Collection

The urban rookery was monitored for approximately 12 weeks in 2020 and to address between-year variation, repeated in 2021. The site was accessed with paddleboards and visits were conducted at dawn to minimize heat stress to the nestlings after the parents flushed. Monitoring commenced 7 days after the first few nesting pairs were observed courting on the islands, allowing enough time for nest building and the first egg to be laid (Bildstein 1993; Heath et al. 2020). Visits to each island were conducted once weekly in 2020, taking at least a 48-h break between each visitation to minimize disturbance. This allowed us to collect sufficient data because ibises bred relatively asynchronously in 2020. In contrast, in 2021, due to the high number of nests initiating simultaneously, breaks between visits were attempted, but rarely occurred. Instead, multiple visits to the same islands during the same week period were required to allow sufficient time to collect all the data. The following metrics were collected: approximate number of nesting pairs in the colony, clutch size of each monitored nest, number of successful nests (producing at least one fledgling), and fledging rates (number of young fledging per nest). Nestlings were considered fledged at 14 days of age, conforming with the protocol established for monitoring the colonies in the Everglades (Frederick, personal communication). The number of nesting pairs and successful nests were approximated via nest checks during visits to the colony using extension ladders that could reach the average height of the trees and by observing the three islands from a vantage point on a nearby street (35 m from the colony) with binoculars. A total of 64 and 67 nests were monitored in 2020 and 2021 respectively from egg laying until either fledging or failure was registered. Target sample sizes were determined based on the stabilization of among-nest variation in natural colonies, starting at n=60 nests (Frederick, personal communication). Whenever possible, trail cameras were placed at locations where one or more nests could be monitored to obtain additional information on causes of mortalities and nest failures. Single pictures were collected using 5-minute detection intervals. The presence of predators (e.g., grackles, crows, rats) and sources of anthropogenic disturbance were also noted during visits.

Nest success, clutch sizes, and fledging rates recorded at the urban colony were compared with results from natural rookeries collected during the same period. Data from natural colonies were collected as part of annual ongoing research conducted by Dr. Peter Frederick. Productivity in the Everglades is measured annually via ground-based observations and most recently, drones (Frederick, personal communication). Nest success was expressed as both apparent (nests with fledged young/nest starts) and as pro-rated for days observed (Mayfield 1961, 1975). Nest success was analyzed according to two distinct definitions. The first defined it as the probability that a given nesting pair would produce hatchlings (i.e., that they will lay at least one egg and it will hatch). The second was the probability that a given nest would produce at least one fledgling. Nest success was also used to assess between-year variation. The effect of habitat type (natural vs. urban) on nest failure was analyzed as a function of time using a logistic exposure model (Shaffer 2004). Habitat type was set as a Boolean variable, where Natural = 0 and Urban = 1. Only the failed nests were used for this analysis in order to compare the probability p that a nest will fail at a given time x in each habitat (1 = Urban, 0 = Natural). Mathematically,

$$p(x) = \frac{e^{\beta_0+\beta_1}}{1+e^{\beta_0+\beta_1}}$$

where β_0 and β_1 are the coefficients computed through the logistic regression. The R package lme4 was used to compute β_0 and β_1 for both 2020 and 2021 data sets (Bates et al. 2015). Differences in clutch sizes from urban and natural rookeries were analyzed with a two-sample t-test.

From the total nests monitored, 36 and 41 chicks in 2020 and 2021 respectively were directly handled and banded. Chicks were captured by hand once a week immediately after hatching until recapture was no longer possible. Ibis nestlings quickly become highly mobile, with capture rates significantly decreasing after their second week of age. For this reason, chicks older than 25 days could not be captured. Age was determined based on size, feather composition and coloration, and bill markings (Bildstein 1993). A total of 39 nestlings from two colonies located in Water Conservation Area 3A (WCA 3A), namely Alley North (26.191096, -80.523351) and Hidden (25.776278, -80.840643), were also handled in 2021. These colonies are approximately 50,000 m^2 and 25,000 m^2 in area and are known to attract up to 50,000 and 5,000 breeding ibis pairs each year, respectively. Vegetation in Alley North is dominated by willows (*Salix* spp.) and the island is surrounded by sawgrass (*Cladium jamaicense*) and cattail (*Typha latifolia*); while Hidden is composed of bald cypress trees (*Taxodium distichum*) and cocoplums (*Chrysobalanus icaco*) and surrounded by forested swamp. Rookeries in the Everglades were accessed with airboats and visits were conducted for no longer than one hour. The first-hatched nestling of each nest was selected for handling due to its higher probability of fledging and to ensure consistency (Kushlan 1977). A temporary expandable plastic band and a non-toxic water-based dye were used to mark chicks until they were large enough to fit a permanent band. Standard avian morphometric measurements, weight, and body condition scores were collected from each chick. Measurements (weight, wing chord, culmen length, and tarsus width and length) were used to construct growth curves. Body condition scores were determined following two methods. In the first, the pectoral muscle mass was subjectively scored from 1 (emaciated) to 5 (obese). The second was calculated using a scaled mass index by regressing the ratio between a standard morphometric measurement (e.g., tarsus length) and body mass. Tarsus length was chosen since it was the measurement previously observed to show the strongest correlation with mass in ibis (Murray et al. 2018).

In order to compare weight and morphometric measurements of Everglades and urban nestlings, a trendline was created for each set of data (Table 2.3). All parameters except for tarsus width can be accurately ($R^2 > 0.9$) represented by straight lines for nestlings aged between 1 to 23 days (age range for which data for both urban and natural birds could be collected). However, tarsus width is poorly represented ($R^2 < 0.9$) by polynomial functions. Because of this, a logarithmic function was used to fit the tarsus width data. While the estimated daily difference in various parameters between natural and urban ibises can be mathematically quantified by taking the difference between each habitat type’s trendline, in order to compute an overall average difference, two methods were used:

1. Computing the average of the difference between daily discrete data points
2. Computing the difference of the average trend line values (Table 2.3)

To obtain the latter, computing the average value f_{avg} of a continuous function $f(x)$ on a closed interval $[a, b]$ is required and can be done as follows:

$$f_{avg} = \frac{1}{b-a}\int_{a}^{b} f(x)dx$$

which, for straight lines in the form of $f(x) = mx + q$, where m is the slope of the line and q is the y-intercept, simplifies to:

$$f_{avg,line} = \frac{m}{2}(a+b) + q$$

For a logarithmic function in the form $f(x) = k \ln x + h$, where k is a constant multiple and h is the y-intercept, the average simplifies to:

$$f_{avg,log} = (h-k) + \frac{k}{b-a}\ln\left(\frac{b^b}{a^a}\right)$$

provided that $a, b, x > 0$. It should be noted that while using the natural logarithm, i.e., the logarithmic function with base e (Euler's number), any logarithmic base can be readily changed with the so-called change of base formula $\log_b x = \frac{\log_d x}{\log_d b}$, where b and d are the old and new bases, respectively, leading to a change in the constant multiple k.

Although visits were initially planned to take place once a week for each island, because of the numerous activities performed during each visit, each of the three islands was often visited more than once a week. The time spent on site was also a concern once it was not possible to adhere to the one-hour limit established for monitoring colonies in the Everglades. Instead, visits lasted approximately two hours. Because the impacts of researchers could not be quantified, it was not possible to measure if the prolonged presence of researchers at the urban colony compared with the Everglades colonies affected productivity through increased nest abandonments and predation, for example. However, avian behavior in response to researchers was noted and compared with their response to routine human disturbance. Visits were immediately interrupted if predators were observed harassing nestling or stealing nest contents from any wading bird species nesting in the colony.

In 2021, in an attempt to reduce predation from grackles and crows, food was supplemented to those species on a residential lawn approximately 35 m from the islands to distract them during each visit. Permission was obtained from one of the residents to place three plates with wet dog food and bread along the shore on his backyard. The grackles' response to the supplemented food was noted and recorded with a trail camera.

RESULTS

Reproductive Productivity

The urban White Ibis colony in Palm Beach, FL was the breeding site of approximately 100 and 150 ibis nesting pairs in 2020 and 2021, respectively (Table 2.1). Counting the total number of nesting pairs was challenging because many of the nests were built in cryptic locations that were often out of sight from the ground (e.g., on the top of the palm trees where they were hidden within the leaves). The number of nesting pairs reported is therefore an estimation based on what could be observed from within the islands and from the street. A total of 64 ibis nests were monitored between April 15 and July 15 in 2020 and 67 nests between April 8 and June 10 in 2021. Sample sizes were based on the minimum of n=60 target and depended on the number of nests that were within reach and could be monitored. Nest success results from the urban and natural colonies were compiled on Tables 2.1 and 2.2. Clutch sizes from urban and natural colonies were significantly different overall (*P*-value = 0.01) and in 2021

(*P*-value = 0.008), but not in 2020 (*P*-value = 0.2). Significant differences between years were not detected in the clutch sizes of the urban colony (*P*-value = 0.5217).

The coefficients computed in the logistic exposure model indicate that nest failures generally occurred earlier in the nesting period in natural environments, while they occurred later at the urban colony (2020: β_0 = -1.79043 ± 0.56714 SE; 2021: β_0 = -1.97500 ± 0.49525 SE) and that for a randomly selected nest that will fail, its failure is overall far more likely to occur in natural rookeries (2020: β_1 = 0.02656 ± 0.02818 SE, *P*-value = 0.002; 2021: β_1 = 0.01941 ± 0.02443 SE, *P*-value < 0.001). While the same trend can be observed for the coefficients computed for both datasets from 2020 and 2021, the effect is greater when comparing failed nests in 2021 than in 2020. In other words, failed nests were more likely to be located in the natural environment than the urban environment particularly in 2021.

Ibises built over 70% of their nests on palm trees on the periphery of the islands at the urban colony, resulting in the nest distributions depicted in Figure 2.3. The use of the islands differed between years: Island C was mostly unoccupied in 2020, but intensely used in 2021. The synchrony of urban nesting during the breeding seasons of 2020 and 2021 varied: while the arrival of nesting pairs was more evenly distributed throughout the 2020 season, urban ibises nested more synchronously in 2021, with most of the pairs arriving in April and some late nesters coming in late May (Fig. 2.2).

Growth Rates

Recapturing nestlings to collect morphometric measurements was challenging after they reached 2 weeks of age due to their increased mobility. The oldest age at which nestlings were captured were 23 and 25 days for natural and urban colonies, respectively. Therefore, data were usually not collected more than three times for the same individual. Tables 2.4 and 2.5 provide the capture rates of the nestlings and how each age group was represented in our dataset. Recaptures at the natural colony were particularly problematic. In addition to the denser vegetation that helped the birds escape and hide, the nesting period was largely synchronous, particularly at Hidden, which meant that after we finished sampling a group of nestlings, there were no hatchlings available for a new group to be sampled from start to finish. Alley North also became problematic once the dry conditions precluded our access towards the end of the nesting season. As a result, 56% of individual nestlings sampled in the Everglades did not have serial data. Nevertheless, the growth curves constructed demonstrate the lack of a significant difference between the rate of growth of urban and natural birds (Fig. 2.4). The trendlines showed mean differences within 1-3% in rate (Table 2.6). Tarsus width diverged from this trend, showing higher percent differences in the growth rates of urban and natural nestlings. Urban and natural chicks also had similar subjective body condition scores, a mean of 2.12 ± 0.357 and 2.13 ± 0.551 overall, respectively. Scores calculated using the tarsus length to body mass ratio were negatively correlated to age and did not differ between habitat types (Fig. 2.5).

Disturbance

Intense human disturbance was a constant for birds nesting at this urban colony. The daily routine around the site consisted of golf course landscaping from 7-8 AM, a large pond aerator that sits approximately 30 m from Island C that would be turned on at around 8 AM, loud chatter from residents and golfers, transit of golf carts from 8 AM until sunset, and other less frequent activities including: cleaning of the water canal, people fishing by the shore, garbage removal, traffic from the residential area (35 m away from the colony), etc. Overall, the avifauna of the islands did not seem to be affected by daily human disturbances and would continue exhibiting normal behaviors, as if they were accustomed to these activities. However, unusual sources of disturbance provoked a noticeable reaction. For instance, during one of our visits, trains were heard in the background and the noise was loud enough to startle the birds. The rookery is also subject to interference from both international and regional annual golf tournaments, which may have been responsible for nest abandonments in 2021. The nests that failed were located on Island A, located closest to the nearest golf green, and the failures were registered while the tournament was ongoing. Golf balls are also an issue at this site, and they were often found scattered on the ground of all three islands.

Although the birds seemed to be highly habituated to human disturbance, they consistently responded to the presence of researchers. The use of paddleboards was less noisy than the airboats used to access the colonies in the Everglades; however adult birds would still flush upon our arrival. Some would remain to protect their nests and those that did flush would mostly settle on the canopies or on the other islands to wait for our team to leave. Most birds would immediately return to their nests upon our departure. During six of our 30 visits in 2021, nestlings fell from their nests while attempting to escape and hide, which demanded that we prolonged our stay on site to catch and return these chicks. In fact, our visits to the islands surpassed the 1 h weekly protocol established for the natural sites due to routine time-consuming activities such as handling the extension ladders and nestlings. To manage these activities, daily visits of approximately 2 h were necessary, particularly in 2021, when the ibises nested more synchronously. No differences were observed in avian behavior in response to researchers between 2020 and 2021. Boat-tailed Grackles and Fish Crows were observed stealing eggs, harassing nestlings, and scavenging nests in 5 out of 66 total visits (2020 and 2021 combined). Rats were also seen, however active predation or scavenging of ibis nests were not observed.

Food Supplementation

Boat-tailed Grackles were observed to readily respond to the supplemented food and reduce their presence on the islands when food was provided. These birds regularly nest at this site, and thus they were continuously found circulating around the area, however without the supplemented food, they were sometimes observed scavenging nest contents. While the grackles were the first to respond to the food and would eat it entirely, it soon started to attract other species, including Egyptian Geese, Common Gallinules, and even ibises. Grackles would still get the advantage of being the first to arrive and they were not easily intimidated by the other species, which possibly allowed for the success of this strategy.

Potential Sources of Nest Attempt Failures

There were a few other sources of nesting failures observed during this study, namely storms, predation, disease, and poor nest-building skills by breeding ibises. Although ibises select the dry season for breeding, storms were not uncommon, particularly during the season of 2020. A higher number of failures was sometimes observed following intense storms (Fig. 2.6). Nests located in the area were found on the ground and the surrounding vegetation was damaged. In one of such occasions, a healthy nestling was found on the ground during a visit that followed a windy evening, along with its entire nest and dead sibling.

Ibises built nests on high palm trees, which required additional equipment to appropriately secure and angle camera traps over the nests. We determined that setting the equipment over such a large number of nests and regularly monitoring the cameras would have demanded too much time and interfered with the collection of data from our primary objective (i.e., check nests and sample nestlings). For this reason, there were only a few nests that could be monitored with a trail camera. Yet, the records obtained revealed important information about predation. The cameras recorded rats dragging nestlings out of their nests, Blue Jays visiting nests, and a Red-shouldered Hawk predating on one of the nestlings. Distinguishing between predation and abandonment events upon finding an empty nest was challenging, since some predators (specially rats and snakes) are very cryptic and rarely leave signs of predation, as observed through camera trap records. Another factor that could be mistaken as abandonment is disease. During the season of 2020, three nestling mortalities due to West Nile virus were documented (see Chapter 3 for further detail). This event accounted for at least two nest failures. Lastly, a few nests (n=3 overall) were notably poorly built and readily failed.

DISCUSSION

The White Ibis is a species that is increasingly showing resilience to drastic habitat changes such as those influenced by land use changes in south Florida. Despite having specific requirements to breed (i.e., tree islands surrounded by water and alligators), which impose a restriction on suitable habitats for breeding, they have already established a few colonies within urban areas of south Florida. While breeding in these environments may be challenging because these birds must tolerate higher levels of anthropogenic disturbance, urban areas may provide several benefits, such as facilitated access to continuous and abundant food sources (e.g., handouts from the public, landfills, etc.). This study was designed to examine the suitability of urban environments for ibis breeding by comparing the reproductive productivity of an urban colony in Palm Beach, FL to natural rookeries in the Everglades. Despite being subject to high levels of both routine human and research-imposed disturbance, the urban colony exhibited relatively high nest success rates in the two years of data collection (Table 2.2).

There are multiple factors that could have contributed to the high success of this urban colony, most notably the access to a continuous supply of food, which could have conferred the ibises independency from the hydrological cycles for foraging, and a higher resilience to disturbance inherent to urban birds that could have led to fewer nest abandonments. Increased food abundance can provide additional benefits to nestlings, including decreased susceptibility to disease. In fact, consistent adequate nutrition is a factor in improved immune function defense in birds (Lochmiller et al. 1993; Becker and Hall 2014). As a result, fewer mortalities due to disease would occur compared with interrupted access to food. The exploitation of anthropogenic food sources has been previously correlated to a higher reproductive success in urban populations of other similar species such as the Australian Ibis and the European White Stork (Corben and Munro 2006; Massemin-Challet 2006). Further research is needed to analyze the role of diet on urban ibis productivity, particularly whether nestlings are consuming anthropogenic food that could perhaps justify its high nest success. In a separate study, and to determine whether our hypothesis about resource availability may play a role in nest success, we investigated the diet of urban ibis and nestlings through stable isotope analyses.

We failed to detect a difference in the growth rates and body condition scores of urban and natural nestlings (Tables 2.3 and 2.6, Figs. 2.4 and 2.5). Differences in tarsus width were more pronounced, likely an artifact due to significant variation among the different people collecting measurements since measuring this metric was more prone to subjective error. While it is possible that urban and natural chicks had similar growth rates overall, our sample size of natural nestlings was insufficient to make any conclusive statements. In addition to the difficulty in acquiring serial data for the natural nestlings, we were only able to visit the Everglades in 2021 due to the SARS-CoV-2 global pandemic that canceled our visits in 2020. It is recommended that larger sample sizes are collected over multiple years and across multiple colonies to yield more significant results. Nevertheless, the urban colony was able to produce almost 2 fledglings per nest, suggesting that urban chicks are able to grow enough to leave the nest and become independent. An explanation for potentially similar growth rates between urban and natural chicks lies on a perhaps underestimated complexity of their diet. Although we hypothesized that adult urban ibises would largely exploit anthropogenic sources when feeding their young, it is possible that they will still manage to provide them with a balanced diet. In addition to food from landfills, garbage, or handouts from the public, urban ibises do forage in anthropogenic areas for terrestrial invertebrates. A mixed diet composed of processed and natural food, such as fish and invertebrates, would likely allow these birds to provide their nestlings with the calories and macronutrients necessary for growth. Additionally, the distance of the urban rookery from the nearest available foraging natural site is 2 km, which is well within the distance that ibis have been known to fly to acquire food for nestlings in previous studies (Frederick et al. 2009). In fact, a recent study on Wood Storks provided evidence that urban breeders provide a diverse diet to their nestlings: a combination of natural and anthropogenic food (Evans and Gawlik 2020). This strategy allowed these birds to keep their nestlings fed under periods where preferred natural food was scarce. As a result, urban Wood Stork colonies were able to remain productive during periods of poor hydrological conditions, while the productivity of natural rookeries was negatively impacted. Similarly, the study failed to detect a significant difference in the body condition of urban and natural nestlings.

Ibises will nest on multiple types of vegetation, but they will select for higher locations for nest building, usually over water (Williams et al. 2008; Heath et al. 2020). There was abundant above ground habitat for nesting wading birds at the urban colony and low vegetation was scarce. Great Egrets nested at high numbers at this site, and they are known for initiating nesting earlier than the ibises (Frederick and Collopy 1989b). When Great Egrets arrived at the urban colony, they largely occupied the center of the islands. Consequently, the remaining nesting habitat available to ibises was mostly restricted to the periphery of the islands, dominated by palm trees. Additionally, the center of the islands did not have dense vegetation, providing limited habitat for nesting. These factors, in addition to their preference to nest over water, likely explain the nest distribution depicted in Figure 2.3. However, it is still unclear why ibises largely avoided Island C in 2020. Precipitation averaged 163 mm during the breeding season of 2020, which included more frequent storms, while in 2021 it averaged 100 mm (National Centers for Environmental Information 2021). A few episodes of collective nest failures were observed following severe storms, particularly in 2020 (Fig. 2.6). In fact, increased rainfall is an important factor influencing nest success through increased nest abandonments and nestling mortalities. Younger nestlings, i.e., hatchlings, are very vulnerable to harsh weather conditions, as they are more susceptible to developing hypothermia. Additionally, parents may decide to abandon their nest if they deem that the increased rainfall may affect their ability to find food (Semones 2003). Multiple nests were usually found on the ground after a severe storm, in addition to both dead and live nestlings.

The impact of predation was not measured in this study, and it therefore cannot be compared between years and habitat type. However, records from personal observations and trail cameras confirmed the presence of Boat-tailed Grackles, Fish Crows, and Blue Jays, in addition to predation by rats and Red-shouldered Hawks at the urban colony. The alligators at this site seem to offer limited protection against terrestrial predators since, when the water levels of the canal decrease, the islands get temporarily connected to the golf course, providing opportunities for predators to cross. In fact, rats were frequently observed visiting nests throughout the day and based on the trail camera information, were likely an important source of nestling mortality. Another potential source of nest attempt failure and nestling mortality is the inexperience of young parents, which can lead to poor nest building skills and the inability to appropriately feed their young (Sydeman et al. 1991; Nisbet et al. 2002). In fact, we noticed a few (n=3) nests with an extremely fragile structure that were built at unstable locations. High nest densities could lead to increased aggression and stealing of nesting material among breeding ibises, which also yields fragile nests. The failure of these three fragile nests monitored was more likely due to inexperienced builders since there was ample habitat available for ibises to nest at the urban colony.

Although the urban colony was subject to high levels of human disturbance, high reproductive success rates were reported for this site, suggesting that these birds are extremely habituated to anthropogenic disturbance. Unusual sources of human interference would still disturb these birds, such as noises from trains, however they were well-accustomed to the routine of the golf course community. Although we could not quantify whether research-imposed interference influenced our results, because of the high resilience exhibited by these birds, it is likely that we did not produce a pronounced impact on their productivity. Nevertheless, it should still be acknowledged as a potential source of failure of nest initiation and nesting attempt.

Our attempt to reduce nest predation by urban avian predators seemed successful. The data collected for this experiment relied solely on qualitative observations, thus we cannot attest to its efficacy with confidence. However, Boat-tailed Grackles were observed to respond immediately to the food offered. Subsequently, we observed fewer individuals harassing nestlings and scavenging nests during our visits to the urban colony. In fact, supplementary feeding avian predators has been previously shown to reduce predation of avian prey (Redpath et al. 2002). While further studies are needed to quantify the success rates of this strategy, it should be considered as an alternative in studies where predation pressures from opportunistic predators are high upon interference by researchers. Supplemental feeding is a common technique in wildlife management and research, yet it can have important negative consequences such as increasing aggression, altering behavior and movement, and habituating wildlife (Smith et al. 2010; DuBois and Fraser 2013). This type of management should be carefully monitored, and only the minimum necessary amount of food should be provided to avoid causing further negative impacts to the local avian community, such as attracting more predators to the site.

Conclusions and Future Directions

Continuous monitoring of this and additional urban sites is necessary to understand the range of tradeoffs to ibis for breeding in urban environments. Despite the high levels of researcher-imposed disturbance in this study, the urban ibis colony monitored showed high productivity during the two years monitored, however its success rates might be even higher in the absence of researchers. The results presented herein indicate that such rookeries have the potential to be as productive as natural colonies. High levels of habituation to human disturbance seem to be the minimum criteria required for successful nesting attempts at such colonies. The reliance on a continuously abundant source of food is likely the primary driver of the success of these urban colonies. Further studies are needed to address this hypothesis, however, like in urban Wood Stork colonies, a reduced dependence on hydrological conditions for foraging likely support high reproductive productivities for the ibis as well (Evans and Gawlik 2020). Reduced predation is also a potential factor that could be contributing to the high productivity of the urban colony.

The drivers of the high reproductive success registered at the urban colony have yet to be investigated. It is possible that urban ibis breeders are still relying on natural wetlands to forage for example, which would impose a limitation on suitable urban habitats for breeding. However, since urban colonies can be highly productive, they have the potential to eventually become larger and more numerous. They may then act as buffers by offering reliable annual recruitment, making them an additional resource for wading birds in Florida that may contribute to the long-term population stability of the ibis in the future. The potential increasing exploitation of urban environments along with a possible progression in habituation could culminate in the establishment of this species as a nuisance. In fact, other urban wading bird colonies tend to be viewed negatively by the public as a result of the noise, foul smell, waste, and a perceived or real risk for disease spillover, and safety concerns if located near airports. Some colonies housing species such as Cattle Egrets (*Bubulcus ibis*), Little Blue Herons, Snowy Egrets, Great Egrets, Black-crowned Night Herons are already considered a nuisance across multiple US states (Telfair et al. 2000; Grant and Watson 1995; Hattori 2009). Additionally, urban Australian Ibis populations are disliked by the public largely due to their intense scavenging of refuse and their abundant urban colonies. The establishment of the ibis as a nuisance species would likely require management of urban populations, especially their rookeries. Collecting information on the productivity of urban rookeries and the factors that influence it is paramount to inform potential future decisions.

Table 2.1. Summary of the reproductive success of an urban White Ibis (*Eudocimus albus*) colony in West Palm Beach, FL and natural colonies in the Everglades in 2020 and 2021.

Year	**Habitat**	**Colony**	**Estimated Nesting Pairs**	**Number of Nests Monitored**	**Apparent Nest Success (%)**	**Mayfield Nest Success (%)**	**Daily Probability of Survival until**		**Mean Clutch Size**	**Mean Fledging Rate**
							Hatching	**Fledging***		
2020	Urban	Golf Course	100	64	62.7	70.8	0.991	0.989	2.52 ± 0.707	1.98 ± 0.636
	Natural	Tamiami West	500	108	40.7	34.8	0.965	0.979	2.56 ± 0.592	2.11 ± 0.729
	Natural	6th Bridge	9,500	75	70.7	73.7	0.990	0.994	2.28 ± 0.758	1.82 ± 0.601
2021	Urban	Golf Course	150	67	73.1	76.4	0.994	0.990	2.52 ± 0.699	1.86 ± 0.606
	Natural	Hidden	3,000	112	70.5	77.0	0.993	0.991	2.62 ± 0.586	1.51 ± 0.675
	Natural	Alley North	30,000	160	60.6	55.1	0.974	0.996	2.10 ± 0.485	1.69 ± 0.506

*All numbers based on nests that survived past the incubation period.

Table 2.2. Summary of the productivity of an urban White Ibis (*Eudocimus albus*) colony monitored in 2020-21 and the previously reported nest success of natural wetland ibis rookeries from 1972 – 2021.

Study	**Year**	**Habitat**	**Location**	**Estimated Nesting Pairs**	**Mayfield Nest Success (%)**	**Mean Clutch Size**
Kushlan 1977	1972-73	Natural	Interior Everglades			2.45 ± 0.670
Rudegeair 1975	1973-74	Natural	Interior Central Florida			2.53
Frederick and Collopy 1988	1986	Natural	Interior Everglades		20.4	2.72 ± 0.534
Frederick and Collopy 1988	1987	Natural	Interior Everglades		48.9	2.53 ± 0.527
Frederick 1995	1989	Natural	Lake Okeechobee		40.5	2.70 ± 0.480
Frederick 1995	1990	Natural	Lake Okeechobee		47.8	2.60 ± 0.620
Frederick 1995	1991	Natural	Lake Okeechobee		34	2.50 ± 0.570
Frederick 1995	1992	Natural	WCA 3		16	2.24 ± 0.572
Frederick 1995	1995	Natural	WCA 3		12.6	2.36 ± 0.771

Semones 2003	2001	Natural	Interior Everglades	17,162	5.4	2.50 ± 0.601
Semones 2003	2002	Natural	Interior Everglades	31,580	44.5	2.56 ± 0.650
Crozier and Gawlik 2003b	2003	Natural	Interior Everglades	12,383	17.3	
Cook and Kobza 2009	2009	Natural	Interior Everglades	43,415	95	
Cook and Kobza 2010	2010	Natural	Interior Everglades	9,047	75	
Cook and Kobza 2011	2011	Natural	Interior Everglades	13,599	43	
Cook and Kobza 2012	2012	Natural	Interior Everglades	7,972	30.7	
Cook 2013	2013	Natural	Interior Everglades	22,226	19.2	
Cook 2014	2014	Natural	Interior Everglades	13,485	48.1	
Cook 2016	2015	Natural	Everglades	28,139	36.7	
Cook and Baranski 2017	2016	Natural	Everglades	12,271	47-59	
Cook and Baranski 2018	2017	Natural	Everglades	22,995	67.5	
Cook and Baranski 2019	2018	Natural	Everglades	100,784	47.5	
Cook and Baranski 2020	2019	Natural	Everglades	21,667	19	
This study	**2020**	**Natural**	**WCA 3**	**9,500**	**73.7**	**2.28 ± 0.758**
This study	**2020**	**Natural**	**Northern Everglades National Park**	**500**	**34.8**	**2.56 ± 0.592**
This study	**2020**	**Urban**	**West Palm Beach**	**100**	**70.8**	**2.52 ± 0.707**
This study	**2021**	**Natural**	**WCA 3**	**3,000**	**77.0**	**2.62 ± 0.586**
This study	**2021**	**Natural**	**WCA 3**	**30,000**	**55.1**	**2.10 ± 0.485**
This study	**2021**	**Urban**	**West Palm Beach**	**150**	**76.4**	**2.52 ± 0.699**

Table 2.3. Trendlines calculated from morphometric measurements of natural and urban (2020 and 2021) White Ibis (*Eudocimus albus*) nestlings. Each equation illustrates the relationship between nestling age (x-axis) and growth (y-axis).

Measurement	**Urban 2020**	**Urban 2021**	**Natural 2021**
Weight (g)	30.124x + 3.0075 ($R^2 = 0.9616$)	27.921x + 31.884 ($R^2 = 0.9414$)	27.9620x + 25.434 ($R^2 = 0.9434$)
Culmen Length (mm)	2.3999x + 14.64 ($R^2 = 0.9846$)	2.3496x + 15.713 ($R^2 = 0.9779$)	2.3922x + 16.364 ($R^2 = 0.9612$)
Tarsus Length (mm)	3.0966x + 17.594 ($R^2 = 0.9686$)	2.6058x + 21.697 ($R^2 = 0.9512$)	2.8325x + 20.879 ($R^2 = 0.9543$)
Tarsus Width (mm)	1.5871ln(x) + 3.1181 ($R^2 = 0.7386$)	2.7151ln(x) + 2.3909 ($R^2 = 0.8678$)	1.5285ln(x) + 3.6594 ($R^2 = 0.599$)
Wing Cord (mm)	8.9202x + 2.9697 ($R^2 = 0.9844$)	8.9502x + 6.9437 ($R^2 = 0.988$)	8.9185x + 7.4381 ($R^2 = 0.9785$)

Table 2.4. Percentage of total captures of urban and natural White Ibis (*Eudocimus albus*) nestlings captured per age group to collect weight and morphometric measurements (culmen length, tarsus length, tarsus width, and wing cord).

Habitat	**Year**	**Birds (1-7 days-old) captured (%)***	**Birds (8-14 days-old) captured (%)***	**Birds (15-21 days-old) captured (%)***	**Birds (> 21 days-old) captured (%)***
Urban	2020	39	33	21	7
Urban	2021	39	28	27	6
Natural	2021	24	42	30	4

*Percent from total captures (urban 2020 [n=94], urban 2021 [n=114], natural 2021 [n=67]).

Table 2.5. Capture rates of urban and natural White Ibis (*Eudocimus albus*) nestlings.

Habitat	**Year**	**N**	**Captured Once (%)**	**Captured Twice (%)**	**Captured Three Times (%)**	**Captured Four Times (%)**
Urban	2020	36	17	22	44	17
Urban	2021	41	17	22	39	22
Natural	2021	39	56	18	23	3

Table 2.6. Average percent difference in the growth rates of urban and natural White Ibis (*Eudocimus albus*) nestlings. Each value was calculated using $\frac{natural - urban}{natural} \times 100\%$.

	Weight	**Culmen Length**	**Tarsus Length**	**Tarsus Width**	**Wing Cord**
Average between discrete points (%)	-3.77	1.75	1.10	-9.25	-0.44
Average from trendline (%)	-1.70	2.80	1.70	-7.68	1.90

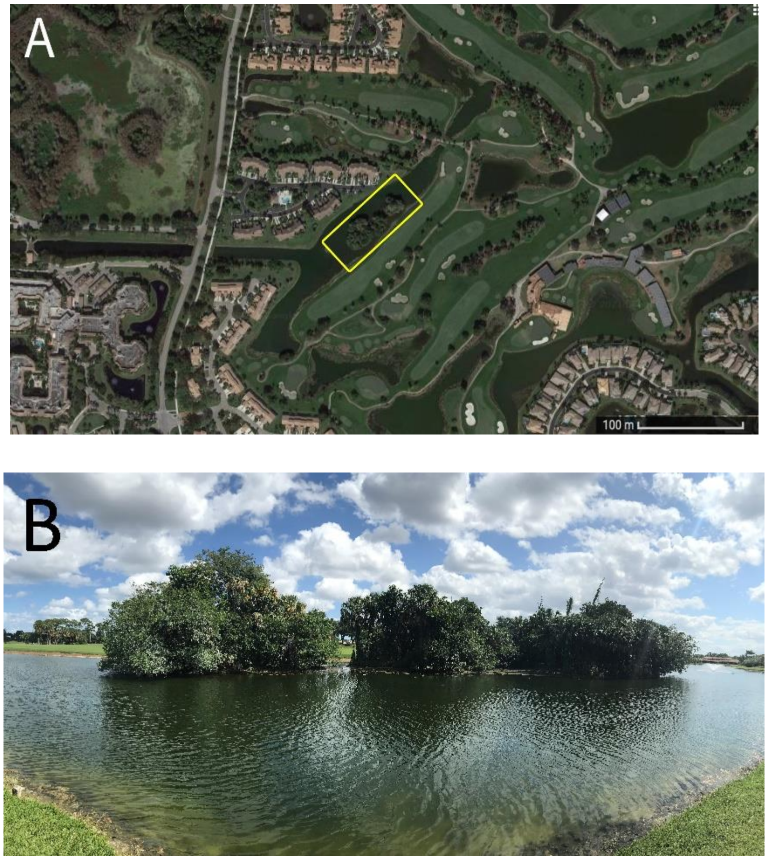

Figure 2.1. Aerial view (A) and front view (B) of an urban White Ibis (*Eudocimus albus*) rookery in West Palm Beach, FL.

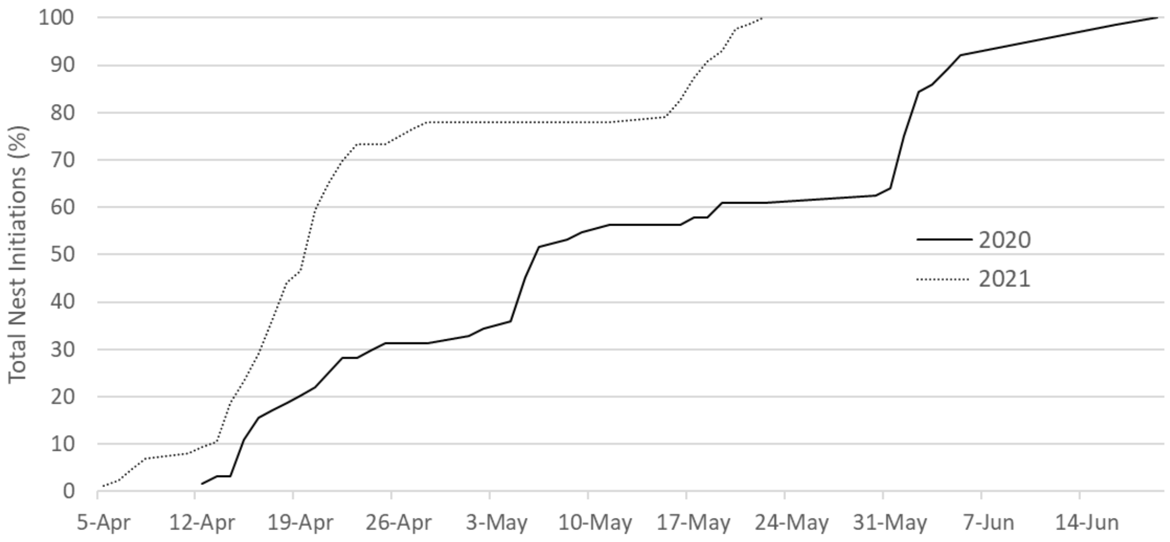

Figure 2.2. Temporal distribution of nest initiations registered at an urban colony of White Ibises (Eu*docimus albus*) in 2020 and 2021. Data for 2021 included 19 nests that were initiated in late May but were not monitored.

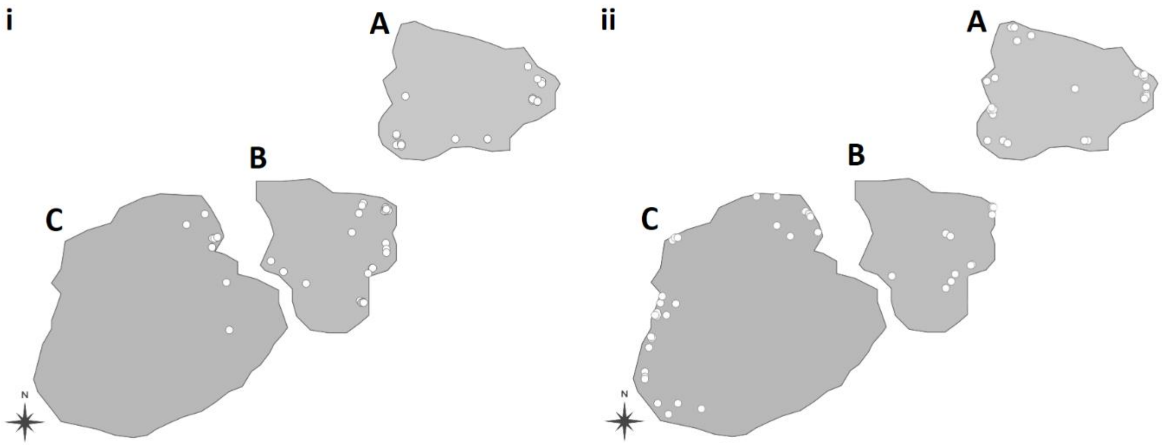

Figure 2.3. The spatial distribution of monitored White Ibis (*Eudocimus albus*) nests on an urban colony comprised of three islands in West Palm Beach, FL varied between 2020 (i) and 2021 (ii). Adjacent islands are labeled A, B, or C for identification.

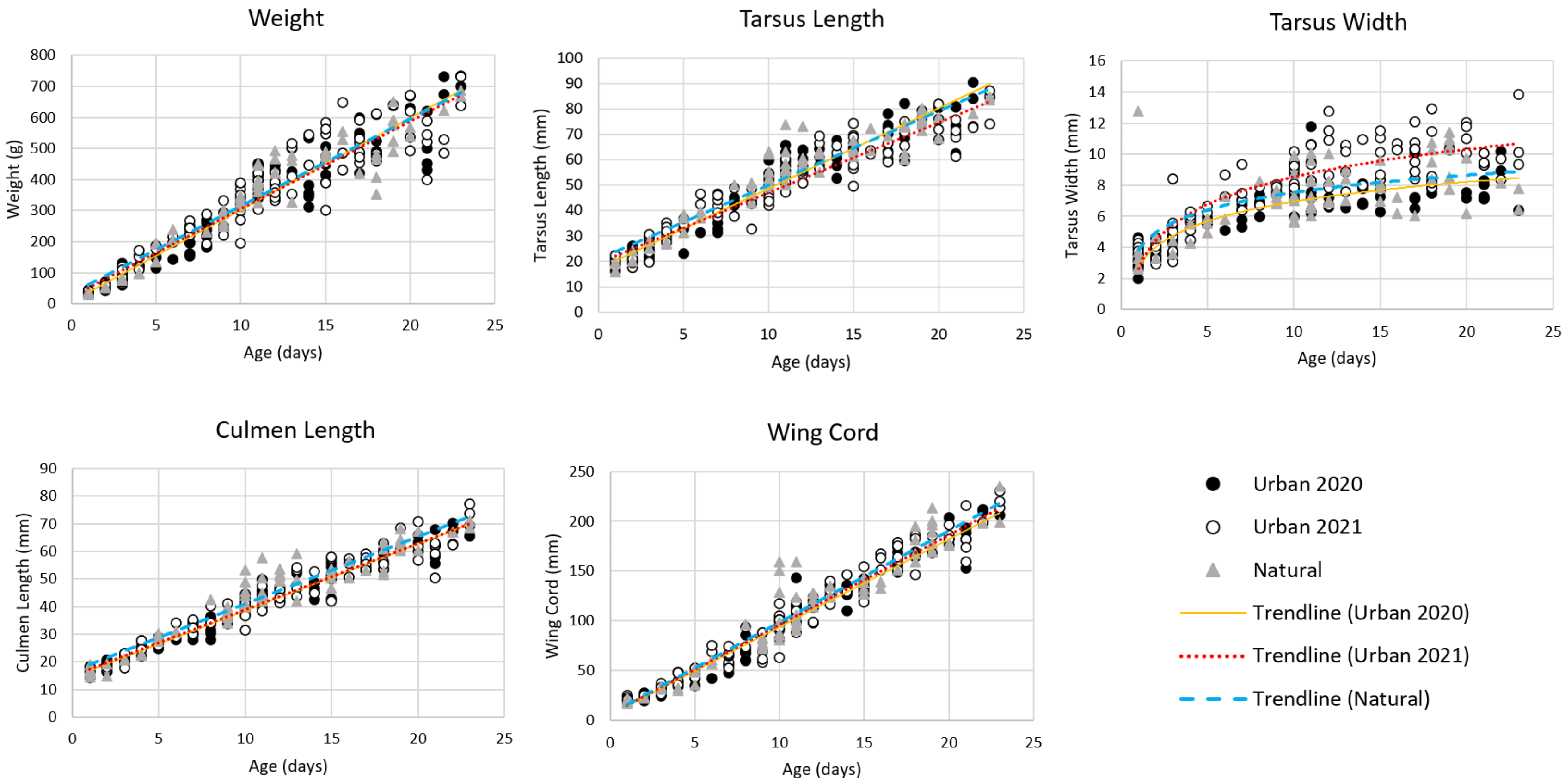

Figure 2.4. The growth curves of different morphometric measurements collected weekly for urban (n=77) and natural (n=39) White Ibis (*Eudocimus albus*) nestlings from hatch until capture was no longer possible (~23 days). Values observed for body weight, culmen length, tarsus length, and wing cord fit under a linear trendline with $R^2 > 0.9$, while a logarithmic trendline fit best for tarsus width ($R^2 > 0.6$).

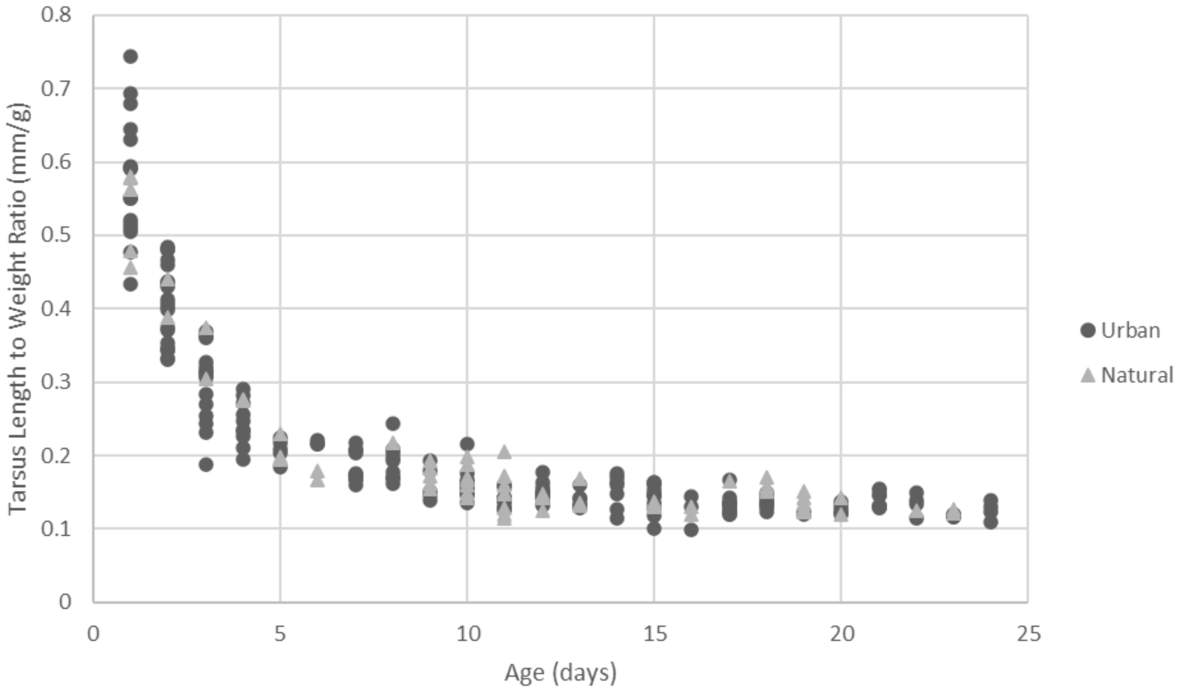

Figure 2.5. Mean body condition calculated by regressing tarsus length to body mass ratio (mm/g) for urban and natural White Ibises (*Eudocimus albus*) in south Florida.

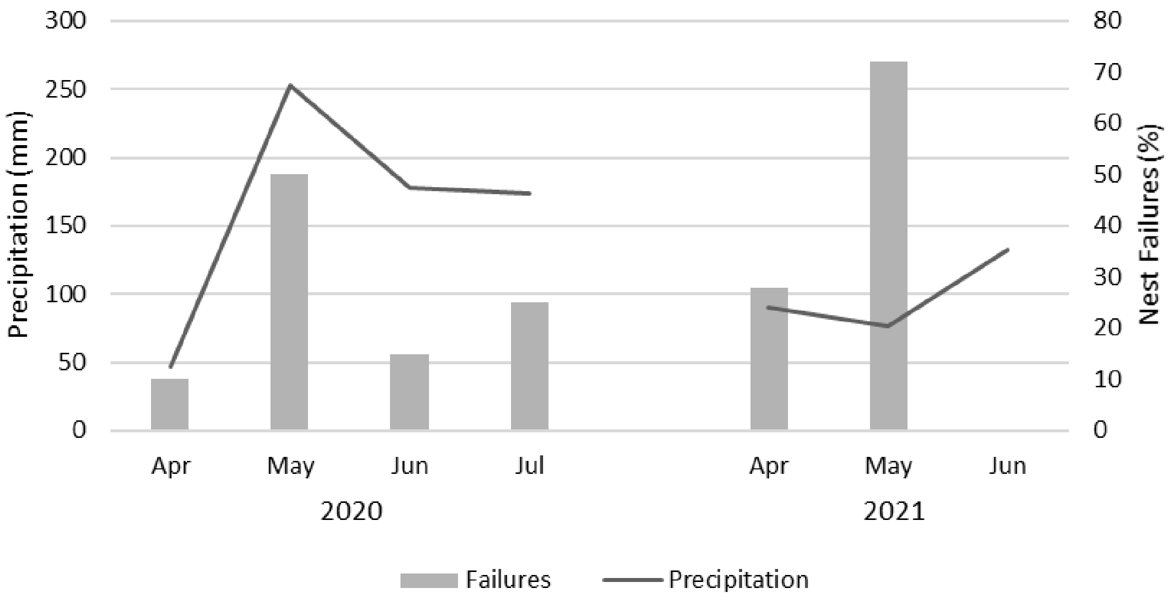

Figure 2.6. Monthly precipitation and failure of nest attempts recorded in 2020 and 2021 at an urban colony of White Ibises (*Eudocimus albus*). Precipitation data was retrieved from the Palm Beach Gardens weather station located at (26.8261, -80.1488).

CHAPTER 3

WEST NILE VIRUS INFECTIONS IN AN URBAN COLONY OF AMERICAN WHITE IBISES (*EUDOCIMUS ALBUS*) IN SOUTH FLORIDA[2]

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[2] Seixas, J. S., Hernandez, S. M., Kunkel, M. R., Weyna, A. A. W., Yabsley, M. J., Shender, L., Nemeth, N. M. Submitted to *Journal of Wildlife Diseases*, 03/03/21.

ABSTRACT

West Nile virus (WNV) is pathogenic in a wide range of avian hosts and is endemic in much of North America. This virus is responsible for population declines of some Passeriformes. We describe a WNV-associated mortality event in White Ibis (*Eudocimus albus*) nestlings. The White Ibis is a species inherent to the Everglades ecosystem that has recently begun nesting in urban areas. An urban ibis colony in south Florida was monitored from March-July in 2020 as part of an ongoing study. Nestling carcasses were collected opportunistically and sent to the Southeastern Cooperative Wildlife Disease Study, University of Georgia within 24 hours for diagnosis. Three ibis mortalities were confirmed to be caused by WNV infection based on histopathology, immunohistochemistry, and reverse transcription polymerase chain reaction. Serial plasma samples collected weekly from 36 healthy chicks of the same urban rookery were tested for WNV-neutralizing antibodies via plaque reduction neutralization test. Antibody titers from three chicks from which serial samples were collected waned over time, suggesting maternal antibody transfer. Ibis mortalities were consistent with a spike of WNV activity in this region of Florida. WNV may be an important seasonal cause of mortality for wading bird nestlings.

BODY

Since its introduction, West Nile virus (WNV; genus *Flavivirus*; family *Flaviviridae*) has become well-established in many North American bird populations and is a significant public health concern. Transmission in temperate regions peaks in the summer and early fall due to increased mosquito vector activity (Apperson et al. 2004). The avian host range of WNV includes > 300 species in the US (Centers for Disease Control and Prevention 2016), yet susceptibility to infection and clinical outcomes varies widely (Pérez-Ramírez et al. 2014). While the potential impacts of WNV in some raptor and passerine species are recognized (Dusek et al. 2010; Kilpatrick and Wheeler 2019), infection in free-ranging waterbirds has not been extensively documented. Nestling mortalities due to WNV have been reported in free-living egrets, herons, and White Pelicans (*Pelecanus erythrorhynchos*; Reisen et al. 2009; Johnson et al. 2010). Conducting nestling mortality investigations in mixed-species colonies is difficult due to reduced accessibility and researcher-imposed limitations such as time spent on the colony (Frederick et al. 1993; Kuiken et al. 1999).

White Ibises (*Eudocimus albus*) in south Florida have increasingly colonized anthropogenic habitats as a response to natural wetland loss (Frederick and McGehee 1994; Hernandez et al. 2016). Commonly reported causes of ibis nestling mortality include predation, starvation, severe weather conditions (e.g., storms), and parental nest abandonment (Adams and Frederick 2009; Heath et al. 2009). The mortality of ibis nestlings was investigated as part of a project on urban rookery productivity. Continuous surveillance of mixed-species colonies, particularly those within high-human densities, for WNV activity is also useful for public health (Johnson et al. 2010). We report a WNV-associated mortality event in White Ibis nestlings and subsequently investigated the exposure of other White Ibis chicks in the rookery to WNV and its relevance to the reproductive productivity of urban colonies and adjacent human populations.

The study site was an urban wading bird rookery in West Palm Beach, FL (26.825079, -80.149621) located on three adjacent islands (ranging in area 478-1312 m^2) situated in a canal that traversed through a golf course community (Fig. 3.1). These islands attract approximately 300 breeding pairs of Ardeids and Phalacrocorcids and between 100-200 pairs of ibises and were monitored from April-July 2020. The colony is also frequented by grackles (*Quiscalus quiscula* and *Quiscalus major*) which predate and scavenge nests. Each island was visited once a week every other day to minimize disturbance. A total of 64 nests were checked during each visit as part of project measuring the productivity of the urban colony. On June 19, two nestlings (10-13 days-old) were found dead (314A) or moribund (314B; died while handling) in a single nest. On July 13, an additional nestling (18 days-old; 359) was found moribund and alone inside its nest (Fig. 3.2). It was transferred to a wildlife hospital and was euthanized. Carcasses were shipped overnight on ice packs within 24 hours to the Southeastern Cooperative Wildlife Disease Study (University of Georgia) for diagnostic evaluation. Necropsy, with preservation of representative tissues from all major organ systems in 10% neutral buffered formalin, immunohistochemistry, standard histological processing, and hematoxylin and eosin staining were performed at the Athens Veterinary Diagnostic Laboratory (Athens, GA). The cause of death of the three nestlings was determined to be WNV infection based on histopathology, immunohistochemistry, and additional ancillary testing (virus isolation or reverse transcription polymerase chain reaction [RT-PCR]). West Nile virus was isolated from pooled tissue samples (heart and/or brain and kidney) from 314B, and WNV RNA was detected by RT-PCR in 314A and 314B. No viruses were isolated from tissues from 314A or 359; and WNV RNA was not detected in tissues from 359. Nestling 314A was in poor nutritional condition, but 314B and 359 were in good nutritional status. All three nestlings had variable lymphoplasmacytic myocarditis with myocardial necrosis; 359 also had fibrinoid necrosis (Fig. 3.3). Nestling 359 also had perivascular, heterophilic nephritis with tubular necrosis, lymphoplasmacytic proventriculitis and perineuritis, hepatocellular lipidosis, and 314A had concurrent lymphoplasmacytic pneumonia. Immunolabeling for WNV antigen was most prominent and abundant in the cytoplasm of cardiomyocytes of 314A and 314B (Fig. 3.4); both nestlings also had occasional labeling in the renal tubular epithelium, and adrenal gland chromaffin cells of 314B. Cardiomyocytes rarely labeled in 359, where occasional macrophages in circulation exhibited cytoplasmic labeling, which was also occasionally in ventricular epithelium (Fig. 3.5). An additional White Ibis (dead) and one Tricolored Heron (*Egretta tricolor*, moribund, later euthanized) also found on the islands on May 20 tested negative for WNV infection.

To better assess WNV dynamics in the rookery, serologic testing was performed on the monitored ibis nestlings (Fig. 3.1). A total of 36 nestlings (oldest chick of each nest) were handled once weekly from hatch to ~20 days post-hatch to collect standard morphometric measurements, blood and fecal samples. Blood (≤ 1% body weight) was collected from the jugular vein and maintained cold while in the field. Plasma was separated via centrifugation within 4h of collection, then stored at -80ºC. Serial plasma samples were tested for WNV-neutralizing antibodies via plaque reduction neutralization test (PRNT; Beaty et al. 1995), as described by Allison et al. (2004), except those cultures were inactivated on day 5 postadsorption with 10% buffered formalin and stained with 0.25-1% crystal violet for plaque visualization. The starting dilution was 1:10, and titers were expressed as the reciprocal of the highest plasma dilution that neutralized ≥ 90% WNV plaque forming units (PFU, $PRNT_{90}$) when compared with control wells. Samples with ≥ 90% neutralization were co-titrated (serial 10-fold dilutions) for WNV and St. Louis encephalitis virus (SLEV) to determine the causative virus (with ≥4-fold higher $PRNT_{90}$). Samples with ≥10 WNV titer and < 10 SLEV titer were considered seropositive for WNV (Komar et al. 2001). Four of the 36 nestlings had WNV-neutralizing antibodies. With the exception of 314B (died immediately after capture), the chicks that died of WNV had not been selected for monitoring, and thus had no blood collected. Nestlings neighboring 359, including its sibling (Fig. 3.2), were seronegative. Seronegative chicks in early samples did not test positive at later time points. Titers ranged from 10 to ≥ 320 and waned with age (Table 3.1), suggesting maternal WNV antibody transfer and decline. Maternal antibodies can be protective and persist for up to several weeks in House Sparrows (*Passer domesticus*) and domestic chickens (*Gallus gallus domesticus*; Nemeth and Bowen 2007; Nemeth et al. 2008). Protective titers for White Ibises are unknown, thus even birds with acquired antibodies may be susceptible to infection and clinical disease. Because ibis chicks are highly mobile and difficult to recapture, we were unable to document the full duration of maternal antibody persistence. One nestling (Bird 36) was captured only once, thus its complete WNV serostatus is unknown.

This report provides evidence that White Ibis nestlings are susceptible to WNV-associated disease and mortality. Twenty-three additional nestlings disappeared from their nests or were found dead throughout the study, including the sibling of 314A and 314B, and the sibling and neighbors of 359; however, these carcasses were unsuitable for diagnostic evaluation due to advanced postmortem autolysis. It is therefore possible that additional WNV-associated mortalities occurred, as supported by WNV surveillance data provided by the Florida Department of Health (FDOH), Tallahassee, FL from several counties in 2020. Over 400 WNV-positive sentinel chickens were documented (25 from Palm Beach County). Human case numbers spiked, with 50 symptomatic cases reported (5 from Palm Beach County, June-August). Nineteen positive mosquito pools were detected across Florida. Surveillance by FDOH was suspended from March 22 – June 14 due to the SARS-CoV-2 pandemic (FDOH 2020). Extended monitoring of the urban ibis colony is needed to elucidate whether WNV-associated nestling mortalities are common at this location or if they were a result of increased virus activity in 2020. Continuous investigation of mortalities at urban rookeries is useful for alerting public health authorities on the pathogens that might be circulating among local human populations.

This study detected a 11% seroprevalence of WNV in ibis nestlings of an urban colony in south Florida. These antibodies likely reflect passive antibody transfer from seropositive mothers, not mosquito-borne WNV infections, as titers quickly waned over time (Table 3.1). Nevertheless, mortalities occurred, which may indicate that ibis chicks are highly susceptible to fatal WNV disease. Additionally, these maternal antibodies suggest that adults may be less susceptible to WNV-associated disease, based on survival, seroconversion, and successful breeding. Potential impacts of WNV in other rookeries are unknown. The breeding season of White Ibises (April-July) overlaps with peak mosquito activity, increasing the potential for WNV mortality to late-born nestlings. Given the dense mosquito populations in wetlands, it is likely that birds breeding in natural rookeries are also exposed to WNV. As chicks are likely more susceptible to clinical disease and mortality, potential detrimental impacts of WNV on ibis populations would likely be restricted to declines in fledging rates.

White Ibises are routinely found in urban areas across south Florida. They aggregate in parks and landfills where they are provisioned with food; readily coming into contact with mosquitoes circulating among human populations. Our finding of WNV-associated nestling mortalities in an urban colony adjacent to residences highlights the importance of continuous testing and surveillance of WNV in wild birds.

Table 3.1. West Nile virus neutralizing antibody titers of the four seropositive White Ibis (*Eudocimus albus*) nestlings of 36 tested via plaque reduction neutralization test (PRNT), and the three nestling mortalities from an urban colony in West Palm Beach, FL.

Bird ID	Hatch Date (2020)	Age in days (WNV $PRNT_{90}$ titer)
6	May 4	2 (80), 10 (20)
7	May 6	2 (80), 11(10), 17 (10)
27	June 22	2 (≥320), 14 (20)
36	July 6	9 (160)
314A*	June 9	10 (n.d.)
314B*	June 6	13 (n.d.)
359*	June 25	18 (n.d.)

*indicates nestlings that died of WNV as confirmed by histopathology, immunohistochemistry and/or RT-PCR.

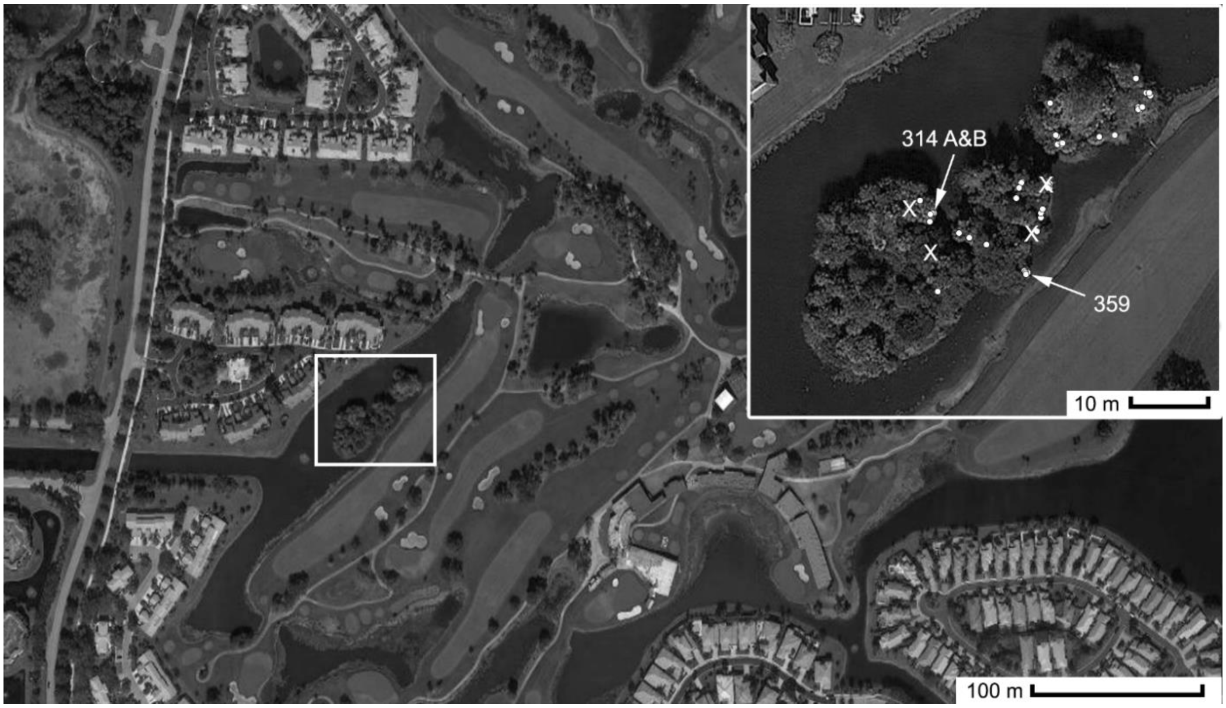

Figure 3.1. Satellite view of an urban colony of White Ibises (*Eudocimus albus*). Nests monitored in 2020 are marked by the dots. Nestling mortalities due to West Nile virus disease were labeled with the nestling IDs and are indicated by the arrows.

Figure 3.2. Moribund White Ibis nestling (*Eudocimus albus*; 359; A) found exhibiting clinical signs of West Nile virus (WNV) disease inside its nest on July 13, 2020 in West Palm Beach, FL. Two additional nestlings from 359's nest (B; indicated by an arrow) and an adjacent nest were found dead but with severe postmortem autolysis.

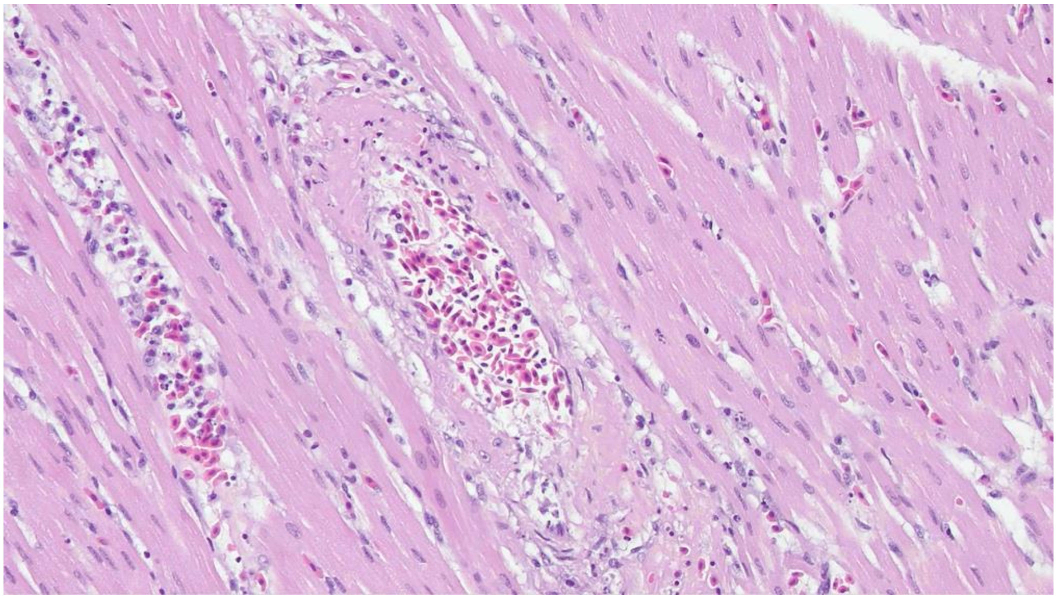

Figure 3.3. Transmural fibrinoid necrosis in an arteriole wall within the septum of White Ibis (*Eudocimus albus*) nestling 359. Hematoxylin and eosin. 20x.

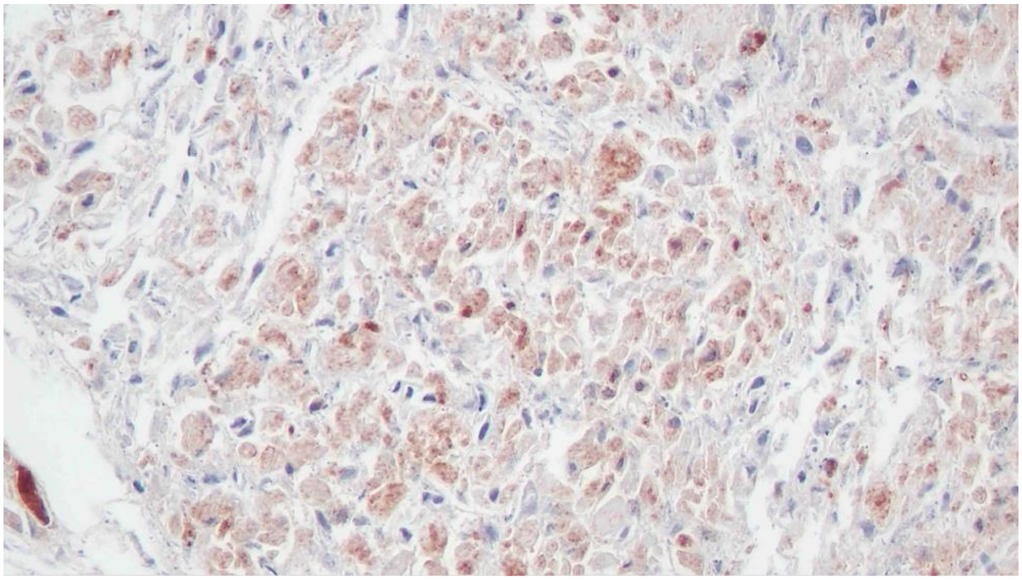

Figure 3.4. Intense cytoplasmic immunolabeling for West Nile virus (WNV) antigen within many degenerated or necrotic cardiomyocytes in the heart of White Ibis (*Eudocimus albus*) nestling 314A. 40x.

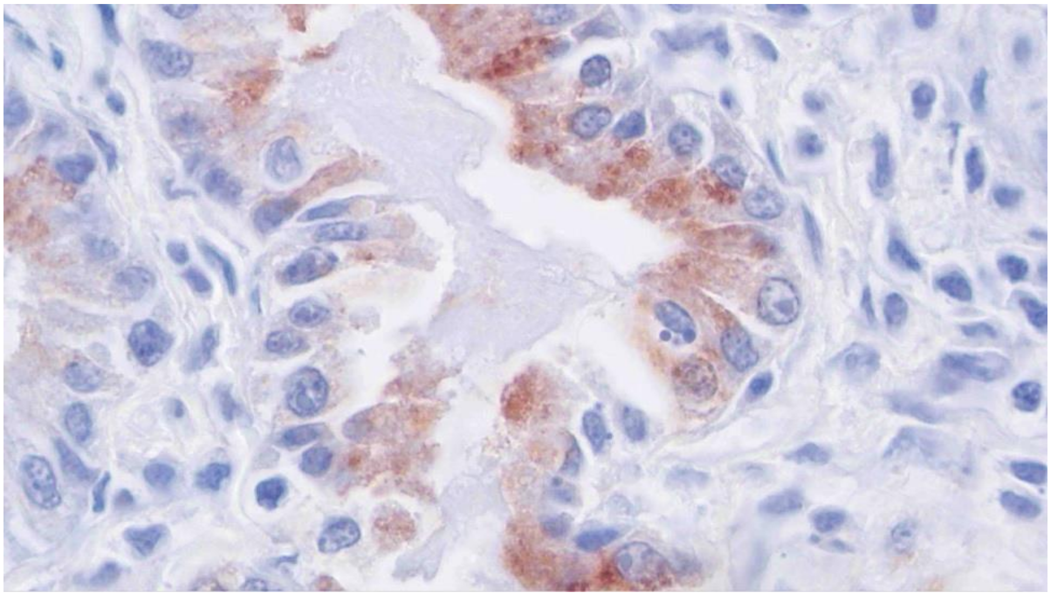

Figure 3.5. Cytoplasmic immunolabeling for West Nile virus (WNV) antigen within numerous epithelial cells along the ventricular mucosa in White Ibis (*Eudocimus albus*) nestling 359. 100x.

CHAPTER 4

CONCLUSIONS

Like the Australian Ibis (*Threskiornis molucca*) and the European White Stork (*Ciconia ciconia*), the White Ibis (*Eudocimus albus*) is becoming increasingly urbanized. However, one of the key obstacles for its progressive adaptation is the ability to breed in anthropogenic environments and establish urban residency, allowing ibises to become independent of natural habitats throughout their annual cycle. The results presented herein provide evidence that ibises have the potential to breed successfully in urban environments. Although ibis breeding grounds are limited to islands surrounded by alligators, the abundance of artificial wetlands in Florida that fulfill these requirements may provide ample habitat for the ibis to establish novel colonies. Continued research on urban colonies is needed to more effectively contrast the productivity of urban and natural environments and better understand the factors contributing to the success of urban breeding grounds. Studies that extend beyond two years of data collection are preferable to effectively address between-year variations on ibis breeding and more accurately describe nest success. Progressive monitoring of the adaptation of this species to anthropogenic environments allows us to make predictions on whether it has the potential to become a nuisance and inform the need for future management. Additionally, monitoring these urban wading bird colonies provides valuable information on the diseases that might be circulating among the local human communities.

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