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American White Pelicans Hand Raised until Fledging and Examination of the Trematode Infection Bolbophorus Damnificus in these Birds

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American White Pelicans hand raised until fledging and examination of the trematode infection *Bolbophorus damnificus* in these birds

By

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American White Pelicans hand raised until fledging and examination of the trematode infection *Bolbophorus damnificus* in these birds

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Because little is known about juvenile American White Pelicans (*Pelecanus erythrorhynchos*) this study was conducted to gather more information on disease, general ecology and growth of American White Pelicans from hatching to fledging.

In July 2011, American White Pelican regurgitate samples from North and South Dakota sub-colonies were collected/analyzed in preparation for a captive trial. Nutrient content compared between the colonies was found to be significantly different. Concentrations of Immunoglobulin Y and A in regurgitate samples were significantly different between colonies.

A captive trial began 29 May 2012 and ended 30 July 2012, in which 16 American White Pelicans were hand raised from hatching to fledging. During the captive trial, various growth parameters, intake and fecal output were examined to determine the effect of the parasite *Bolbophorus damnificus* in 8 infected and 8 non-infected (parasite free) pelicans.

Growth data collected on *B. damnificus* infected (n = 8) American White Pelicans was compared to previously mentioned parasite-free pelicans (n = 8) to determine effects

of the parasite. There were no differences between groups for culmen length (P= 0.214), tarsal length (P = 0.306), body weight (P = 0.884) or intake (P = 0.963). There was also no effect of the parasite on body temperature.

Towards the end of the captive trial, several pelicans both on (n = 16) and off (n = 11) trial became naturally infected with West Nile Virus. Clinical symptoms ranged from lethargy and/or wing droop to total paralysis. Progression of disease is detailed in two well-defined case studies with additional information included on clinical signs, physiological parameters, and a review of the pathology of disease for other infected birds.

Key words: American White Pelican, *B. damnificus,* energetics, morphometric, West Nile virus

DEDICATION

I dedicate this to my husband, family, co-workers and close friends who have provided me with patience, support and guidance through this great endeavor. I would also like to dedicate much of this work to my committee member and mentor Linda Pote who will be greatly missed.

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CHAPTER I

INTRODUCTION

American White Pelicans (*Pelecanus erythrorhynchos*) are large, migratory, fish-eating birds for which there is still an abundant amount of information to be learned (Knopf and Evans 2004). In general, information on basic ecology for most pelican species is lacking. Specifically, information related to immunology, metabolism, growth, morphology, behavior, and disease is limited for fledgling American White Pelicans (hereafter AWPE). This study hopes to enhance information related to each one of the aforementioned areas, focusing primarily on juvenile pelicans from hatching to fledging. Additional information on adult pelicans will also be discussed, but since less is known about juvenile AWPE, they will be the focus of this study.

The first part of the study examined the nutrient content and quantity of Immunoglobulin Y and A (IgY and IgA) contained within regurgitate fed to AWPE chicks. Additionally regurgitate was examined to determine whether its contents were complete enough or too digested to contain the infective stage (metacercariae) of the trematode parasite *Bolbophorus damnificus* which can occur in fish consumed by wild parent AWPE. Prior to this study a nutrient analysis of regurgitate fed to chicks has not been performed for AWPE. These data are useful for formulation of a diet for captive held AWPE and for further examining preferences of items fed to growing AWPE chicks (Ferguson et al. 2011). The nutrient analysis of regurgitate fed to chicks could be

compared to the nutrient analysis of fish consumed by adult AWPE to determine what differences exist in requirements of young and adult AWPE. The potential passive transfer of IgY and IgA through regurgitate has not previously been reported for AWPE. Determining concentrations of IgY and IgA is important for understanding immunology of AWPE. These data could also be used by researchers to create a more comprehensive understanding of AWPE health and disease response (McDade 2003).

The second part of the study included a captive trial that lasted for ~9 weeks, the amount of time Schaller (1964) reported for AWPE to fledge (62 days). AWPE eggs were collected, hatched, and 16 were maintained till fledging (~ 9 weeks) in order to better understand growth, morphology, behavior and disease. Chick growth was measured by recording daily intake, body weight and temperature, and every three days culmen and tarsal length. Changes in morphology such as when eyes opened, eye color, growth of down, etc. were also recorded, which could be useful for determining the age of AWPE chicks. Understanding growth of AWPE chicks will enhance captive and wild management of AWPE in addition to being useful for energetics and damage management models. During the captive trial data were also collected noting changes in behavior from hatching to fledging and noting changes in behavior when birds were introduced to disease.

During the captive trial, 8 of 16 pelicans raised in captivity were infected with *Bolbophorus damnificus* (hereafter *B. damnificus*) to determine if the parasite had a detrimental effect on the pelican, which was determined by comparing growth between parasite infected and parasite free pelicans. *Bolbophorus damnificus* is a trematode parasite of which AWPE have been confirmed the definitive avian host (Kinsella et al.

2004). AWPE perpetuate the spread of this parasite between aquaculture ponds causing economic losses of $27 million per year in Mississippi (Wise et al. 2008). Although there have been numerous studies examining the impacts and prevention of the parasite *B. damnificus*, little is known about the effect of the parasite on fledgling AWPE. By infecting half the pelicans with the parasite it was possible to determine the effects of the parasite on young AWPE, in addition to demonstrating an early age at which birds may be successfully infected. Since the effects of the parasite *B. damnificus* have not been previously studied in combination with data collected on immunology, this information could be useful to those studying evolution of adaptations to fight disease in AWPE (McDade 2003).

During the captive trial, the metabolism of fish consumed by chicks (hatching to fledging) was also estimated by examining the nutrient content of fish as well as the fecal composition of chicks following feeding. Metabolism of growing pelicans has not been previously reported and is also important in examining nutrient requirements of AWPE chicks whether in reference to captive or wild AWPE. Metabolism data (similar to regurgitate data) could be used by researchers to further examine life history and foraging strategy and better explain how AWPE have adapted over time (Carey 1996, Orians and Pearson 1979, Roger and Smith 1993).

Lastly 11 of 16 captive trial pelicans and 10 of 11 additional pelicans raised alongside captive trial pelicans (to be used later in another disease study) became naturally infected with West Nile Virus (hereafter WNV). A natural infection of WNV has never been reported in naïve captive AWPE (aside from treatment birds in this study infected with *B. damnificus*). There is still much to be learned about WNV in AWPE,

however the progression of disease and clinical symptoms reported in this study is unique and is being reported for the first time. These data may allow researchers to better handle potential outbreaks by knowing early signs of WNV in captive or wild settings (Owens and Bennet 2000).

The overall objective of this study is to gain information on AWPE from hatching until fledging. While there is a basic foundation of information available on fledgling AWPE, much is still missing. Much of the information collected during this study has not been previously reported for AWPE and is essential to better understanding the ecology (e.g., growth, morphology, metabolism, immunology, behavior, and disease) of AWPE. Data collected in this study will serve to improve both captive and wild management/conservation of all pelican species. Information gained in this study will also contribute to a better understanding of the evolution of AWPE as a species in relation to life history, foraging strategy, breeding strategy, and genetics.

This research was conducted under United State Department of Agriculture, National Wildlife Research Center, Animal Care and Use Committee study protocol QA-1794 and applicable federal and state permits.

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CHAPTER II

IMMUNOGLOBULIN CONCENTRATION AND NUTRIENT ANALYSIS OF REGURGITATE/FECES COLLECTED FROM AMERICAN WHITE PELICANS (*PELECANUS ERYTHRORHYNCHOS*)

Introduction

Passive transfer of Immunoglobulin Y and A (IgY and IgA) is a life history strategy for piscivorous birds because it enhances chick survival (McDade 2003). Little is known about this adaptive behavior, therefore it is important to determine if and how much immunoglobulins are passively transferred to pelican chicks during rearing. In addition to examining IgY and IgA concentrations in regurgitate, little is known about the nutrient content of regurgitate matter fed to young pelicans and whether there are differences in metabolism and digestion of fish in young versus adult American White Pelicans (*Pelecanus erythrorhynchos*). Understanding nutrient requirements of young pelicans will enhance captive rearing and help to provide answers to other questions involving disease, nutrition and basic ecology.

American White Pelicans (hereafter AWPE) are large aquatic birds which migrate from the northern United States and southern Canada to the southern United States, Central America, and Mexico; spending summer months in the northern climate and winter months in southern climates. American White Pelicans have been reported to travel distances of 96 to 240 km to forage (Johnson and Sloan 1976; Knopf and Kennedy

1980). Typically AWPE consume a variety of fish species but may occasionally consume crayfish and salamanders (Knopf and Evans 2004). Fish and crustacean species reported to be consumed by AWPE include carp (*Cyprinus spp*.), perch (*Perca spp.*), trout (*Oncorhynchos spp.),* sunfish (*Lepomis spp.)* and crayfish (*Cherax spp.)* among many others (McClements et al. 2003). Preferences for different species of fish have been reported for the AWPE. Ferguson et al. (2011) reported AWPE to selected small ~75 g carp over ~350 g larger catfish during the month of May. American White Pelicans not only showed preferences for fish species but also utilized nutrients from an all carp diet more efficiently than from a 50:50 mixture (catfish:carp) or an all catfish diet in a study performed by Ferguson et al. 2011. Derby and Lovvorn (1997) reported that AWPE preferred to consume suckers (*Castastomus spp*.) compared to trout (*O. mykiss*) even when trout were more abundant and readily available.

American White Pelicans typically regurgitate a soft pellet of non-digested material whereas cormorants (similar to pelicans) often regurgitate bony pellets (Duke et al. 1976; Ferguson et al. 2011). It is believed that cormorants regurgitate these pellets to eject bulky, non-nutritious material consumed in the diet (Duke et al. 1976). Adult cormorants will often regurgitate larger bones; however, young chicks rarely regurgitate bone possibly to retain more minerals to their diet (Van Dobben 1952). Therefore AWPE chicks, similar to cormorants, may regurgitate less bone. It may be easier for young cormorants and pelicans to digest/break down bone since they are consuming regurgitate (already partially digested material). Young cormorants and pelicans additionally can only consume smaller more digestible pieces of bone due to their size, which may explain increased digestibility of dry matter (Dunn 1975; Brugger 1993). A study conducted by

Brugger (1993) revealed cormorants, similar to pelicans, digest certain species of fish more efficiently; for example, when cormorants were fed Gizzard Shad (*Dorosoma cepedianum*) no bony indigestible material was regurgitated. However, cormorants regurgitated bony material when they consumed Channel Catfish (*Ictalurus punctarus*) and Bluegill *(L. macrochirus*). Much of the regurgitated material by cormorants was partially digested, indicating that adults may obtain energy from these fish before feeding the fish to their young (Dunn 1975). Although consumption of fish by both cormorants and pelicans in a captive setting may not mimic feed intake in the wild, additional studies are needed to determine if AWPE are similar to pelicans in the digestion of feed.

Other factors affecting AWPE food/prey intake may relate to familiarity with foraging sites, social facilitation, and frequency of feeding (Junor 1965, 1975; Brugger 1993; King 2005). In captivity AWPE may consume approximately 800 to 1,500 g of fish daily, which is at least 10% of their body weight on an as-fed basis (Guillet and Furness 1985; Johnsgard 1993). In the wild, AWPE have been reported to feed from reservoirs, estuaries, rivers, and fish ponds (King 2002). While consuming a wide variety of fish, AWPE may digest fish species differently, as reported for cormorants (Brugger 1993). In captivity it is common to feed live fish to AWPE. However, AWPE similar to other fish eating birds (Jackson et al. 1987; Brugger 1993) can be trained to consume fresh dead or frozen fish. This can be advantageous for zoos feeding AWPE because acquiring and storing frozen fish is easier than supplying fresh live fish.

Limited information exists on content of regurgitate matter fed to young birds and the metabolism of several fish species consumed by AWPE, especially during a period of growth. In addition to knowing little about the nutrient content of regurgitate matter fed

to growing chicks, there is a lack of information about whether there is any transfer of passive immunity. The objectives of this study were to analyze the nutrient content of regurgitate matter, to describe the potential passive transfer of IgY and IgA to chicks through regurgitate matter, and to describe differences in nutrient metabolism of growing AWPE from hatching to fledging, approximately 9 weeks of age from North and South Dakota consuming 4 types of fish.

Materials and methods

This research was conducted under United State Department of Agriculture, National Wildlife Research Center, Animal Care and Use Committee study protocol QA-1794 and applicable federal and state permits.

Wild sample collection

During early July 2011, regurgitate matter was collected from wild AWPE adults and young chicks from colonies at Chase Lake, ND and Bitter Lake, SD. Samples were collected from food and stomach contents regurgitated on the ground by individual birds. Sub-colonies where regurgitate samples were collected were less than 1 mile apart.

To avoid collecting multiple samples from the same bird, only one large sample within close proximity to other similar samples was collected. Regurgitating is a defense mechanism employed by these birds when stressed. Therefore, regurgitate matter was collected as expelled by chicks or adults. Regurgitate matter on the ground was collected manually using small garden trowels, spoons, or sample cups. Over 100 samples of regurgitate (wild chicks and parents) from multiple sub-colonies were collected and placed into labeled sealable plastic bags. Sub-colonies were differentiated as clusters of

nests and AWPE over bare ground separated from other groups by vegetation. Plastic bags were kept on ice while transported back to Mississippi State University. Regurgitate samples were then dried at 60ºC in a forced air oven and compiled for each sub-colony. Dried regurgitate samples were ground by passing through a 2 mm screen in a Thomas Wiley Mill® (Author H. Thomas, Philadelphia, PA). All regurgitate samples were analyzed for dry matter, organic matter, neutral detergent fiber, fat and crude protein (AOAC 2003) and gross energy was determined using an isoperibol oxygen bomb calorimeter (Parr Instrument Co., Moline, IL).

Regurgitate samples were analyzed by the Mississippi State Chemistry Lab (AOAC 2003) for concentrations of IgA and IgY. Two serum samples that were collected from wild AWPE in Belzoni, Mississippi were used to validate testing for comparison against IgY and IgA samples collected in wild AWPE regurgitate. Prior to drying regurgitate samples several ~10 gram portions of each wet sample were randomly selected and compiled for each sub-colony. Selection of ~10 gram samples included using a numbered grid (placed over each sample) along with a random number generator. For samples that contained whole/partially digested fish, a ~10 gram sample of each was cut using a knife. Compiled wet samples were mixed in a blender and then spun in a centrifuge at 1228 x g for 8 minutes before the supernatant was collected for analysis. The information gathered from regurgitate samples was used to formulate a balanced diet for captive AWPE chick rearing as part of a much larger energetics trial.

Captive sample collection

The following year (late May 2012) viable AWPE eggs were collected from similar locations in which previous regurgitate samples were collected from wild birds

(Chase Lake, ND and Bitter Lake, SD colony sites). Fifty eggs from each colony were transported back to and incubated at the USDA, Wildlife Services, National Wildlife Research Center Mississippi Field Station's biosecurity level 2 laboratory on Mississippi State University campus until hatching. Eggs hatched between 29 May and 2 June. Sixteen chicks were randomly selected for a captive energetics trial and were housed in a secure lab in individualized cages (40.6 x 43.2 x 69.9 cm) allowing feces to be collected every few days. As chicks grew (~3 weeks of age) they were transferred to an outdoor research aviary and maintained in individual metabolism pens (115.6 cm x 58.4 cm x 147.3 cm) specifically designed for AWPE.

American White Pelicans used in the captive energetics trial were fed ad libitum Channel Catfish (*I. punctatus*), specific pathogen free (SPF) Channel Catfish (*I. punctatus.*), Gizzard Shad *(D. cepedianum)* or Menhaden (*B. patronus*). The same species of fish were offered to each pelican during each feeding with 4 feedings occurring each day. For the first week pelicans were fed 4 times a day, three times per day for the next 2 weeks and from ~3 to 9 weeks of age were fed twice daily, once in the morning and once in the afternoon. Daily samples of the fish offered and weekly composited fecal samples were collected for 9 weeks, as AWPE progressed from hatching to fledging. Collection pans were placed below each individual cage to facilitate collection of feces. Fecal samples (~6 for each bird) were collected weekly to bi-weekly and compiled for each individual pelican. Nutrient metabolism was determined by calculating the amount of nutrients fed using intake of all fish species and subtracting those excreted in feces. Fish and fecal samples were dried at 60^{o}C in a forced air oven and were ground to pass through a 2 mm screen in a Thomas Wiley Mill® (Author H. Thomas, Philadelphia, PA).

All samples were analyzed for dry matter, organic matter, neutral detergent fiber, acid detergent fiber, fat and crude protein (AOAC, 2003) and gross energy was determined using an isoperibol oxygen bomb calorimeter (Parr Instrument Co., Moline, IL).

Statistical Analysis

Regurgitate data were subjected to analysis of variance (ANOVA) using R® (Version 2.15.2). Values were reported as means ± standard error and a $P < 0.05$ was considered as statistically significant. Individual pelican regurgitate was considered the experimental unit. The response variable was nutrient content of regurgitate samples compiled by sub-colony, with each state (North Dakota or South Dakota) being the explanatory variables. When means differed ($P < 0.05$) they were separated using Fisher's protected least significant difference. Comparisons between North and South Dakota fecal data were made using an ANOVA in R® (Version 2.15.2). Values were reported as means ± standard error and a $P < 0.05$ was considered as statistically significant. A coefficient of variation was calculated in Microsoft Excel for each individual colony (North Dakota and South Dakota) and as a group to compare variation of food consumed. To accurately determine the concentrations of antibodies within the samples, modified direct (serum) and indirect (regurgitate) ELISA tests were devised. It was necessary to devise a novel ELISA test due to the uncertainty of tested IgA and IgY ELISA commercial kits regarding non-chicken avian species (Cray and Villar 2008; Martinez et al. 2003; Crowther 2001). The intra-assay coefficients of variation of IgY and IgA were 7.0% and 2.0% respectively, with no reportable inter-assay variation as only one test was performed. A T-test was used to determine differences in IgY and IgA concentrations between North Dakota ($n = 5$) and South Dakota ($n = 3$) sub-colonies.

Results

One hundred and three total regurgitate samples were obtained from 3 South Dakota and 5 North Dakota sub-colonies. Differences existed between the regurgitate samples collected among sub-colonies in North Dakota and South Dakota. American White Pelicans in South Dakota regurgitated samples contained more organic matter (P = 0.012), crude protein (P = 0.001) and energy (P = 0.034); however, they contained less neutral detergent fiber (P = 0.014) and acid detergent fiber (P = 0.005; Table 2.1) than pelicans in North Dakota. Dry matter content and fat content were similar between samples collected from the two states. Differences within each state's sub-colonies could not be determined as samples were compiled prior to analysis, but some general trends did exist. The nutrient values for the 3 South Dakota sub-colonies seemed to have a narrower range of variation than the 5 North Dakota sub-colonies (Table 2.2). Pelicans in North Dakota had a large variation in neutral detergent fiber (CV = 52.60) and acid detergent fiber (CV = 58.56) among sub-colonies. South Dakota colonies had a moderate variation in acid detergent fiber (CV = 35.01) and crude protein (CV = 32.83). Overall acid detergent fiber (CV = 83.41) was the most variable nutrient for both colonies (Table 2.2). While regurgitate samples may often be partially digested, many of the AWPE in South Dakota were observed consuming giant tiger salamanders (*Amblystoma tigrinum*) and crayfish (*Astacoidea*). Crustacean, amphibian and a variety of fish species were observed in regurgitate matter: bass (*Pecichthyidae*), catfish (*Ictaluridae*), drum (*Sciaenidae*), herring (*Clupeidae*), minnows (*Cyprinidae*), pike (*Esocidae*), perch (*Percidae*), sticklebacks (*Gasterosteidae*), suckers (*Castostomidae*), and sunfish (*Centrarchidae*).

Immunoglobulin A and Immunoglobulin Y concentrations in regurgitate samples were averaged among sub-colonies within each state. Average concentrations of IgY and IgA in AWPE regurgitate from South Dakota (n= 3) averaged 4.67 ± 1.14 ng/mL and 18.95 ± 2.52 ng/mL, respectively (Table 2.3). Average concentrations of IgY and IgA in North Dakota (n = 5) AWPE regurgitate samples averaged 1.58 ± 0.93 ng/mL and 1.62 ± 1.11 ng/mL, respectively (Table 2.3). A T-test was used determine that concentrations of IgY (P = 0.017) and IgA (P = 0.002) were significantly higher for regurgitate samples collected from South Dakota sub-colonies (n = 3) than North Dakota sub-colonies (n = 5).

Growing AWPE chicks that were allowed ad libitum access to one species of fish at each feeding consumed 83.5% Menhaden (*B. patronus*), 8.5% Gizzard Shad (*D. cepedianum*), 5.1% SPF Catfish (*I. punctatus*) and 2.9% Channel Catfish (*I. punctatus*; Table 2.4). No differences for AWPE nutrient metabolism were detected between growing birds from North and South Dakota (Table 2.5).

Discussion

There are several factors that may influence differences in regurgitate including: the availability of foraging sites (Johnson and Sloan 1976), diet of fish species consumed (Crivelli 1981, McClements et al. 2003, Glahn and King 2004), differences in climate (Brugger 1993), and duration of food item retained in the stomach prior to regurgitation.

Differences between North Dakota and South Dakota regurgitate samples could be attributed to the fact AWPE may travel long distances in order to forage (Johnson and Sloan 1976; Knopf and Kennedy 1980). The narrow range of variation among the 3 sub-colonies in South Dakota may indicate that birds in this region may have been sharing a

common food resource. Parent pelicans in South Dakota sub-colonies had low values for neutral detergent fiber and acid detergent fiber, meaning their diet was much more digestible and had more energy than fish consumed by pelicans in North Dakota (Table 2.2). Perhaps parent pelicans chose to consume the more digestible, readily available and energy efficient food source rather than travelling a long distance to another food source. The much wider range of variation observed for the 5 sub-colonies from North Dakota may indicate that resources near the colony were limited and that AWPE had to travel greater distances to forage in a variety of potholes, rivers and estuaries. Acid detergent fiber and neutral detergent fiber values were greater (Table 2.2) for pelicans in North Dakota, meaning their resources were less digestible and lower in energy which also supports the theory that pelicans may have had to travel to alternative food resources to obtain enough nutrients and energy. Consuming a variety of food resources in different locations may also explain why there is a larger variation in acid detergent fiber and neutral detergent fiber for pelicans in North Dakota. Species of fish and crustaceans consumed by AWPE included carp (*Cyprinus spp*.), perch (*Perca spp.*), trout (*Oncorhynchos spp.),* sunfish (*Lepomis spp.)* and crayfish (*Cherax spp.)* among many others (McClements et al. 2003), all of which vary in nutrient composition themselves in addition to consuming a variety of different diets. Fish such as carp are generally omnivorous bottom feeders consuming many benthic insects, crustaceans and detritus (Crivelli 1981), while other fish species such as channel catfish reportedly consume larval aquatic insects, terrestrial insects, zooplankton, crustaceans, and vertebrates such as toads and fish (Glahn and King 2004). These differences might help account for why regurgitate samples were so variable within and between sub-colonies.

The duration of food items retained within the stomach may also have affected the composition of a regurgitate sample. The length of time food is retained influences nutrient assimilation and absorption of nutrients (Hilton et al. 1998). Pelicans that have retained food items longer than other pelicans may digest more nutrients prior to regurgitation, resulting in a regurgitate samples with reduced nutritive value. As AWPE are known to travel long distances to forage (Johnson and Sloan 1976; Knopf and Kennedy 1980), availability of, or distance travelled to foraging sites would directly impact the length of time a food is retained within the stomach. Additionally, size and composition of each prey item consumed would impact rate of digestion (Hoar et al. 1979).

Concentrations of IgY and IgA in AWPE regurgitate have not been previously reported, therefore little is known about the potential passive transfer of immunity between adults and chicks. Concentrations of IgY previously reported by Lebacq-Verheyden et al. (1974) in chicken saliva were 0.28 mg/mL (280,000 ng/mL), several times the amount collected from AWPE regurgitate samples in North and South Dakota. While we would expect saliva concentrations to be similar to regurgitate concentrations, only regurgitate samples were analyzed from the AWPE in this study. Differences between North Dakota and South Dakota IgY and IgA concentrations could be due to a dilution of values attributed to collection during rain. However, it is notable that concentrations of IgA in AWPE regurgitate collected from South Dakota (18.95 ng/mL) were significantly greater ($P = 0.002$) than IgA collected in North Dakota regurgitate (1.62 ng/mL). More studies are needed to determine normal concentrations of immunoglobulins in pelican serum and regurgitate, and how these may be passively

transferred to their young to influence immunity. Still, evidence suggests passive transfer of antibodies may occur through regurgitate (saliva) which may provide immunity to developing chicks during the first few weeks of their life (Hamal et al. 2006), something previously unreported in young AWPE. Pelicans raised in captivity without passive transfer of immunity from parents through regurgitate may be more susceptible to disease, therefore determining baseline concentrations may be important for future study/improvement of captive management.

Although no differences existed in North Dakota (n = 8) and South Dakota (n = 8) captive raised AWPE for nutrient utilization of the four fish species fed during the captive energetics trial, overall nutrient metabolism by growing AWPE was relatively efficient (Table 2.4). Nutrient metabolism of catfish, carp and a 50:50 mixture has been previously reported in adult AWPE (Ferguson et al. 2011). Nutrient metabolism for dry matter, organic matter, crude protein, neutral detergent fiber, fat and energy in growing AWPE (hatching to fledging, ~9 weeks) was elevated above those values previously reported for all diets fed to adult AWPE (Ferguson et al. 2011), which suggests growing AWPE may metabolize nutrients more efficiently than adult AWPE (Table 2.5). The observed differences in the ability to metabolize nutrients between the growing and adult AWPE may be related to the difference in age, as well as diet composition.

The objective was reached by determining immunoglobulins can potentially be passively transferred from adult to chick through regurgitate during chick rearing by determining concentrations of IgY and IgA in regurgitate samples collected from pelican colonies in both North Dakota (n = 5) and South Dakota (n = 3). Additionally the nutrient content of regurgitate collected from both North and South Dakota was analyzed and

discussed for each state. Characterizing the composition of regurgitate matter serves to better understand requirements of nestling AWPE. Secondarily, differences in nutrient metabolism of growing AWPE taking part in a captive energetics trial was examined on a weekly basis and was determined to be not significantly different between pelicans from North Dakota (n = 8) and South Dakota (n = 8). Detailing nutrient metabolism of growing AWPE will further enhance the understanding of nutritional and immunological requirements for growing AWPE, which is necessary to properly raise young AWPE in captivity. Future research may include determining energetic requirements of and morphological development of young fledged AWPE for the first year after they leave the colony.

Table 2.1 Nutrient analysis of regurgitate samples collected from several American White Pelican *(Pelecanus erythrorhynchos)* sub-colonies at Chase Lake, ND and Bitter Lake, SD 7-10 July 2011.

		DM* basis					
State	**DM*, %**	**OM*, %**	**CP*, %**	**NDF*, %**	**ADF*, %**	**FAT, %**	**Energy, kcal/g**
SD†	32.36	81.58	55.83	17.52	2.62	18.35	5.02
ND†	32.30	62.10	25.52	43.48	33.30	19.41	3.95
SEM†	3.70	4.29	4.08	5.94	5.52	1.86	3.09
P† =	0.991	0.012	0.001	0.014	0.005	0.667	0.034

*DM = dry matter, OM= organic matter, CP= crude protein, NDF= neutral detergent fiber, ADF= acid detergent fiber

†SEM = Standard error of the mean, SD = South Dakota, ND = North Dakota, P = p-value

Table 2.2 Nutrient analysis of regurgitate samples collected from several American White Pelican *(Pelecanus erythrorhynchos)* sub-colonies at Chase Lake, ND and Bitter Lake, SD during 7-10 July 2011.

			DM* basis					
State	**SUBC***	**DM*, %**	**OM*, %**	**CP*, %**	**NDF*, %**	**ADF*, %**	**FAT, %**	**Energy kcal/g**
SD	1	23.67	80.17	55.96	9.05	3.57	16.74	4.83
SD	2	39.65	81.30	52.73	16.17	3.44	20.40	5.10
SD	3	33.76	83.25	58.79	27.33	0.85	17.90	5.13
ND	4	40.53	73.14	37.43	27.94	15.98	22.32	4.67
ND	5	33.33	64.92	30.03	39.09	28.66	20.46	4.26
ND	6	32.71	66.85	22.13	43.84	34.82	23.05	4.27
ND	7	27.39	52.60	22.34	50.66	41.13	14.33	3.32
ND	8	27.57	52.97	15.67	55.88	45.89	16.87	3.20
SD CV*	1-3	24.97	1.91	5.43	52.60	58.56	10.19	3.29
ND CV*	4-8	16.63	14.54	32.83	24.83	35.01	19.12	16.41
Overall CV*	1-8	18.34	17.58	46.07	48.82	83.41	15.93	17.17

* SUBC= sub-colony, DM = dry matter, OM= organic matter, CP= crude protein, NDF= neutral detergent fiber, ADF= acid detergent fiber, CV= coefficient of variation

Table 2.3 Average concentrations of spun regurgitate IgY and IgA in American White Pelican regurgitate samples collected from colonies at Chase Lake, ND (n = 5) and Bitter Lake, SD (n = 3) during 7-10 July, 2011.

Sample ID	**IgY (ng/mL)**	**SD***	**IgA (ng/mL)**	**SD***
South Dakota	4.67	1.14	18.95	2.52
North Dakota	1.58	0.93	1.62	1.11

* SD= Standard deviation

Table 2.4 Nutrient analysis and proportion of fish species (n = 4) fed to growing captive American White Pelican chicks (*Pelecanus erythrorhynchos*) during 31 May through 30 July 2012.

		DM* basis						
Sample ID	**DM* %**	**OM* %**	**CP* %**	**NDF* %**	**ADF* %**	**FAT, %**	**Energy kcal/g**	**% Diet**
Menhaden	28.8	83.19	59.78	9.7	0.97	24.65	5.18	83.5
Gizzard Shad	23.8	83.87	60.84	8.58	1.16	26.14	5.50	8.5
SPF Catfish	29.48	82.75	54.35	34.83	2.91	30.09	5.25	5.1
Farmed Catfish	28.74	80.77	51.76	29.96	2.7	29.97	5.26	2.9

*DM = dry matter, OM= organic matter, CP= crude protein, NDF= neutral detergent fiber, ADF= acid detergent fiber

Table 2.5 Nutrient metabolism of 4 different fish species fed to growing American White Pelican chicks (n = 16; *Pelecanus erythrorhynchos*) during hand rearing, 31 May to 30 July 2012.

		DM* basis					
State	**DM*, %**	**OM*, %**	**CP*, %**	**NDF*, %**	**ADF*, %**	**FAT, %**	**GE*, %**
SD†	64.27	74.73	46.12	95.54	84.97	87.80	85.31
ND†	65.56	76.67	50.75	95.00	86.04	90.77	86.12
SEM†	2.06	2.02	4.60	0.80	1.65	1.95	0.98
P =	0.665	0.510	0.488	0.641	0.652	0.298	0.569

*DM = dry matter, OM= organic matter, CP= crude protein, NDF= neutral detergent fiber, ADF= acid detergent fiber, GE= gross energy

† SEM = Standard error of the mean, SD = South Dakota, ND = North Dakota

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CHAPTER III

GROWTH OF AMERICAN WHITE PELICANS (*PELECANUS ERYTHRORHYNCHOS*) FROM HATCHING TO FLEDGING

Introduction

There is limited information available on the growth and morphology of American White Pelicans (*Pelecanus erythrorhynchos,* hereafter AWPE) from hatching to fledging. Accumulating knowledge on growth and morphology of juvenile pelicans will specifically enhance captive rearing in addition to being useful for conservation and energetics modeling.

American White Pelicans are large piscivorous birds that migrate across North America. They typically spend summer months in the northern United States and southern Canada, migrating to southern United States, Mexico and Central American in the winter months. AWPE (~1 year +) body size ranges from 127 to 170 cm long, with a bill (culmen) length measuring ≥310 mm for males and ≤309 mm for females (Dorr et al. 2005). Apart from bill length, females and males have a very similar physical appearance although females will often be slightly smaller (Johnsgard 1993).

AWPE may fledge as early as 62 days (Schaller 1964), with fledging rates reported to range from 0.21 to 1.23 birds per nest (Sloan 1973). Knopf (1976) reported a 70% mortality rate for chicks from 1 day to 3 months of age due to various causes including starvation, harassment, nest abandonment, hypothermia, and disease. Adult

pelicans may feed their young as frequently as 4 times a day, but as chicks mature, feeding frequency decreases (Johnsgard 1993). Pelican chicks are born altricial, greatly affecting thermoregulatory ability and independence. Natal down first appears on about day 6, with plumage length increasing from 0.1 to 1.9 mm from days 6 to 8, and increasing to about 11 mm by day 14 (Daniels 1997). Abraham and Evans (1999a) reported that AWPE reach modest thermoregulatory capabilities by day 7 post-hatching, with enhanced development of self-thermoregulation at about 16 days. Young AWPE may rely on vocalizations to solicit additional brooding warmth from parents (Evans 1992). Other studies have shown that thermoregulation becomes well developed in other large Pelecaniformes at similar ages, at ~20-25% of adult mass and by ~16-26% of the way through the nestling period (Abraham and Evans 1999b).

Maintenance diets for an AWPE average ~10% of body weight (800 to 1,500 g) of fresh fish daily (Johnsgard 1993, Ferguson et al. 2011). Total feed needed to raise a hatchling pelican to fledging has been estimated to be 68.1 kg on an as-fed basis (Hall 1925) with breeding adults having increased energetic demands requiring 1,800 g of fish daily (as-fed basis) or as much as 40% of their body weight.

In a 4 year study conducted by Schrieber (1976), tarsus length, culmen length and body weight data were collected for several Brown Pelicans (*Pelecanus occidentalis*). Tarsus lengths typically reached full length by 5 weeks with their culmen continuing to grow until fledging. Body weights reached maximum by 45 to 60 days of age with Brown Pelicans losing up to 600 g of body weight prior to fledging. Culmen length and body weight have been previously used to predict Brown Pelican age, such as the following equation developed by Palacio (2001): Estimated Age (weeks) = Culmen Length (cm) x

0.3501-0.0184. Schrieber (1976) estimated that ~50,000 g of fish would be required to raise a Brown Pelican from hatching to fledging.

Kendeigh et al. (1977) developed formulas used to determine how much energy is needed for daily existence of adult cormorants and pelicans depending on body mass (M:g): g existence energy EE (kJ*day-1) = $17.34M^{0.5444}$ when ambient temperature was 0ºC and EE = $4.472M^{0.6637}$ when ambient temperature was 30ºC, with body masses of 6,500 g for pelicans and 2,000 g for cormorants respectively. Derby and Lovvorn (1997) developed bioenergetic model estimates of energy requirements of 0.232 kJ/g of fish for an 8,900 g Great White Pelican (*Pelecanus onocrotalus*) and 0.262 kJ/g of fish for a 6,500 g AWPE. These values may under-estimate requirements extrapolated from Great White Pelicans in energetic cages; as it is known that intake of captive and wild birds may differ due to several factors such as frequency of feeding, social facilitation, and stress (Junor 1972).

American White Pelicans over the last few decades have lost foraging and nesting habitat due to factors such as soil erosion, flooding, drought, decreasing shorelines and human intrusion (King 2005). With this decrease in habitat, pelicans have sought out new food resources, often resulting in predation of commercial catfish where available. In the Mississippi Alluvial Valley, commercial aquaculture of Channel Catfish (*Ictalurus punctatus*) is of great economic importance. One common avian predator of commercial catfish is the AWPE (King 2005). Predation of catfish by AWPE has resulted in great economic losses in addition to several other negative impacts such as AWPE perpetuating the spread of a digenetic trematode *Bolbophorus damnificus* resulting in additional annual losses in Mississippi reported at $27 million (Wise et al. 2008).

The objective of this study is to describe the growth of AWPE from hatching to fledging using data collected on body weight, intake, excrement, culmen and tarsal length. Additional information on morphometric development will also be detailed. The assessment of energetic requirements of young pelicans will provide more information regarding consumption and diet, further detailing unknown information about the growth of pelicans. Data on growth and morphology will improve both captive and wild management of AWPE, in addition to providing essential information that could be used for predicting age, energetics and general ecology.

Materials and methods

During late May 2012, 100 viable AWPE eggs were collected from Chase Lake, ND and Bitter Lake, SD colony sites. Eggs were transported in coolers lined with protective dryfast foam padding and were kept warm (37.5^{o}C) using a heating pad and a digital thermometer. Eggs were also moistened every 4 hours using a spray bottle filled with water. Eggs were incubated at the USDA, Wildlife Services, National Wildlife Research Center's biosecurity level 2 laboratory on the Mississippi State University campus. Eggs were warmed to 37.5^{o}C using an incubator (Sportsman Cabinet Egg Incubator 1502 equipped with a ~19 liter water reserve system) and maintained at 60% humidity. Eggs were monitored daily and began to hatch immediately after returning, with the first pelican hatched on May 29 2012. Thirty six eggs were allowed to hatch over the next 3 days and were maintained for ~one week before randomly selecting 16 pelicans for a captive energetics trial. An equal number of pelicans were selected from each major breeding colony (half North and half South Dakota) in order to have a larger representative sample and additionally to determine if differences existed between these

colonies. Of the 16 pelicans selected for the captive energetics trial only the 8 control birds will be discussed in this paper (4 from North Dakota, 4 from South Dakota) as the other 8 pelicans were artificially infected with a digenetic trematode *Bolbophorus damnificus* to determine its effects on growing AWPE. The captive trial took place at the Mississippi Field Station where pelicans were raised from hatching through fledging (~9 weeks).

As the eggs began piping the room temperature was reduced to ~36.0^{o}C and monitored using a digital thermometer. A dehumidifier was used to keep the humidity of the room at ~60%, also measured by a digital monitor. Once chicks emerged from the eggs, they were not fed for 12-24 hours to allow the nutrients in the placental lining of the egg to be absorbed. Chicks were then placed in plastic coated wire cages 0.5 m x 0.5 m x 0.8 m equipped with heating lamps covering ~30% of the cage 12-24 hours post-hatching. Cages were also equipped with black foam pads ~25 cm long and 15 cm wide to allow chicks a softer alternative to wire flooring and to reduce potential foot problems. Additional chicks not selected for use in the trial were euthanized using carbon dioxide following the 2007 AVMA Guidelines on Euthanasia. A diet was formulated using information gathered from a previous study looking at the nutrient content of regurgitate matter fed to growing pelicans (T. Ferguson unpublished data). Fish were cut up and (or) thawed prior to each feeding. Pelican chicks were fed an ad libitum diet composed of Channel Catfish, specific pathogen free (SPF) catfish (*Ictalurus punctatus.*), Gizzard Shad *(Dorosoma cepedianum)* and Menhaden (*Brevoortia patronus;* T. Ferguson unpublished data).

Chicks began consuming small cut up pieces of fish (~2 to 4 grams in size) ~24 hours after hatching. Chicks were bottle fed water prior to and following each feeding. Chicks often vocalized prior to feeding then would quiet down and sleep following full satiation of both fish and water. Once chicks were accepting whole fish at ~3 weeks of age they were transported to the USDA/WS National Wildlife Research Center's outdoor research aviary on the Mississippi State University campus. Each chick was banded and placed in an individual metabolism pen customized for energetics work measuring 115.6 cm x 58.4 cm x 147.3 cm. Each pen was additionally equipped with a heat lamp covering ~30% of the pen, which the pelican could move in and out of to keep warm. The pelicans remained inside the pens until they were ~9 weeks old (fledging).

Feeding frequency was eventually decreased from 4 to 2 feedings; for the first week pelicans were fed 4 times a day, for the next 2 weeks 3 times a day, and from ~3 weeks of age and older pelicans were fed twice daily (once in the morning and once in the afternoon). Pelicans were weighed once daily in the morning, prior to feeding. To measure body weight, individual pelicans were removed from their cages and placed in a large pre-weighed bin secured on a scale. Intake was recorded daily with culmen and tarsal lengths measured every 3 days. Culmen and tarsal measurements were taken by removing the pelican from the pen and having one person hold the bird while another measured (to the nearest 0.02 mm) using a dial caliper or (to the nearest mm) using a steel rule. Each pelican was assigned a fecal collection pan that was placed underneath wire flooring of each metabolism pen and feces were collected at 2 week intervals. Feces were collected by scraping fecal matter from each collection pan into pre-weighed plastic

bags. Fecal collection pans were then cleaned and reweighed prior to the next collection period.

Data were collected daily for body weight and intake. Data on culmen length and tarsal length were collected every 3 days in addition to collecting fecal data every 1-2 weeks. All data were entered into a spreadsheet and both standard deviation and means were calculated for all birds (n = 8) on a weekly basis except for fecal data, which was averaged over the entire trial. Standard deviation and means for hatching and final weights, culmen lengths, tarsal lengths were also reported for all pelicans (n = 8).

Predictive models were also created for daily body weight, daily intake, culmen length and tarsal length to allow for further growth analysis. For intake and culmen data models were compared with REG procedures in SAS using R^2 to index fit. For body weight and tarsal data models were compared using the NLIN procedure in SAS. The Gompertz model best fit daily body weight and tarsal data (Figure 3.1 and 3.4). Daily intake was best predicted using a polynomial model (Figure 3.2) and culmen length was best predicted using a linear model (Figure 3.3). Predictive models are portrayed in figures as thicker black lines in addition to other data.

This research was conducted under United State Department of Agriculture, National Wildlife Research Center, Animal Care and Use Committee study protocol QA-1794 and applicable federal and state permits.

Results

All values reported in the results section are means $\pm$ SD. Hatchability of eggs used in the trial from both North Dakota and South Dakota was 100%. No differences

existed for parameters intake, body weight, culmen length and tarsal length between pelicans from North or South Dakota colonies.

The average total amount of fish (all 4 species) consumed per bird over 62 days averaged 50,314 g. The average intake of all AWPE for each week is reported in Table 3.1. Intakes peaked during week 6 at 1,255.96 g ± 170. 01 g and week 7 at 1,238.11 g ± 254.82 g. Intake as a percentage of body weight ranged from 8.54% to 42.73%, averaging 26.31% over the entire trial. The average daily intake of growing AWPE (n = 8) is plotted in Figure 3.2. Each AWPE (n = 8) averaged a fecal output of 8,342 g over the 62 day period. During week 9 there was a decrease in intake (Figure 3.1) and body weight (Figure 3.2) for all pelicans.

During the trial, AWPE (n = 8) averaged an initial body weight of 107.38 g ± 10.73 g, ranging from 94 g to 123 g at hatching (Figure 3.1). Final body weights of pelicans by the end of the trial averaged 5,890.75 g ± 845.23 g ranging from 4,828 g to 7,189 g. Peak body weight for AWPE averaged 6,727.63 g ± 1,033.75 g and occurred during week 8 on different days for most birds. Following peak body weights there was a reduction in weight for all birds after day 50.

AWPE culmen lengths (n = 8) averaged 21.20 mm ± 1.08 mm at hatching and ranged from 19.52 mm to 23.16 mm (Figure 3.3). Culmen lengths for fledged AWPE averaged 234.86 mm ± 15.94 mm, ranging from 216 mm to 259 mm. American White Pelicans tarsus length at hatching averaged 21.00 mm ± 1.13 mm, ranging from 19.52 mm to 22.18 mm (Figure 3.4). Final tarsal lengths averaged 124.97 mm ± 7.71 mm, ranging from 111.31 mm to 133.7 mm.

A timeline was created (Table 3.2) to describe the physical characteristics, behavioral characteristics and obstacles overcome during hand-rearing. Examples of such characteristics include initial feather development (June 4-6), dropped egg tooth (June 7-9) and commencement of gular fluttering (June 10-19).

During the last 2 weeks of the trial 5 of 8 pelicans tested positive for WNV by one or all of the following: histomorphological disease consistent with WNV infection (a full complement of major organs was examined in each case), reverse transcriptase PCR detection for WNV (pooled brain, heart, kidney, liver, spleen), or complement fixation serological testing. Although 5 of 8 pelicans were confirmed WNV positive, the effect of WNV infection on the trial was considered statistically non-significant.

Discussion

The plotted daily body weights of AWPE (n = 8) show a range of variation for growing chicks. There is expected to be variation among chicks during development but some activities may have affected their growth as a whole. For example on day 23 (June 22) body weights were affected by the move of pelicans from an indoor biosecurity level 2 facility to an outdoor research aviary, explaining the slight dip in body weights at that time (Figure 3.1). Otherwise, AWPE growth increased nearly linearly until peaking during days 50-55 of the trial. A similar growth pattern was observed in a study conducted by Schreiber (1976) looking at the changes in weight with age in nestling Brown Pelicans (Figure 2.1 in Schreiber 1976). Similar to Schreiber (1976) as AWPE grew weights became more variable with maximum body weights reached around 50-55 days. There were also similarities between body mass growth curves of Red-throated

Loons (*Gavia stellata*) and pelicans; however unlike pelicans there was no slight decrease in body mass towards fledging (Rizzolo et al. 2015).

Adult AWPE (n = 12) have been previously reported to average a weight of 5,939 g with a standard error of ± 387.2 g in an average of pre and post-trial weights (Ferguson et al. 2011). By the end of the trial fledged AWPE (n = 8) weighed an average of 5,890.75 g ± 845.23 g, well within the average reported for adults. However, at peak body weight fledgling AWPE averaged 6,727.63 g ± 1,033.75 g which exceeds the previously mentioned average of 5,939 g ± 387.2 g (Ferguson et al. 2011). Similar observations were made in Brown Pelicans (Schreiber 1976) where nestlings prior to fledging were reported to be heavier than adults. In Figure 3.1, the last week (from week 8 to week 9) prior to fledging, AWPE decreased in weight losing ~900 g of body weight. Body weights of Brown Pelicans followed a similar trend with pelicans losing up to 600 g of body weight just prior to fledging (Schreiber 1976). Although Brown Pelicans lost less body weight (up to 600 g) they generally weigh less than AWPE to begin with, therefore the ratio of weight loss relative to overall body weight of each pelican species is similar. This is likely due to factors such as increased energetic demands preparing for flight.

It is also notable that was a slight divergence of pelicans into 2 different weight groups, with heavier pelican's typically having longer culmens. Because it was determined that divergence of body weight was not due to origin, it is believed differences may be related to sex as males are typically heavier with longer culmens. Unfortunately the sex of all pelicans on trial was not determined during necropsy, therefore the hypothesis males were heavier than females cannot be tested.

The amount of fish consumed per pelican from hatching to fledging averaged 50,314 g over 62 days (Table 3.1). Estimates on intake may be conservative due to pelicans being confined to energetics cages during the trial. Schrieber (1976) estimated that Brown Pelicans would require ~50,000 g of fish from hatching to fledging, a very close comparison to the average observed in this trial for AWPE from hatching to fledging at 50,314 g. Other intake estimates made by Hall (1925) at 68.1 kg (68,100 g) on an as-fed basis (hatching to fledging) were greater than data from this trial. Maintenance diets for adult AWPE have been previously estimated to be ~10% of body weight, 800 to 1,500 g (Johnsgard 1993; Ferguson et al. 2011). Although peak intakes from week 6 and 7 are within the normal range for adult maintenance (Ferguson et al. 2011), the percentage of body weight being consumed is greater than the maintenance requirement of 10%. In fact, intake as a percentage of body weight ranged from 8.54% to 42.73%, averaging 26.31% over the entire trial, remaining almost entirely above adult maintenance requirements. During this period of growth it is expected that there will be increased energetic demands observed similar to increased energetic demands during breeding (Hall 1925). By the end of the trial (week 9) AWPE chicks had fledged and intake as a percentage of body weight for all birds had decreased to near adult maintenance levels (8.54%). AWPE intake decreased ~615 g from week 8 to week 9, just prior to fledging, and may be due to several factors including natural phenomena or environmental stress. Heat stress may suppress appetite in bird species (Donkoh 1989) where as WNV infection may cause birds to become too immobile to swallow fish (M. Sovada personal communication).

The average daily intake of growing AWPE (n = 8) is plotted in Figure 3.2. Although pelicans were fed multiple fish species ad libitum, variability in intake may be affected by several factors such as fish preference (Ferguson et al. 2011) and stress (Junor 1972). As previously mentioned young AWPE were transferred from an indoor facility to an outdoor facility on June 22 (day 22). Intake after the transfer declined from ~1,100 grams to ~500 grams (Figure 3.2) but increased back to ~1,100 g 2-3 days later, showing pelicans are sensitive to stress. The more variable intake of pelicans after being transferred to the outdoor facility may be due in part to the fluctuation in environmental conditions; averaging ~12 ºC hotter during the day than at night. The conditions in the indoor facility were maintained at a constant level throughout each day. During the last 2 weeks of the trial increased variability in intake may have been due to several pelicans testing positive for WNV (F. Cunningham personal communication) or exercising their flight muscles in preparation fledging.

Additionally, AWPE individual fecal output averaged 8,342 g over the 62 day period. As feces were collected weekly and AWPE were maintained outdoors, it is likely that some of the water content of the feces evaporated between collections, leaving a conservative estimate of fecal output.

AWPE culmen lengths were measured every 3 days (Figure 3.3). Culmen growth was essentially linear for all 9 weeks of the trial, similar to nestling Brown Pelicans (Schreiber 1976). Similarly culmen growth in the Red-throated Loon increased at a near linear rate from 0-20 days of age but experienced a slight rate decrease in towards day 50 (Rizzolo et al. 2015). Culmen lengths for AWPE >7 months old were previously reported at ≥310 mm for males and ≤309 mm for females (Dorr et al. 2005). In this trial culmen

length for fledged AWPE only averaged 234.86 mm ± 15.94 mm indicating an AWPE culmen will continue to grow past fledging. It is notable that AWPE with longer culmen lengths were often heavier than AWPE with shorter culmen lengths.

Rapid tarsus growth occurred from day 2 to day 30 (June 2 and June 30, respectively), peaking around day 30. After day 30, growth slowed but variability of tarsal length among birds increased, similar to observations made by Schreiber (1976) in Brown Pelicans and Rizzolo et al. (2015) in Red-throated Loons. Average tarsal length of adult male AWPE has been reported by Dorr et al. (2005) as 120.4 mm ± 0.4 mm (range 105-137 mm) and for adult female AWPE 111.0 mm ± 1.2 mm (range 100-120 mm). Juvenile tarsal lengths (including both sexes) at the end of the trial averaged 124.97 mm ± 7.71 mm, ranging from 111.31 mm to 133.7 mm. The average for all juvenile pelicans was slightly more than the average reported by Dorr et al. (2005) for adult male AWPE indicating pelican tarsi were either fully grown or perhaps may slightly decrease in size reaching adult maturity.

Beginning around 10 days of age, 2 AWPE did exhibit severe but temporary splaying of legs, but in these cases the growth of the tarsus did not seem to be affected. As AWPE were fed ad libitum, and growth during the first few weeks of life is rapid, it's possible that chicks did not develop enough leg strength to support their body weight and may have consequently become splay-legged. In wild AWPE colonies splay-legged young have been observed (T. King, personal communication) however it is not certain whether most of those young will eventually right themselves or perish. During the captive trial AWPE that became splay-legged were aided through a flexible structure that forced young to hold their feet underneath them. This structure was made of duct tape,

and was wrapped around chick's leg slightly above the tarsus. The tape was double sided (not sticking to leg of bird) and extended across from one leg to the other at each birds shoulder width. The tape was replaced every 2 days to accommodate the chicks' rapid growth. All splay-legged young recovered after ~1 week.

To further detail growth of the AWPE, a morphometric timeline was created (Table 3.2). Nearly all pelicans exhibited peeling and redness during the first few weeks. It is not known whether this occurs due to rapid growth or another contributing factor. Humidity in the room was kept around ~60% and temperature was held constant. Additional measures were taken to sooth the dry cracking skin by wrapping the young chicks in a wet cloth containing a mild amount of aloe while being fed, however this did not seem to have any observable effects on the chick. All pelicans exhibiting skin peeling/cracking and associated redness healed within a week of onset and no further skin abnormalities were observed. No differences in growth or intake existed between wrapped and unwrapped birds nor between those that required duct tape structure placed on their legs and those whom did not.

Several other notable physical developments and behaviors were also reported in Table 3.2. AWPE chicks developed natal down by day 6 as predicted by Daniels (1997). Newly hatched chicks remained extremely vocal until after feedings when they would usually quiet and fall asleep. Similar to Evans (1992), only 1 to 2 hours passed before they again would begin vocalizing. Thermoregulatory abilities were not a target of study during this trial; however, AWPE chicks were transferred to the outdoor facility around 3 weeks of age with thick down. Most pelicans became rather docile when being handled, whereas a few maintained wilder instincts; such as biting or trying to avoid human

contact. As pelicans were handled often, it is believed that several may have imprinted on the main caretaker. Pelicans were more co-operative with the main caretaker when weighed and fed, therefore it is important for future researchers to consider using one main caretaker during captive rearing of AWPE chicks. In addition to making handling of birds easier, levels of stress in chicks may be reduced if one main caretaker is used.

Lastly as growth analysis is an important part of avian physiology, 4 predictive growth models were created. The Gompertz models best described the growth in mass (daily body weight) and tarsus whereas daily intake and culmen length were best predicted using a polynomial and linear models, respectively. As previously discussed pelicans experienced a reduction in intake and body weight during the last 2 weeks of the captive trial, therefore predictive intake and body weight models may only be accurate during the first 7 weeks of the trial. Predictive modeling will allow researchers to more accurately estimate the age of both captive and wild young AWPE in addition to enhancing previously unknown information on growth and physiology of the AWPE from hatching to fledging.

Conclusion

As AWPE continue to lose habitat, in addition to having high mortality rate prior to fledging (King 2005, Knopf 1976), it is important to consider future management of this species by further detailing information about growth, caregiving and behavior. The objective was met as this trial further detailed information on the growth and feed consumption of AWPE from hatching to fledging and set a guideline for captive hand rearing. Information obtained in this trial may also have several other applications. Intake data from young AWPE may help enhance energetics models by determining if intake of

first year pelicans is greater than adults, and may help in refining increased energetic demands on adults associated with chick rearing. Intake and fecal output data may also be useful in refining damage management strategies used to reduce AWPE predation and disease transmission at aquaculture facilities and on natural resources by allowing targeting of specific age groups. Quantification of feces may also allow managers to estimate potential parasite transfer to ponds. Management of aquaculture facilities may include a risk-cost analysis of disease/predation compared to preventative measures. Predictive models may be used for comparing or predicting growth of various other bird species in addition to detailing previously unknown information on growth curves of AWPE. Culmen length and body weight models may be combined to develop a more precise equation predicting age of AWPE similar to that of Palacio (2001). Data collected on intake and body weight may also be used to develop daily maintenance energy formulas for growing pelicans, providing a more accurate estimation of the additional energetic requirements of adult AWPE during chick rearing (Kendeigh et al. 1977).

Table 3.1 Weekly body weight, intake, and intake as a percentage of body weight for captive American White Pelicans *(Pelecanus erythrorhynchos)* from hatching to fledging (n = 8) from 29 May through 30 July 2012.

	Body weight (grams)		**Intake (as-fed) (grams)**		
Week	AVG[1]	STD[1]	AVG[1]	STD[1]	% BWT[1]
1	146.73	51.86	51.74	19.40	35.26
2	627.66	300.67	268.20	44.39	42.73
3	1851.71	409.22	599.64	87.00	32.38
4	3048.48	435.15	865.93	101.67	28.41
5	4240.38	583.93	1208.41	126.20	28.50
6	5376.14	674.63	1255.96	170.01	23.36
7	6153.70	827.54	1238.11	254.82	20.12
8	6533.77	977.95	1145.41	204.97	17.53
9	6207.75	925.37	530.07	126.17	8.54

[1] AVG = average, STD= standard deviation, % BWT= intake as a percentage of body weight

Table 3.2 Morphometric timeline for captive raised American White Pelicans (*Pelecanus erythrorhynchos*; n = 8) from hatching to fledging 29 May to 1 Aug. 2012.

Days Post Hatching	Morphometric Timeline
Day 1-3	All pelicans hatched, and vocalizing
	Limited mobility, naked, black eyes, white toenails
	Weights ranged from 88 to 141 g
	Fed 4 times from 8 am to 10 pm
Day 4-6	Some skin peeling, toenails black tipped
	Eye colors beginning to form
	Slight black pigment on bill
Day 7-9	Pelican eye colors: blue, dark brown and light brown
	Down developing on outer tips of humeral and alar feather tracks
	Black pigment over 30-60% of face and bill
	Toenails ~70% black
	Some pelicans exhibiting cracking of skin and associated redness
Day 10-12	Down developing on dorsal and ventral feather tracks
	Dropped egg tooth
	Some pelicans becoming splay legged
Day 13-22	Down developing along capital feather tracks
	Gular fluttering and preening observed
	Pelicans consumed whole fish ~280 grams
	Free movement of neck and wings
Day 22-28	Pelicans covered in down feathers 1-4 mm in length
	Blue eye colors separates into a dark and light blue
	Birds biting at body down feathers
	Pelicans beginning to stand upright and walk
Day 29-31	Secondary feather development over wings 2-4 cm in length
	Black tipped primary feather development 1-3 cm in length
	Culmen of some pelicans show a slight bend
Day 32-39	Primary (black-tipped) and secondary feather development 2-8 cm in length
	Pelicans biting at primary and secondary feather development on wings
Day 39-52	Beginning to develop small tuft of feathers in coronal region of head
Day 52 – Day 62	Full development of body and flight feathers
	Pelicans practice flapping wings in cages

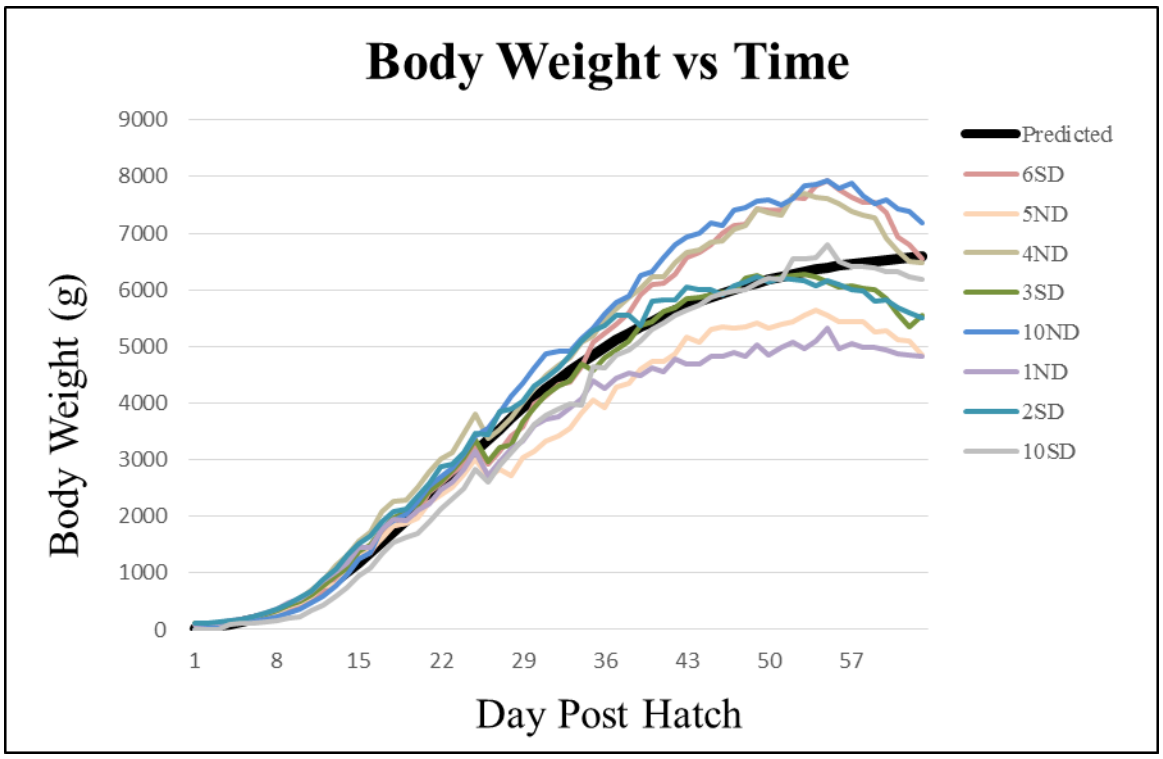

Figure 3.1 Body weight of North Dakota (ND) and South Dakota (SD) captive raised American White Pelicans *(Pelecanus erythrorhynchos*; n = 8) from hatching to fledging 31 May to 1 Aug. 2012 and the Gompertz predicted model.

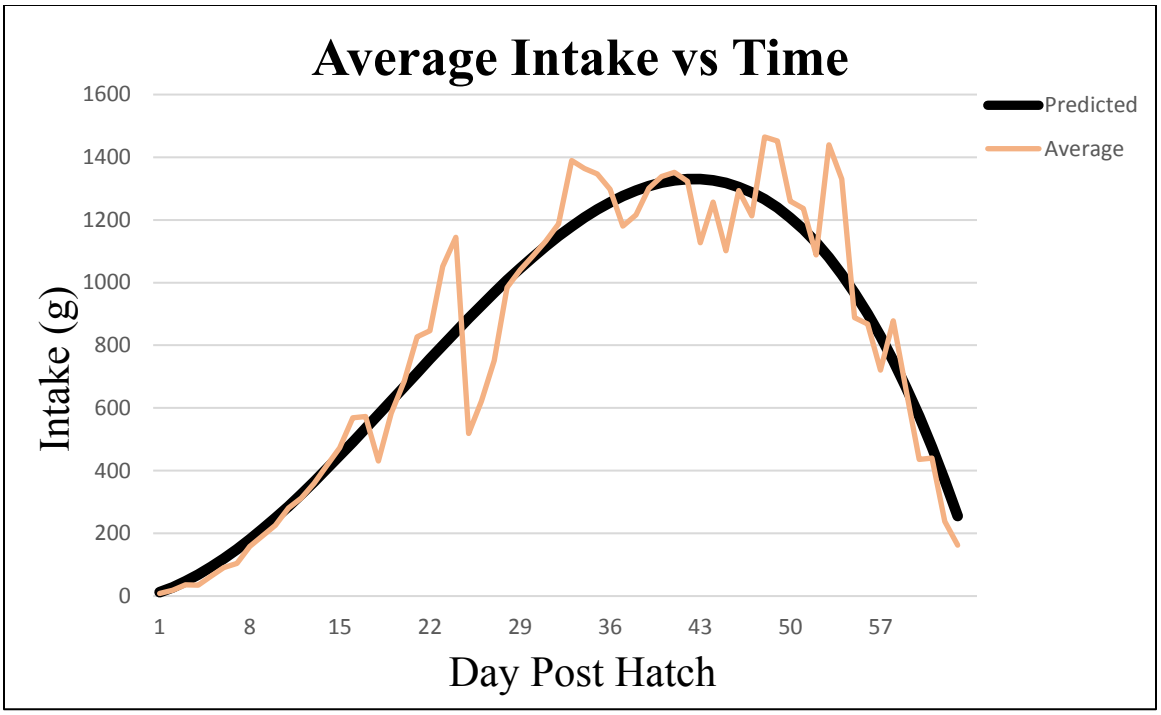

Figure 3.2 Average intake for North Dakota (ND) and South Dakota (SD) American White Pelicans (*Pelecanus erythrorhynchos*; n = 8) from hatching to fledging, 31 May to 1 Aug. 2012 and the polynomial predictive model.

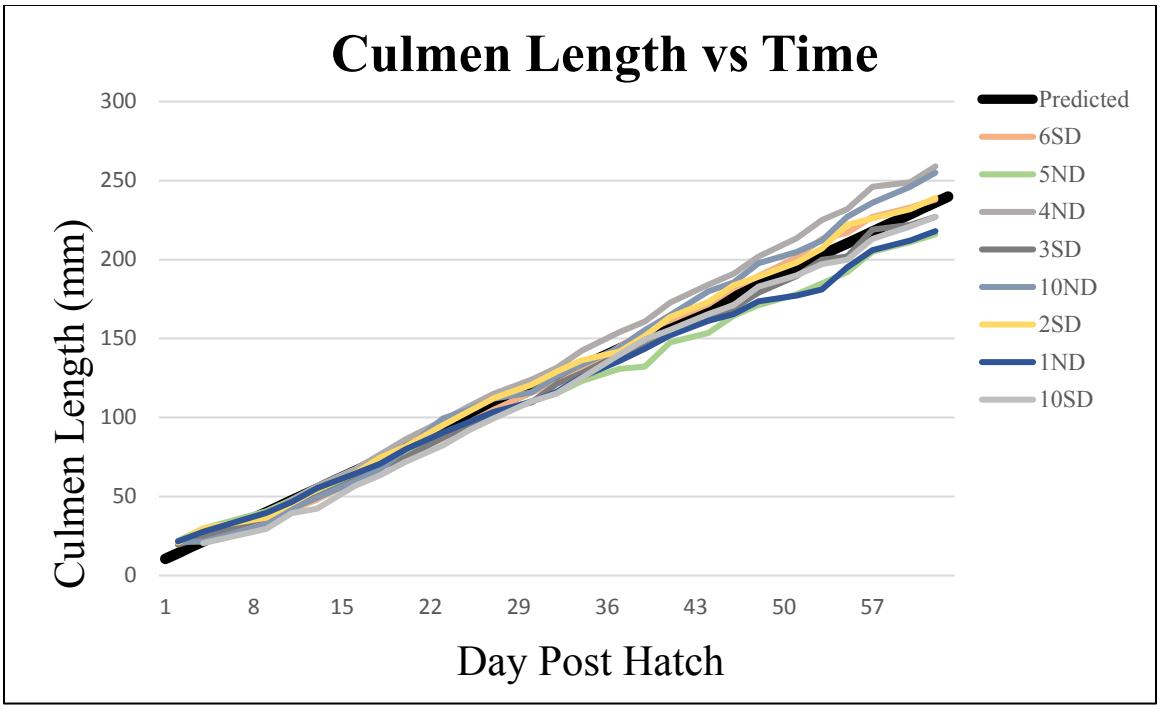

Figure 3.3 Culmen length for North Dakota (ND) and South Dakota (SD) captive raised American White Pelicans (*Pelecanus erythrorhynchos*; n = 8) from hatching to fledging 31 May to 1 Aug. 2012 and the linear predictive model.

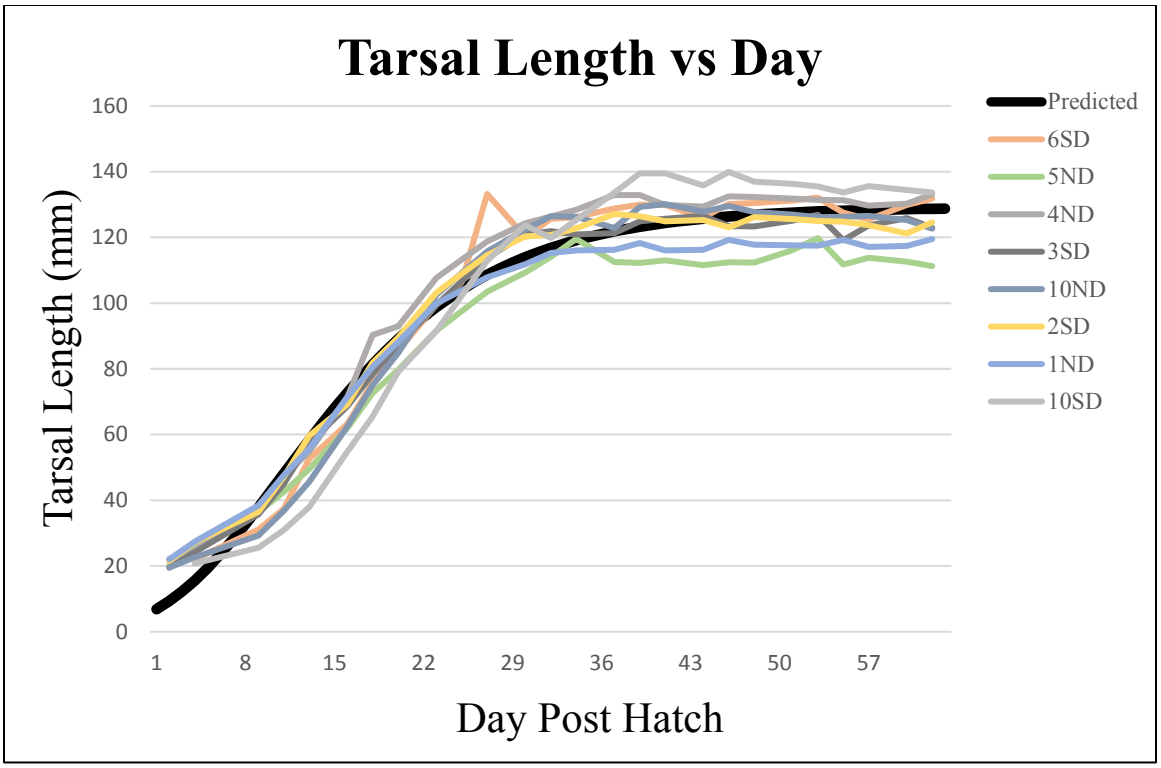

Figure 3.4 Tarsal length for North Dakota (ND) and South Dakota (SD) captive raised American White Pelicans (*Pelecanus erythrorhynchos*; n = 8) from hatching to fledging 31 May to 1 Aug. 2012 and the Gompertz predictive model.

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CHAPTER IV

IMPACT OF *BOLBOPHORUS DAMNIFICUS* ON AMERICAN WHITE PELICANS (*PELECANUS ERYTHRORHYNCHOS*) DURING A PERIOD OF GROWTH

Introduction

In the Mississippi Alluvial Valley (Mississippi Delta), commercial aquaculture of Channel Catfish (*Ictalurus punctatus*) is of great economic importance. Ponds were first established in 1965 (Wellborn 1988) and have since undergone extensive expansion. As commercial catfish ponds have increased in number so have numbers of various piscivorous bird species (Glahn and King 2004, Overstreet and Curran 2004). One common avian predator of commercial catfish is the American White Pelican (*Pelecanus erythrorhynchos*; King 1997, 2005). In one day a flock of 250 American White Pelicans (hereafter, AWPE) may consume $3,000 worth of catfish, an average of 2750 catfish/day valued at $1.54 kg (Glahn and King 2004). In addition to causing great economic loss in catfish production through predation of catfish, AWPE have also been identified as the definitive host for *Bolbophorus damnificus* (*B. damnificus*), a digenetic trematode (Kinsella et al. 2004; Overstreet and Curran 2004, Doffitt et al. 2009; Yost et al. 2009). AWPE have been reported to perpetuate the spread of this trematode between aquaculture facilities with devastating effects to the commercial catfish industry.

In a study conducted by Doffitt et al. (2009) 2 AWPE were artificially challenged with 182 and 156 *B. damnificus* metacercaraie, the adult stage of *B. damnificus* in the AWPE. They consumed 12-14 infected fish containing the metacercaraie over a period of

7 days. Both AWPE shed eggs 3 days post infection, with one of those pelicans continuing to shed intermittently during the rest of the trial (~5 weeks). Necropsies were performed for both birds and no negative impacts of the *B. damnificus* parasite were reported (Doffitt et al. 2009). Natural infection of *Bolbophorus spp*.in adult AWPE have been routinely reported (Overstreet and Curran 2005,) with parasite loads as high as 118± 233 (Kinsella et al. 2004). Captive adult AWPE have been previously infected with the *B. damnificus* trematode in 3 different trials, however effects of the trematode on pelicans have not been reported (Doffitt et al. 2009, Yost et al. 2009, Overstreet et al. 2002). The number of helminth species (including *B damnificus*) examined by Kinsella et al. (2004) in dead AWPE ranged from 3-17 (average 11). Most of those birds examined were reported to have died from acute illness or trauma, however evidence of regurgitation prior to death was noted (Kinsella et al. 2004). Negative impacts of similar digenetic trematode *Ascocotyle (phagicola) longa* have been reported in the avian host *Ardea cocoi* Linnaeus (Aves, Ciconiiformes, Ardeidae) by Barros et al. (2002). *Ascocotyle (phagicola) longa* is similar to *B. damnificus* in that it matures in the small intestine later shedding eggs that will also go on to infect a snail intermediate host. Negative impacts observed by Barros et al. 2002 include ataxia, undernourishment, reduced muscular mass of sternum and lesions in the gastrointestinal tract of the infected definitive avian host. Digeneans *Phagicola longus* and *Mesostephanus appendiculatoides*, (both also occurring in the AWPE) have been reported to distort host tissues and produce and inflammatory response in Brown pelican, but the effects often depend on the number of parasites present in addition to other factors such as a secondary bacterial infection (Overstreet and Curran 2005). Nestling brown pelicans 4–5 weeks old from Louisiana contained an

average of 1,112 *Phagicola longus* per bird of along the small intestine and ceca, many in the mucosa and lamina propria. The parasite was attached to the villar tips and occasionally penetrated the epithelium (Humphrey et al. 1978, Greve et al. 1987).

Significant losses in catfish production have been reported in the Delta of Mississippi, with annual losses of $27 million attributed to *B. damnificus* infection of catfish in aquaculture facilities (Wise et al. 2008). Severe to moderate outbreaks of *B. damnificus* reduced economic returns to a point where they could not cover the cost of production (Wise et al. 2008). Ponds with low prevalence rates (1-33%) reduced economic returns by 61% (Wise et al. 2008). In another study conducted by Wise et al. (2013) the effects of sub lethal and chronic trematode infection impacts on fish production were studied. Mild infection reduced feed consumption, fish production, and economic returns (Wise et al. 2013). However, once the snail (*Planorbella trivolvis*), the source of trematode infection (cercaria) was removed, growth rate, weight gain, feed consumption and feed efficiency were equal or greater than trematode negative fish (Wise et al. 2013), meaning once metacercarial cysts are fully developed there may be little impact on performance. However, negative impacts upon penetration of *B. damnificus* cercaria into the fish and its development to the mature metacercariae are associated with morbidity and mortality. Hemorrhaging, kidney tubule necrosis, kidney inflammation and poor growth rates have been reported in infected catfish (Doffitt et al. 2009, Overstreet et al. 2002, Terhune et al. 2002, Yost et al. 2009). Although *B. damnificus* infection has been reported to be detrimental to catfish, little is known about the effects of natural infection in AWPE. Artificial infections of *B. damnificus* in adult AWPE did not indicate any negative effects of the parasite, even when high doses of

infected catfish were consumed (Doffitt et al. 2009, Yost et al. 2009 and Kinsella et al. 2004). Because *B. damnificus* had previously been reported to reduce growth and or negatively impact health of various host species (Doffitt et al. 2009, Overstreet et al. 2002, Terhune et al. 2002, Yost et al. 2009) it was necessary to investigate effects of B. *damnificus* on the definitive host, the AWPE. In order to determine whether the parasite *B. damnificus* had any effects on American white pelicans growth, various parameters such as daily intake, daily body weight, culmen and tarsal lengths were measured and compared between 8 parasite infected (treatment) and 8 non-infected (control) birds.

There is no current information on *B. damnificus* in growing AWPE from hatching to fledging (~ 9 weeks of age) and the impact on metabolism at this age. The 2 primary objectives were to determine if young AWPE could become infected with *B. damnificus* within the first two weeks of life, and to determine if there are any detrimental effects of infection on the birds from hatching to fledging. This research has implications for commercial aquaculture production as well as conservation and management of AWPE.

Materials and methods

During late May 2012, 100 viable AWPE eggs were collected from Chase Lake, ND and Bitter Lake, SD colony sites. Eggs were transported in coolers lined with protective dry fast foam padding and were kept warm (37.5^{o}C) with a heating pad using a digital thermometer. A spray bottle filled with water was used to moistened eggs every ~4 hours. Upon arrival eggs were incubated at the USDA, Wildlife Services, National Wildlife Research Center's biosecurity level 2 laboratory on Mississippi State University campus. Eggs were incubated at 37.5^{o}C (Sportsman Cabinet Egg Incubator 1502

equipped with a 5 gallon water reserve system) and maintained at 60% humidity. Eggs were monitored daily and began to hatch immediately (29 May 2012). Eggs from both North Dakota and South Dakota hatched at a rate of 100%. Thirty six eggs were allowed to hatch over the next 3 days and were maintained for ~1 week. Of the first 20 pelicans hatched sixteen were selected randomly for this captive trial. An equal number of pelicans were selected from each major breeding colony (8 from North and 8 from South Dakota) in order to have a larger representative sample. The *B. damnficus* challenge took place at the Mississippi Field Station where pelicans were raised from hatching through fledging (~9 weeks). The parasite trial began on 29 May 2012 (known in Figures as day -2) and ended on 30 July, 2012.

When the eggs began piping the room temperature was reduced to ~36.0ºC and monitored using a digital thermometer. A dehumidifier was used to keep the humidity of the room at ~60%, also measured by a digital monitor. Once chicks emerged from the eggs, they were not fed for 12-24 hours to allow the nutrients in the placental lining of the egg to be absorbed. Chicks were then placed in plastic coated wire cages 0.5 m x 0.5 m x 0.8 m equipped with heating lamps covering ~30% of the cage 12-24 hours post-hatching. Cages were also equipped with black rubber foam pads ~25 cm long and 15 cm wide to allow chicks a softer alternative to wire flooring to reduce potential foot problems. Additional chicks not selected for use in the trial were euthanized following the 2007 AVMA Guidelines on Euthanasia. A diet was formulated using information gathered from a previous trial looking at the nutrient content of regurgitate matter fed to growing pelicans (T. Ferguson unpublished data). Fish were cut up and (or) thawed prior to each feeding. Pelican chicks were fed an ad libitum diet composed of Channel Catfish

(*Ictalurus punctatus*), specific pathogen free (SPF) catfish (*Ictalurus punctatus*), Gizzard Shad *(Dorosoma cepedianum)* and Menhaden (*Brevoortia patronus;* T. Ferguson unpublished data).

During the first week pelicans were fed 4 times a day, for the next 2 weeks 3 times a day, and from ~3 weeks of age and older pelicans were fed twice daily (once in the morning and once in the afternoon). Once chicks were accepting whole fish at ~3 weeks of age (June 22) they were transported to the USDA/WS National Wildlife Research Center's outdoor research aviary on the Mississippi State University campus. Each chick was banded and placed in an individual pens measuring 115.6 cm x 58.4 cm x 147.3 cm. Pens were additionally equipped with a heat lamp covering ~30% of the pen, which allowed the pelican to move freely under the heat lamp. The pelicans were housed in the pens until they were ~9 weeks old (fledging).

Sixteen pelicans originally designated for the parasite trial were divided into two groups, uninfected controls (n = 8) and birds infected with the digenetic trematode *B.* damnificus (n = 8). To determine the effect *B. damnificus* had on infected AWPE food intake, body weight, culmen length, tarsal length and temperature were examined. Pelicans were weighed once daily in the morning, prior to feeding. Intake was recorded daily, culmen and tarsal lengths were measured to the nearest 0.02 mm every 3 days. Culmen and tarsal measurements were taken by removing the pelican from the pen and having one person hold the bird while another measured using a dial caliper (to the nearest 0.02 mm) or using a steel rule. Additionally half of each group, 4 uninfected controls and 4 parasite infected pelicans were implanted subcutaneously with IPTT-300 (Bio Medic Data Systems) temperature monitoring devices between the shoulders as per

manufacturer guidelines. Temperatures were recorded beginning on 2 July through the end of the trial (also known as weeks 6-9) to determine whether parasite infected pelicans regulated temperature differently than parasite free birds.

Two parasite exposures successfully infected the pelicans (n = 8) with the *B. damnificus* trematode. Pelicans at ~9 days of age were infected with the *B. damnificus* trematode (8 June 2012). Metacercariae (18 each) were surgically excised from *B. damnificus* specific pathogen free (hereafter, SPF) infected catfish. Excised metacercariae were fed by gavage to pelicans using a stainless steel bulbed inoculum syringe, prior to feeding SPF catfish (Doffitt et al. 2009, Yost et al. 2009). Fecal samples were collected for all birds (n = 16) and examined for trematode eggs for 6 days post infection using a modification of the fecal sedimentation technique described previously in Doffitt et al. (2009) and Yost et al. (2009). A second challenge was done with the same treatment birds with *B. damnficus* positive fish when pelicans were ~22 days of age (21 June 2012). Prior to feeding a subsample of the infected fish was taken and it was estimated that the fish were infected with approximately 29 metacercariae per fish (range 27-32). Feces for treatment birds (n = 8) were collected in the 6 days following exposure and were first confirmed *B. damnificus* positive using modified sedimentation technique (Doffitt et al. 2009, Yost et al. 2009). Eggs were molecularly confirmed to be *B. damnificus* using species specific primers protocols developed in Griffin et al. 2010. *Bolbophorus damnificus* eggs (2-3) collected from each treatment bird (n = 8) were also confirmed morphologically between 123–129 μm long (Overstreet et al. 2002, Doffitt 2011). Sedimentations were also performed 6 days post challenge for all control birds (n = 8) confirmed negative, meaning no shedding of trematode eggs occurred.

This research was conducted under United States Department of Agriculture, National Wildlife Research Center, Animal Care and Use Committee study protocol QA-1794 and applicable federal and state permits.

Statistics

To determine the effect of the *B. damnificus* infections on growing pelicans data were analyzed using R© (Package 2.15.3). The control group was considered parasite free with the treatment group as parasite infected. Parameters compared were food intake, body weight, culmen length, tarsal length and body temperature (probe), weekly, for over a period of 9 weeks. Several models were compared using the package "lmer" in R© and model fit was assessed using Akaike's Informative Criterion (AIC: Field et al. 2012). The information-theoretic method was used for model selection with numerous potential explanatory variables, as well as their interactions (Anderson and Burnham 2002). All models contained fixed effects (day, week, parasite, probe, state) and random effects (Bird ID) measured over time. Probe in the model represents temperature implant. State represents the origin of the pelican: North Dakota or South Dakota. There was an additional fixed effect for only the temperature model known as feeding (temperature was taken twice a day following feeding). After comparing all models, the model with the lowest AIC was selected as the model of best fit. The model was then analyzed using the "nlme" package in R© to determine significance, with p-values of ≤ 0.05 considered statistically significant.

Data collected for control (parasite negative) birds (n = 8) are reported and incorporated into the statistical analysis of this trial for comparison. Since pelican gender

was not determined, the effect of sex is unknown. All values reported in the results section are means ± SD.

Results

Two parasite exposures successfully infected the pelicans (n = 8) with the *B. damnificus* trematode. All treatment birds were confirmed positive after the second inoculation at 3-4 weeks of age. No trematode eggs were observed in the feces of control birds. There was no effect of B. damnificus infection on growth of AWPE from hatching to fledging.

The model selected to best fit the culmen data had an AIC value of 3597.72 (df=16): Culmen<-lme(Culmen~day+week+parasite+probe+state, random=~1|BirdID, data=Culmen). There was no significant effect of parasite (P = 0.214), probe (P = 0.517) or state (P = 0.695) on growth. There was an effect of week for both parasite infected and control pelicans on culmen length (P <0.001). Average culmen length for treatment (n = 8) and control pelicans (n = 8) is linear in shape (Figure 4.1).

The final model selected best representing the tarsal data had an AIC of 3189.96 (df=15): Tarsal<- lme(Tarsal~day+week+parasite+probe+state, random=~1|BirdID, data=Tarsal). There was no effect of parasite (P = 0.306), probe (P = 0.843) or state (P = 0.589). There was an effect of week on tarsal length (P <0.001). Tarsal length growth of treatment and control pelicans is depicted in Figure 4.2.

The final model selected to best represent body weight data had an AIC value of 1803.0 (df=13): BodyWeight<-lme(BW~week+parasite+probe+state, random=~1|BirdID, data=Intake). There was no significant effect of parasite (P = 0.884), probe (P = 0.786), or state (P = 0.914). There was a significant effect of week (P <0.001)

on growth. Daily body weights of treatment (n = 8) and control (n = 8) pelicans peak around the end of week 8 (Figure 4.3).

The final model selected to represent weekly food intake data had an AIC value of 1716.77 (df=14): WeeklyIntake<-lme(WI~week+parasite+probe+state, random=~1|BirdID, data=Intake). There was no significant effect of parasite (P = 0.963), probe (P = 0.564), or state (P = 0.914) but, there was an effect of week (P <0.001). There were no differences between average daily intake for control and treatment pelicans (Figure 4.4).

No difference was observed between the average daily body temperature of treatment and control pelicans (Figure 4.5). The average temperature for treatment pelicans was 39.18 ±0.55ºC. The final model selected to best represent the temperature data had an AIC value of 1123.67 (df=7): Temperature<-lme(Temp~week, random=~1|BirdID, data=Intake). The model of best fit did not include the fixed effects parasite, probe or state. Temperature, only taken in the last 4 weeks of the trial (weeks 6-9) was not significant for week 6 (P = 0.069), week 7 (P = 0.362), week 8 (P = 0.747) or week 9 (P = 0.729).

It should be noted that 11 of 16 pelicans during the last week of the trial became infected with West Nile Virus (hereafter, WNV). Five of the 11 WNV infected pelicans were also infected with the *B. damnificus* trematode. Pelicans displaying clinical signs of WNV during the last week of the trial were confirmed WNV positive (through reverse transcriptase PCR/serology) once the trial concluded. Although these pelicans tested positive for WNV infection, WNV status was not included in the model since its effects were considered statistically non-significant.

Discussion

Initial attempts to infect AWPE were not successful when pelicans consumed surgically excised metacercariae confirmed morphology and molecularily *B. damnificus*. Perhaps the metacercariae alone were partially digested and too damaged to develop into patent adults, or perhaps pelicans were too immature to develop infected. However, the second attempt to infect pelicans was successful when pelicans were fed *B. damnificus* metacercariae contained within the tissue of catfish that had been confirmed both morphologically and molecularly to be *B. damnificus*. Other studies have also had success infecting AWPE using whole catfish infected with the *B. damnificus* parasite (Doffitt et al. 2009, Yost et al. 2009, Overstreet and Curran 2002). Perhaps feeding tissue containing the metacercariae allowed enough protection from digestion that the viability of parasites was increased. All pelican chicks (n = 8) infected with the *B. damnificus* parasite developed patent infections within 2-3 days post exposure, similar to adult pelicans infected by Doffitt et al. (2009). As pelican chicks in the wild typically consume regurgitate (Pinson and Drummond 1993) containing parts or whole regurgitated fish, it is possible they may become infected during chick rearing. This is the first study demonstrating that AWPE chicks can become infected with *B. damnificus* as early as 3 weeks of age. The *B. damnificus* life cycle in ~3 week old chicks in this trial was similar to adult AWPE, with chicks developing patent infection 3 days post challenge and continuing to shed ova.

The effect of the parasite *B. damnificus* on growth of the culmen from during hatching to fledging has not been previously reported. There was an effect of week on culmen length ($P < 0.001$) meaning that growth for pelicans (both parasite infected and

free) each week was different. There was however no significant effect of the parasite (P = 0.214) on culmen growth. However, there remains the possibility that a non-significant effect may have been confounded by some other factor such as sex, which was not examined in this study. Significant growth each week of the trial suggests pelicans whether parasite free or infected grow at a rapid rate from hatching till fledging.

There was no difference between initial or final tarsal length between parasite free and parasite infected birds (P = 0.306) meaning there was no effect of the parasite *B. damnificus* on growth of the tarsus. The growth of the tarsus was significant for both parasite free and parasite infected pelicans by each week (P <0.001). Significant growth between each week indicates rapid growth of the tarsus for parasite infected (and parasite free) pelicans from hatching to fledging. Although the parasite did not seem to diminish rapid growth of the tarsus it is notable that the growth of the tarsus for all birds seemed to level off around week 5 (Figure 4.2). Growth of the tarsus for growing AWPE infected with the trematode *B. damnificus* has not been previously reported.

Initial or final body weights for parasite infected and parasite free pelicans did not differ statistically indicating that the parasite *B. damnificus* did not have a significant effect on body weight. Body weights for parasite infected pelicans are depicted in Figure 4.3. Body weight for both groups increased during the first 8 weeks and declined the last week of the trial. Although there was a decrease in intake and body weight for WNV infected pelicans, there was also a decline in body weight and intake for pelicans that were identified as not infected with WNV, meaning this may also be a naturally occurring phenomenon (Schrieber 1976). It is hypothesized that during this last week pelicans are dropping weight in preparation for flight, or may have increased energetic

demands as they practice flapping. Additional factors affecting intake or body weight may include environment as there were several periods of high heat (~40^{o}C) during the trial. The small irregularity (Figure 4.3) occurring at day 22 (22 June 2012) is likely due to the movement of pelicans from the indoor facility to the outdoor facility. Pelicans are highly sensitive to stress often resulting in regurgitation or refusal to eat (Ferguson et al. 2011).

Intake for parasite infected and parasite free groups is depicted in Figure 4.4. On day 22 (22 June, 2012) there was a sharp decrease in intake for all pelicans as during this time pelicans had been transported to an outdoor facility. Intake during the last week of the trial (day 53 to 60) decreased, but was similar between groups. This may be due to several factors. Conversely, there might be increased energetic demands nearing flight as pelicans increase the frequency of flapping wings as they get older. Intake each week differed significantly as pelicans were growing at rapid rate, and then during the last week, losing weight at a rapid rate. Since there was no effect of the parasite, body weight for parasite infected pelicans did not seem to be diminished nor increased over time, meaning appetite for infected pelicans was not adversely affected.

Body temperatures for uninfected nor parasite infected AWPE (hatching to fledging) have not been previously reported. Body temperatures while not significantly different between parasite infected and parasite free pelicans are both shown in Figure 4.5. Variation in temperature for pelicans ranged from 38.3^{o}C to 40.3^{o}C. Although not considered statistically significant, it is notable that pelicans consuming the parasite infected diet did experience a high temperature during day 54 followed by an immediate drop until day 58 (Figure 4.5). Less variability was reported in uninfected pelicans

overall, including days 54-58 (Figure 4.5). Three of 4 parasite infected/temperature implanted pelicans were later confirmed WNV positive (at the end of the trial). An increase in temperature may indicate that bird is fighting illness (Gray et al. 2012). The larger variation in temperature could be due to pelicans fighting the onset of WNV, differences in exposure, or perhaps prior infection. Although *B. damnificus* infection did not affect daily average temperature, it may have made pelicans more susceptible to secondary disease (Barros et al. 2002). Temperature was not significantly different for week 6 ($P = 0.069$), week 7 ($P = 0.362$), week 8 ($P = 0.747$) and week 9 ($P = 0.729$). This agrees with previous research that the ability of birds to maintain temperature improves with age (Sachi and Jodice 2009). Infection with the *B. damnificus* parasite did not seem to affect the natural development of temperature maintenance by growing pelicans.

Overall growth of the tarsus and culmen along with intake, body weight and temperature data collected on all pelicans was not different between treatment groups. Parasite infected birds did not show reduced growth for any measured parameter. No other negative effects such as ataxia, reduction in muscular mass, reduced growth or undernourishment were observed in infected pelicans as observed in other trematode infected waterbirds (Barros et al. 2002, Overstreet at al. 2002) or other infected fish species (Overstreet and Curran 2005, Terhune et al. 2002, Wise et al. 2013). Perhaps the infection was just not strong enough, or some other factor such as sex may have confounded the data. It is also possible that physical changes or clinical signs may have bene observed if the length of the trial was extended. Although this trial indicates there were no significant effects on growth of the pelican without an internal examination of

tissues following necropsy, it could not be determined whether there was any distortion of host tissues or inflammatory response of infected pelicans (Overstreet et al. 2005).

In addition to providing essential data on the effects of the trematode *B. damnificus* in juvenile pelicans, data collected during this trial on culmen, tarsal, intake, and body weight could also be used to create a model to age pre-fledged chicks. The level of infection of *B. damnificus* in this trial could be considered mild, because pelicans may be exposed to increased levels of this parasite in their everyday environment. Fish infected with the parasite *B. damnificus* parasite have been known to contain numerous metacercariae encysted within their skin (Doffitt et al. 2009). In this trial all infected pelicans developed a patent infection following an average dose of 29 metacercariae. In the wild it is approximated that pelicans may consume up to 1800 grams of fish on a daily basis (Johnsgard 1993). In a captive setting AWPE were reported to consume ~2,000 grams of fish daily (Ferguson et al. 2011). Pelicans consuming catfish from *B. damnificus* infected ponds could consume several infected fish in a day, meaning the degree of infection may be more. This was shown in a study conducted by Doffitt et al. (2009) where pelicans consumed between 12-14 infected fish over a 7 day period, containing up to 182 metacercariae. Although data in this trial suggest that there is no effect of a small level infection of *B. damnificus* on pelicans in this trial, the possibility exists that increased infection may impose negative impacts on growth. Further studies are required to determine the impacts of a greater infection of *B. damnificus* on AWPE whether juvenile or adult.

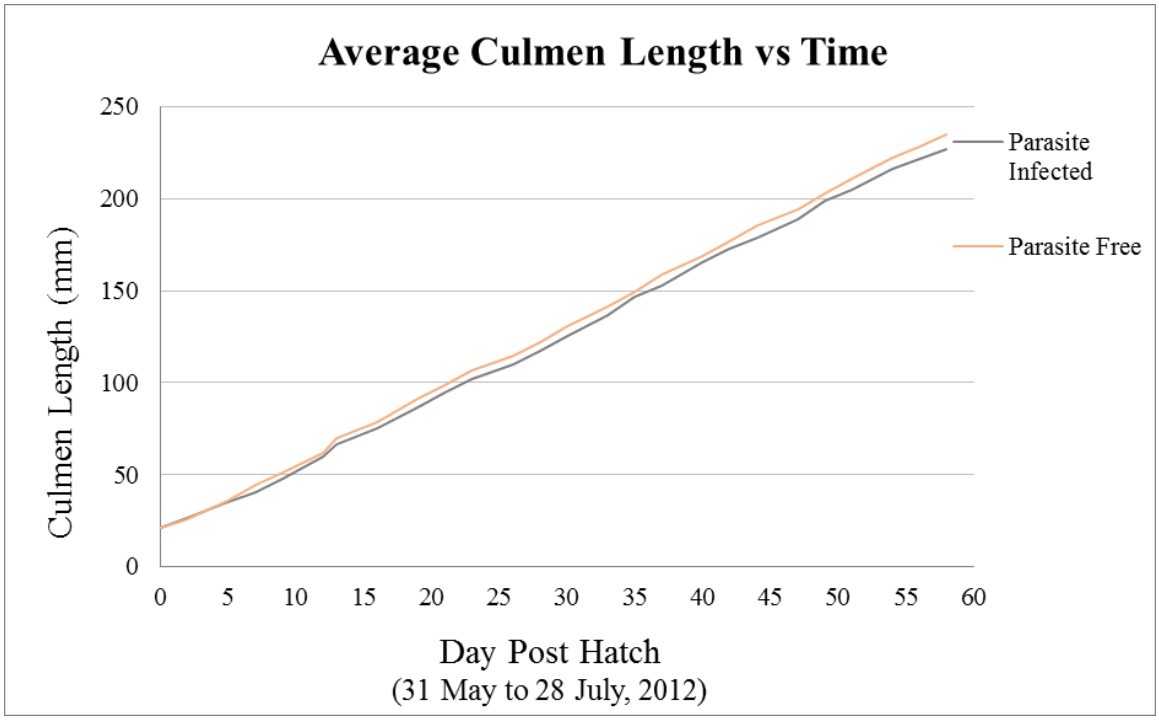

Figure 4.1 Culmen length for parasite infected (treatment) and parasite free (control) American White Pelicans *(Pelecanus erythrorhynchos)* measured from hatching to fledging 31 May to 28 July 2012.

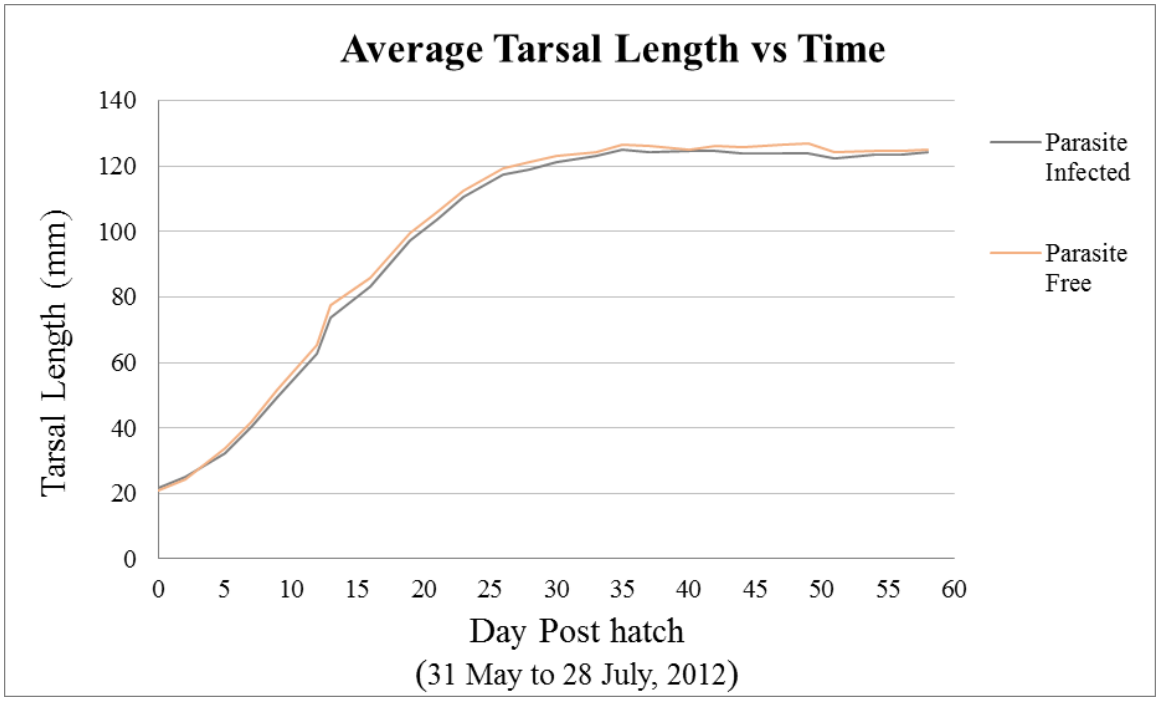

Figure 4.2 Growth of tarsus for parasite infected (treatment) and parasite free (control) American White Pelicans *(Pelecanus erythrorhynchos)* measured from hatching to fledging 31 May to 28 July 2012.

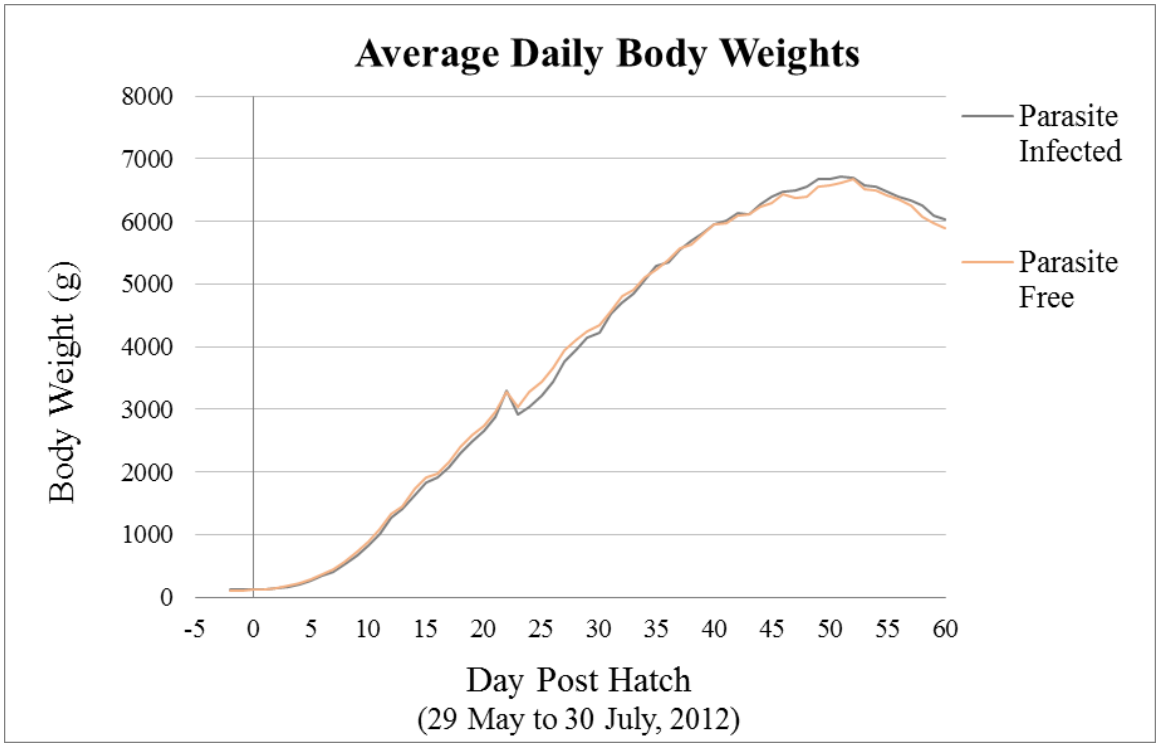

Figure 4.3 Body weights of parasite infected (treatment) and parasite free (control) American White Pelicans *(Pelecanus erythrorhynchos)* measured from hatching to fledging 29 May to 30 July 2012.

Negative days represent pelicans born early, the trial officially began when all birds (n = 8) were hatched (day 0).

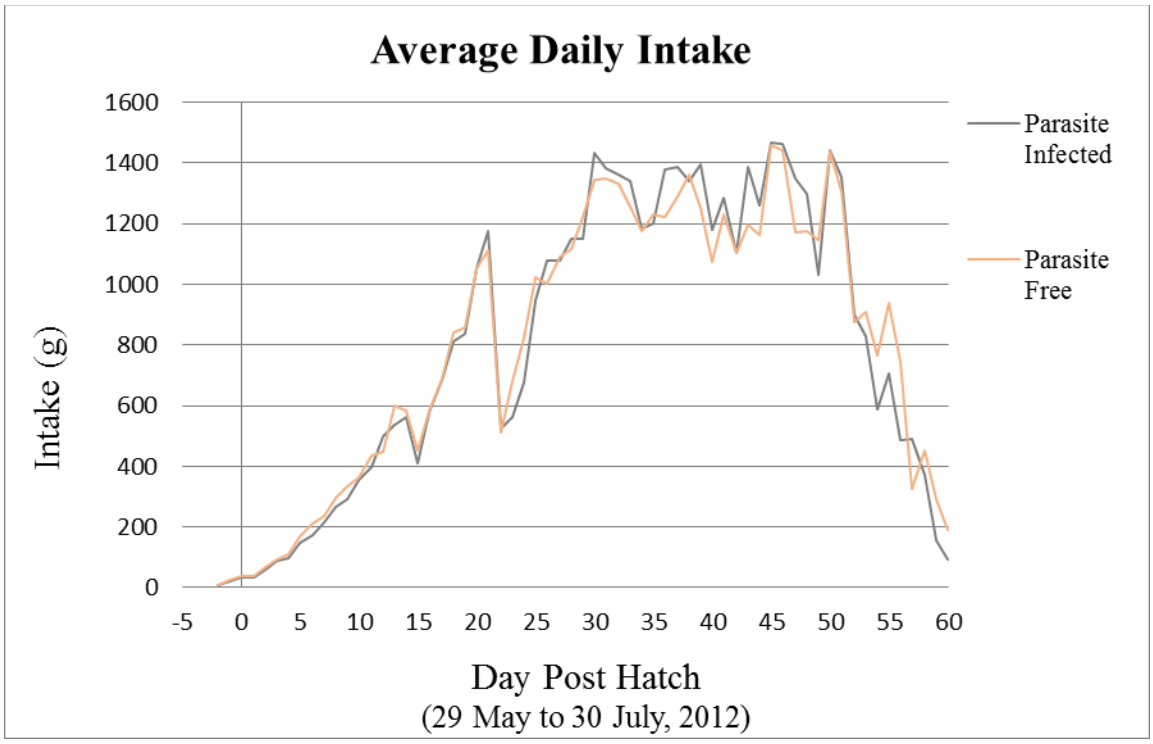

Figure 4.4 Daily intake of parasite infected (treatment) and parasite free (control) American White Pelicans *(Pelecanus erythrorhynchos)* measured from hatching to fledging 29 May to 30 July 2012.

Negative days represent pelicans born early, the trial officially began when all birds (n = 8) were hatched (day 0).

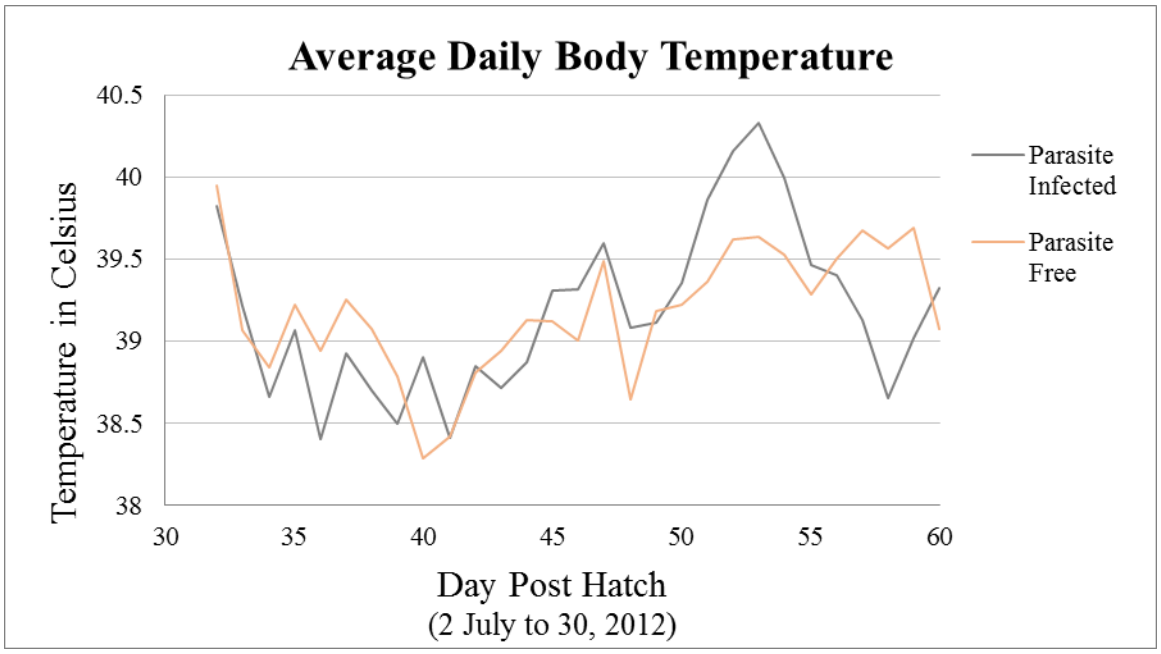

Figure 4.5 Daily temperatures of parasite free (n = 4) and parasite infected (n = 4) American White Pelicans *(Pelecanus erythrorhynchos)* taken from 2 July to 30 July 2012.

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CHAPTER V

NATURAL WEST NILE VIRUS INFECTION IN CAPTIVE RAISED AMERICAN WHITE PELICANS *(PELECANUS ERYTHRORHYNCHOS)*

Introduction

West Nile virus (WNV) reported to infect the wild American White Pelican (*Pelecanus erythrorhynchos*; Johnson et al. 2010) and is a mosquito-borne zoonotic arbovirus belonging to the genus *Flavirirus* and Family Flaviviridae. West Nile Virus first emerged in the eastern North America in New York in 1999 (strain known as NY99) and was first isolated from a dead American Crow (*Corvus brachyrhynchos*; Lanciotti et al. 1999). Since then WNV infection has spread and has now been confirmed in at least 198 species of birds in North America (Komar 2003a). Clinical signs such as lethargy and recumbence, and less frequently, pathological signs such as multi-organ hemorrhage have been reported for various affected bird species (Komar 2003a). Several species of birds infected with WNV have presented with brain hemorrhage, meningoencephalitis, splenomegaly, and myocarditis (Steele et al. 2000; Swayne et al. 2001).

Komar (2003a) reported WNV is most commonly transferred to birds through mosquito bites, and death often results from multiple organ failure as numerous cell types are damaged in various tissues. Transmission of WNV from bird to bird has been documented in captive populations of chickens, crows and geese (Langevin et al. 2001; McLean et al. 2001; Banet-Noach et al. 2003; Austin et al. 2004). Komar (2003a)

demonstrated that in a lab setting 3 bird species (Blue Jays, Black-billed Magpies and Ring-billed Gulls; *Cyanocitta cristata, Pica hudsonia, Larus delawarensis*, respectively) can transmit WNV through direct contact (Komar 2003a). Strong evidence suggests WNV may be vertically transmitted in mosquito hosts (Miller et al. 2000), with vertical transmission confirmed in 3 different Culex species by Goddard et al. (2003). Intrauterine WNV transmission has also been reported in one young human female with the onset of disease occurring in week 27 of gestation, however 74 other women infected with WNV during pregnancy gave birth to apparently healthy infants (Hayes et al. 2005). Probable transmission of WNV through breast milk was also reported in 2002 in a 40 year old female, infected post-delivery, with WNV nucleic acid detected in her infant (Hayes et al. 2005). Although vertical transmission of WNV has been reported in several non-avian species, little evidence suggests that WNV is vertically transmitted in AWPE. Although unlikely, vertical transmission of WNV in avian species was suspected by Komar et al. (2003b) as he reported a low-level persistent infection in the ovary of a common grackle 11 days after termination of detectable viremia. However, no evidence currently exists to suggest that AWPE transmit WNV vertically.

Although WNV has been confirmed in numerous bird species, much is unknown about the clinical and physiological changes associated with infection (Komar 2003a). American White Pelicans exposed to West Nile Virus in the northern Great Plains colonies experienced great losses from 2006-2008 with mortality rates ranging from 25% to 36% (Sovada et al. 2008). Because many American White Pelicans and other bird species are greatly affected by WNV it is important to further detail limited information available regarding the progression of the disease.

American White Pelicans (hereafter AWPE) are large aquatic birds that spend summer months in southern Canada and the northern United States and migrate to Central America, Mexico, and the southern United States during the winter. Observation of WNV in AWPE was first documented in 4 major colonies located in the northern Great Plains in 2002: Chase Lake National Wildlife Refuge (NWR) in North Dakota, Bitter Lake (Waubay NWR) in South Dakota, Medicine Lake NWR in Montana, and Marsh Lake in Minnesota (Rocke et al. 2005). Unusually high numbers of dead pelican chicks were observed by Sovada et al. (2008) at these colonies in 2002 and 2003, and were later attributed to the arrival of WNV in the region. Juvenile pelican mortality was estimated to have increased until fledging during mid-July from 4% to as high as 44% (Johnson et al. 2010). The AWPE dying from WNV are typically older chicks ranging from 4 to 12 weeks of age (most 6 to 8 weeks old) and 20-74% of fledgling pelicans sampled in various northern Great Plains colonies presented with antibody titers to WNV (M. Sovada personal communication). In the Mississippi flyway WNV has been reported to be the greatest mortality factor for AWPE causing 4 significant die offs in this region resulting in an estimated 5,464 mortalities (Rocke et al. 2005).

AWPE infected with WNV may exhibit a variety of clinical signs such as torticollis, reduced mobility, and ataxia (Johnson et al. 2010). The number of days from infection to clinical signs in the AWPE are unknown. Johnson et al. (2010) reported pelican deaths were observed on average 23 days prior to onset of human clinical symptoms, meaning pelicans may serve as indicators for human disease. In young AWPE the greatest viral loads of WNV were detected in brain, heart, skin, and feather pulp, with skin as the most efficacious tissue for detection (Johnson et al. 2010). The high incidence

and rapid spread of WNV in northern Great Plains colonies suggests that AWPE may transmit the virus from bird to bird; however, reports have confirmed the primary route of transmission to be vectored through mosquitos (M. Sovada Personal communication).

In July 2012 a WNV outbreak occurred during a captive pelican trial conducted at the USDA WS National Wildlife Research Center (NWRC) aviary on the Mississippi State University campus. The WNV was confirmed in 21 of 27 naive hand-reared AWPE through either reverse transcriptase PCR detection, complement fixation serological testing, or by histological changes ascribed to WNV. Because little is known about disease dynamics within pelicans, the objective of this trial was to describe the clinical signs of WNV and progression of disease in captive-held AWPE with a special emphasis on 2 case studies.

Study area and methods

During late May 2012, viable AWPE eggs were collected at Chase Lake, ND and Bitter Lake, SD colony sites (50 from each colony) and incubated at the USDA, Wildlife Services, NWRC's BSL 2 laboratory on the Mississippi State University campus until hatching.

Pelicans were raised as part of a captive energetics/parasite trial describing conducted at the USDA, Wildlife Services, NWRC's aviary. Eggs hatched from 29 May to 2 June 2012 and chicks were removed from incubators and placed in individual pens (0.5 m x 0.5 m x 0.8 m) and maintained at 36ºC. Each week the room temperature was decreased by 2 °C as birds developed thermoregulatory abilities. Each pen was equipped with a heat lamp (warming ~30% of the pen) to ensure an additional heat source was available. At ~3 weeks of age (22 June 2012) pelicans were transferred to the NWRC

outdoor research aviary. For the purpose of this trial the transfer of AWPE to the outdoor research aviary will be known as day 0, representing potential initial exposure to WNV (Table 5.1). The research aviary was comprised of a cement pad with drains and a metal roof supported by poles surrounded by security fencing and covered by aviary netting. No other animals were housed inside the research aviary at this time; however, small birds or insects could pass through the netting. Within approximately a 2 km radius of the aviary several other animal species were prevalent such as cattle, horses, poultry, fish, and free-ranging wild birds. An adjustable shade cloth provided limited temperature regulation. Of the 27 pelicans raised in captivity, 16 were further selected for a trial involving energetics and were placed in individual metabolism pens specifically designed for pelicans measuring (115.6 cm x 58.4 cm x 147.3 cm). Eight of the 16 pelicans were implanted subcutaneously with IPTT-300 (Bio Medic Data Systems) temperature monitoring devices between the shoulders as per manufacturer guidelines. The remaining 11 pelicans were kept at the same facility and were divided into 5 larger pens measuring 3 x 3 x 2 m. Four contained 2 birds, and one pen contained 3 birds. The larger pens were equipped with water tanks (132 x 79 x 36 cm) with ramps into those tanks. Tanks were emptied and refilled with fresh water daily. Each group pen and individual pen was equipped with a heat lamp allowing the chicks to brood as needed.

Pelicans were fed, ad libitum, farm-raised Channel Catfish (*Ictalurus punctatus*), specific pathogen free (SPF) channel catfish, Gizzard Shad *(Dorosoma cepedianum)* and Menhaden (*Brevoortia patronus*). Specific pathogen-free catfish were obtained from the SPF fish facility at Mississippi State University's College of Veterinary Medicine. Local farm-raised catfish were obtained from a nearby aquaculture facility and were euthanized

and fed immediately to the pelicans. Also, Menhaden and Gizzard Shad were obtained frozen from nearby commercial fisheries, thawed, and fed to the pelicans. Daily body weights, food intakes, ambient and body temperatures (n = 8) were recorded.

This research was conducted under United States Department of Agriculture, National Wildlife Research Center, Animal Care and Use Committee study protocol QA-1794 and applicable federal and state permits.

West Nile Virus in American White Pelicans

Twenty-one pelicans out of 27 became naturally infected with WNV (Table 5.2). Mosquitos and mosquito larvae (*Culex sp.)* were observed in drains located in the outdoor facility where the AWPE were kept and these areas were treated once with 10.31% *Bacillius thuringiensis israelensis* (Bti) following the end of the captive trial to eliminate mosquito populations (S. Lemmons personal communication). Following reports of WNV positive AWPE the Mississippi Department of Health sampled mosquitos in several locations including a location near the facility where AWPE were kept. Mosquitos were however tested at 4 and 8 weeks after the outbreak occurred and all mosquitos tested negative for WNV (Mississippi Department of Health personal communication). Although several pelicans displayed moderate to severe clinical symptoms and were later confirmed positive for WNV, only 2 of those pelicans had also been implanted with a temperature device. Therefore, a timeline was created detailing the progression of disease in those 2 infected pelicans (PCR confirmed) using information gathered on food intake, body weight and temperature.

During the time pelicans became naturally infected with WNV, 16 pelicans were participating in a captive trial studying the effects of the parasite *Bolbophorus*

damnificus. In order to control confounding factors, West Nile Virus was added into the overall model used to determine the effects of the parasite on various growth parameters and was determined to be non-significant. Several pelicans (group-housed) that became infected were not participants in the current trial and therefore individual bird data on growth parameters was not available. In some instances data that were collected on infected pelicans could not be analyzed due to low numbers of experimental units and inability to account for confounding factors (Table 5.2). Therefore, only observational data were collected on clinical symptoms and progression of disease.

Clinical Symptoms and General Progression

Symptoms were first observed in birds at 6 to 7 weeks of age in 3 group-housed birds on day 22 (calendar days listed in Table 5.1) after being moved outdoors. Initial observable clinical signs AWPE displayed were lethargy and wing droop. Of the 3 initial group-housed pelicans displaying clinical symptoms, 2 shared a pen, and another was housed in a nearby pen shared with an uninfected bird. After 24 hours of displaying lethargy and wing droop, all 3 AWPE showed a 20-50% reduction in food intake. During the period in which AWPE first began to exhibit clinical symptoms, the temperatures within the facility ranged from 16 to 40ºC, averaging 28ºC with an average humidity of 60%. Elevated temperatures of ~40ºC occurred twice during the trial for a period of 2-3 days each. During the first period of high heat no observation of wing droop or lethargy was made, however reduced intake was reported. Within 24 hours of a second period of high heat (~40ºC) was the first observation of clinical symptoms including wing droop and lethargy. Approximately 48 hours after the initial onset of clinical signs, one AWPE was unable to walk and was ataxic. At this time, 25 days after being moved outdoors, all

3 pelicans exhibiting clinical symptoms were euthanized and necropsied, where a presumptive diagnosis of WNV infection was made based on clinical symptoms.

Over the next several days additional AWPE exhibited similar and additional symptoms to the first 3, including 1 bird that excreted bloody material from the cloaca. Group-housed AWPE generally became infected first, followed by several pelicans housed in individual pens. There did not seem to be any pattern of infection observed among birds. Again on day 25, coinciding with euthanasia of the first 3 birds, 2 more non-trial birds housed in larger pens exhibited similar symptoms.

During days 30-38 several other pelicans in the group-housed pens and smaller pens began displaying wing droop accompanied by lethargy (Table 5.1). Wing droop appeared to be related to fatigue or reduced energy likely incurred by acquiring the WNV infection. AWPE could physically raise their wings up to a more normal position when startled, but would soon droop their wings. Within 24-48 hours after onset some pelicans were observed dragging their wings across the floor, causing wing abrasions. In conjunction with the reduced intake and wing droop other clinical signs were observed such as torticollis, ataxia, agitation and lethargy. Two pelicans appeared unable to straighten their necks which likely affected their food intake. Others had difficulty maintaining their balance and were ataxic within large and small pens. Agitation was usually observed during feedings where pelicans would become increasingly aggressive towards the handler often snapping at their hands but refusing to eat food. During periods other than feeding, pelicans were quiet and immobile, often resting in one area. At this stage AWPE exhibiting clinical symptoms were euthanized.

Four group-housed WNV infected AWPE experiencing an acute form of infection, progressed quickly to a severe state and shared similar symptoms with the other infected birds such as lethargy, wing droop, and reduced appetite. Within 48-72 hours they were unable to walk or raise their heads and were subsequently euthanized. Any AWPE that lost > 15% body weight and (or) displayed moderate-severe neurological symptoms was deemed unable to recover and was euthanized (NWRC protocol QA-1794 IACUC guidelines). AWPE typically survived 5 to 10 days post onset of clinical signs before being euthanized, but in the 4 cases exhibiting more severe clinical signs pelicans were euthanized within 72 hours of the onset of clinical signs. In the following week, due to rising concern for the pelican's welfare and to limit further exposure to mosquitos, the captive energetics/parasite trial was terminated and all remaining birds (n = 11) were moved to a secure indoor facility at the Mississippi State University College of Veterinary Medicine. AWPE were placed in individual pens (1-2 birds per pen; 1.2 x 2.7 m) in an environmentally controlled building maintaining a constant temperature of 21-24°C. As the indoor facility was completely enclosed (BSL 2) it limited any further exposure to infected mosquitos. Approximately 70% of AWPE were refusing fish prior to the move to the indoor facility but following the acclimation period (~24 hours) seemed to improve and once again began to consume a small portion of fish (200 to 600 g/day). Approximately 4 days after being moved to the secure indoor facility many of the pelicans (~95%) again began to refuse food. As most AWPE seemed to be declining in condition, it was decided to euthanize the remaining AWPE on 8 August 2012 to prevent any further suffering.

Progression of Disease in Case Studies

Two pelicans (7SD and 2ND) used in the captive energetics/parasite trial implanted with temperature implants began showing clinical signs on day 27 and 30, respectively. In both cases 1 day prior to observation of clinical symptoms there was a reduction in intake. Initial clinical symptoms observed in both birds included wing droop and lethargy, followed by increased agitation and aggression during feeding occurring within 72 hours of initial onset of clinical symptoms. Intake for Pelican 7SD decreased beginning on day 26 from 1112 g to 358 g on day 31, an overall reduction of intake of ~68% (Table 5.3). Pelican 7SD also experienced progressive weight loss of 550 g, beginning on day 27 ending on day 32 when it was euthanized at its final weight of 5696 g (Table 5.3). Additional clinical signs manifested in Pelican 7SD by day 32 including torticollis of the neck and immobility. At this point it was decided that Pelican 7SD along with 2 other pelicans displaying immobility would be euthanized. Pelican 2ND began showing clinical signs such as wing droop and lethargy on day 30. Intake for Pelican 2ND was reduced from 1303 g on day 29 to 407 g on day 30, eventually being reduced to 0 g on day 37. Overall from day 29 to day 37, there was a 100% reduction in intake. Pelican 2ND experienced a progressive weight loss of 681 g, beginning on day 31 ending on day 38 (Table 5.3). Again following immobility and neurological symptoms, this pelican was euthanized on day 38 at a final body weight of 5224 g.

Daily body temperatures were recorded for pelicans 2ND and 7SD. Normal bird temperatures are typically 40.0 ^{o}C $\pm$ 1.5 ^{o}C (Calder and King 1974) with AWPE (n = 12) temperatures reported as 40.48 ^{o}C $\pm$ 0.06 ^{o}C (T. King personal communication). Pelicans 2ND and 7SD experienced a slight elevation in body temperature followed by a decline

in temperature prior to euthanasia (Figure 5.1). Pelican 7SD experienced a temperature of 40.8ºC on day 31 followed by 39.7 ºC on day 32 by which point pelican 7SD had progressed to complete immobility and neurological symptoms, 4 days after initial observation of clinical symptoms. Pelican 2ND experienced its greatest body temperature of 40.7ºC on day 30 and 31 (first days of observable clinical symptoms) making a general decline to a temperature of 36.5ºC on day 37. The following day pelican 2ND was displaying neurological signs such as immobility and in-coordination and was euthanized. The body temperatures for pelicans 7SD and 2ND prior to observation of clinical symptoms (day 27 and 30, respectively) remained slightly below the average of the non-infected pelicans (Figure 5.1).

It is also to be noted that Pelicans 7SD and 2ND were artificially infected with the *B. damnificus* parasite during the captive energetics/parasite trial. Although it was determined that the parasite *B. damnificus* had no significant effect on growth, there is the possibility that *B. damnificus* infection may have made pelicans more susceptible to WNV infection. A repeated measures mixed model was used to compare various growth parameters collected on all parasite infected (n = 8) and parasite free (n = 8) pelican chicks to determine there was no significant effect of infection on growth.

Pathology

Twenty-one of the 27 AWPE tested positive for WNV by one or all of the following: histomorphological disease consistent with WNV infection (a full complement of major organs was examined in each case), reverse transcriptase PCR detection for WNV (pooled brain, heart, kidney, liver, spleen), or complement fixation serological testing. In 2 cases the diagnosis was made by serology or PCR, but without histological

changes that could be ascribed to WNV. In most cases, the major finding was lymphocytic meningoencephalitis and/or myocarditis with cell necrosis, which is typical of WNV infection in birds. Cycle threshold (Ct) values were reported as small as 8.9 and 8.6 and titers reported ranged from 16 to 64 in pelicans that tested positive for WNV. Smaller Ct values indicate more virus is present. No physical lesions were reported externally.

Discussion

Limited information exists in characterizing the effects of a natural WNV infection in naïve captive-raised birds. AWPE in the northern Great Plains, Montana, North Dakota, South Dakota, and Minnesota have been affected by WNV since 2002 and have seen great reductions in numbers of fledging chicks (Johnson et al. 2010). Many of the clinical symptoms displayed by WNV infected birds in captivity are similar to those observed in wild AWPE colonies. Sovada et al. (2008) described several birds also displaying torticollis, immobility, and ataxia. AWPE exhibiting clinical signs from this captive trial were within the 6-8 week age range similar to the age of infected wild pelicans (Johnson et al. 2010). Pelicans at this age may be most susceptible to WNV as passive immunity from the parents (through regurgitation) or egg yolk is reduced and development of natural active immunity is just beginning (Nemeth et al. 2008, Nemeth and Bowen 2007). Previous work has shown that the passive transfer of immunity may be possible through regurgitate (T. Ferguson, unpublished data) however, since pelicans were raised in captivity they may have been more susceptible to WNV infection as no passive immunity was provided through regurgitate as these birds were fed. Additionally, incomplete feather development may make juvenile pelicans more susceptible as fully

developed feathers may serve as a physical barrier to mosquitos. The timing of infection may also be related to peak mosquito breeding season in Mississippi which is typically July to October (Mississippi Department of Health 2010). Both mosquitos and mosquito larvae were observed around the facility, however none tested positive for WNV. No birds (other than pelicans) were observed within the facility. There was a probability that the cohorts of birds were infected by WNV-infected mosquitos. Because pelicans are social birds and colonial breeders sharing close proximity between nests, individually housed birds, although not in physical contact, were within close proximity to each other. The close proximity of pelicans may have resulted in a greater rate of WNV transmission between birds, vectored by mosquitos.

Group-housed pelicans were observed to crèche for warmth and socialization. Although some evidence suggests birds in close proximity may be able to transfer WNV through feces (Komar 2003a), there is no evidence to suggest that group-housed pelicans transmitted WNV through fecal contamination. Although WNV could potentially be spread through feces, in this case it is believed to have been mostly transmitted by a mosquito vector. Pelicans housed within individual pens were at no risk to infect each other through fecal contamination as all feces were collected by pans. Because each pelicans feces were collected underneath their individual pen and the pattern of infection appeared random, it is unlikely that the virus was spread through fecal contamination, although evidence of bird-to-bird transmission in several corvid species has been documented (Kipp et al. 2006). No abnormalities were observed in fecal material, except for blood in the stool of one infected pelican exhibiting severe clinical symptoms.

In this observational study the progression of disease in WNV infected pelicans could be classified into 2 categories with some pelicans experiencing a chronic infection usually resulting in gradual loss of body weight, or as more acute where pelicans were quickly affected by neurological symptoms. Acute and chronic WNV infections have been described by Reisen et al. (2013) in other several other bird species. Pelicans that lost body weight over time would show little interest in feeding, avoid being fed (agitation/aggression), and (or) immediately regurgitate fish following consumption; however, they still maintained mobility. Reduced intake and other observed symptoms were likely caused by WNV, but may have been related to heat stress. Reduced intake has been associated with heat stress in other birds (Donkoh 1989). Beyond reduced intake other behavioral abnormalities during times of extreme heat stress are not well known; therefore behavioral changes observed were initially attributed to prolonged exposure to elevated temperatures. Pelicans that were more severely affected by WNV would often progress through similar but more pronounced clinical symptoms within 72 hours becoming nearly or completely immobile and physically unable to consume fish. Although the date of initial exposure is unknown, the incubation period for WNV can be ≤ 10 days (Komar 2003a); therefore, it is suspected that the first 3 pelicans were likely exposed sometime around/after day 12 of being moved to the outdoor facility. Because feather development at this point was incomplete, pelicans may have been more easily bitten by mosquitos.

Since illness was unexpected, diagnostic methods were chosen by a Board Certified Pathologist/Veterinarian (W. Baumgartner personal communication). Several methods were used to rule out other processes/microbes and to confirm West Nile Virus

as the etiological agent: RT-PCR, serological titers, histopathology and clinical signs. Cycle threshold (Ct) values, a relative measure of the concentration of target in a PCR reaction, were reported as low as 8.9 and 8.6. Values < 20 are strong positive reactions indicative of abundant target nucleic acid in the sample (Mehta et al. 2010). According to Reisen et al. (2013), Ct values <30 indicate acute WNV infection. These low values may also indicate that pelicans could become viremic from 2 to 7 days post exposure (Phalen and Dahlhausen 2004). Titers were used to evaluate immune response and to evaluate the time course of disease. West Nile Virus titers range from ≥1:1280 in many bird species (Dusek et al. 2009). Titers ranged from 1:16 to 1:64 in those pelicans that tested positive for WNV indicating that many pelicans mounted an immunological response to the infection. General symptoms of WNV infection shared by 15 species of birds in various studies included brain hemorrhage, splenomegaly, meningoencephalitis, myocarditis and multiple organ failure (Komar 2003a; Steele et al. 2000; Swayne et al. 2001). Similar histopathological findings were observed in the AWPE that became naturally infected with WNV during this captive trial.

The natural infection is believed to have been introduced by the *Culex tarsalis* mosquito (confirmed to be present at the research facility), however it is not known what mammal or bird species possibly first brought WNV to the area. Several birds commonly observed within a 2 km radius of the aviary are listed in Table 5.4.

It is important to recognize that a variety of bird and mammal species could possibly serve as sources of infection (reservoirs) or competent hosts resulting in transfer of WNV to other animals and humans (Dietrich et al. 2005). Identifying species that may amplify virulence or act as reservoirs for WNV is important when considering how to

reduce transmission to humans. Thousands of AWPE have been infected with WNV on their breeding grounds (Sovada et al. 2008), and many of them that recover migrate south for the winter months. Typically Ct values reported as low as 8.9 and 8.6 indicate that the bird species, in this case juvenile AWPE, may become viremic when infected with WNV. This indicates that wild AWPE could become viremic on the breeding grounds, acting as potential amplifying hosts. Because infection of AWPE on the breeding ground typically occurs several weeks prior to migration (Sovada et al. 2008) most pelicans would likely have become viremic prior to migration.

Most of the pelicans that become diseased with WNV are young, 6-8 weeks old, but pelicans anywhere from 4-12 weeks of age are at greater risk of becoming infected and succumbing to disease (M. Sovada personal communication). Additionally, mosquito season in North and South Dakota peaks during the time in which pelicans are 4-12 weeks of age. AWPE typically fledge around 9 weeks of age (Schaller 1964), and are unlikely to venture far from the colony during peak infection. American White Pelicans usually migrate south between September and October (Knopf and Evans 2004) at which time surviving pelicans would have likely already recovered from WNV (Sovada et al. 2008). Therefore it seems unlikely that AWPE are responsible for spreading WNV beyond the breeding grounds; however, many other species of birds remain competent hosts year round and throughout adulthood. The fact that pelicans may become viremic for a period (2-7 days) indicates there is a need to take precautions to reduce the possibility of disease transmission to humans. Further research is needed to determine the best way to prevent transmission whether through separation of wildlife sanctuaries from

human contact (at breeding grounds), mosquito control, increased awareness, or by other means.

To help prevent transmission of WNV sources of the infection need to be identified. Since the original source of infection is unknown (although likely vectored by the *Culex tarsalis* mosquito) other routes of infection including vertical transmission was investigated. It is not believed that WNV is vertically transmitted to AWPE for few reasons. For example, northern Great Plains colony chicks do not show any sign of disease prior to the emergence of the *Culex tarsalis* mosquito and severity of the disease in the northern Great Plains colonies parallels mosquito abundance (M. Sovada personal communication). Similar to hens and common passerine species, it is suspected that maternal antibodies may protect chicks from WNV during the first few weeks post hatching but eventually dissipate, explaining why pelican chicks become infected as early as 4 weeks of age (Nemeth et al. 2008, Nemeth and Bowen 2007). Vertical transmission has not been detected in other avian species (Nemeth et al. 2008, Nemeth and Bowen 2007) and is therefore unlikely occurring in AWPE. Although evidence suggests that vertical transmission in AWPE is unlikely, it cannot be completely disproven. Therefore, further research is required to determine if vertical transmission does occur in various avian species.

Five out of 21 pelicans that became infected with WNV had been previously infected with the trematode *Bolbophorus damnificus* including case study pelicans 2ND and 7SD, but were otherwise considered naïve to disease. The remaining 16, both group-housed and captive trial pelicans that became infected with WNV, were completely naïve to disease. This research is the first reported instance in which hand-reared pelicans have

become naturally infected with WNV in a captive setting, in which clinical symptoms, and progression of disease have been documented. Our data illustrate that healthy pelican chicks are very susceptible to WNV and may experience rapid progression of disease. Future research to determine risk factors and a further characterization of histopathology and tissue samples of pelicans affected by WNV are necessary for a further understanding of this disease.

Table 5.1 Timeline linking West Nile Virus onset of clinical symptoms to days post suspected exposure for American White Pelicans (*Pelecanus erythrorhynchos*) during 29 May to 30 July 2012.

Days Outside/Exposed	Date	Event
-24	29-May	Pelicans hatched
0	22-June	Pelicans moved outside
22	14-July	Pelicans A073, A231, A318 show symptoms
25	17-July	Pelicans A073, A231, A318 euthanized
27	19-July	7 SD: first clinical symptoms
30	22-July	2 ND: first clinical symptoms
32	24-July	7 SD: euthanized
38	30-July	2 ND: euthanized
47	8-August	All remaining pelicans euthanized

Table 5.2 West Nile Virus positive and negative group-housed (n = 11) and captive trial (n = 16) American White Pelicans (*Pelecanus erythrorhynchos*) with and without temperature probes during 29 May to 30 July 2012.

	Group-housed pelicans (n = 11)	**Captive trial pelicans (n = 16)**
West Nile Virus positive	10	11
West Nile Virus negative	1	5
Temperature probe	-	8
No temperature probe	-	8
Temperature probe WNV positive	-	5[1]
Temperature probe WNV negative	-	3[2]

1 Three of 5 pelicans were also infected with *Bolbophorus damnificus*
[2] One of 3 pelicans was also infected with *Bolbophorus damnificus*

Table 5.3 Average Food intake (g) and body weight (g) for 14 captive American White Pelicans (*Pelecanus erythrorhynchos*) and 2 case studies 2ND and 7SD, during the time in which pelicans began showing clinical symptoms of West Nile Virus, 15-30 July, 2012

	2ND[1]		**7SD[1]**		**Average[2]**	
Date	Intake (as fed)	Body weight	Intake (as-fed)	Body weight	Intake (as-fed)	Body weight
15 July	1244	5681	1169	6075	1502	6401
16 July	1412	5689	1039	5895	1484	6551
17 July	1176	5739	1122	6098	1275	6513
18 July	1456	5740	1112	6154	1230	6554
19 July	710	5780	900	6246	1129	6703
20 July	1315	5708	1310	6179	1458	6723
21 July	1303	5793	1229	6182	1339	6761
22 July	407	5905	76	5947	981	6796
23 July	371	5680	358	5696	940	6667
24 July	366	5368	NA	NA	746	6599
25 July	321	5378	NA	NA	918	6521
26 July	30	5315	NA	NA	703	6442
27 July	22	5203	NA	NA	466	6368
28 July	402	5290	NA	NA	442	6227
29 July	0	5224	NA	NA	255	6089

[1] NA= information not available
[2] Average (n = 14) excludes pelicans 2ND and 7SD

Table 5.4 Bird species observed within 2 km of the American White Pelican (*Pelecanus erythrorhynchos)* research aviary, May-July, 2012.

American Crow (*Corvus brachyrhynchos*)
American Kestrel (*Falco sparverius*)
American Robin (*Turdis migratorius*)
Blackbirds (*Turdus merula*)
Black Vulture (*Coragyps atratus*)
Bobolink (*Dolichonyx oryzivorus*)
Blue Grosbeak (*Passerina caerulea*)
Blue Jay (*Cyanocitta cristata*)
Brown-headed Cowbird (*Molothrus ater*)
Canada Goose (*Branta canadensis*)
Carolina Wren (*Thryothorus ludovicianus*)
Cattle Egret (*Bubulcus ibis*)
Common Grackle (*Quiscalus quiscula*)
Common Ground Dove (*Columbina passerina*)
Eastern Belted Kingfisher (*Megaceryle alcyon*)
Eurasian Collared Dove (*Streptopelia decaocto*)
Eastern Kingbird (*Tyrannus tyrannus*)
European Starling (*Sturnus vulgaris*)
Great Blue Heron (*Ardea herodias*)
Great Egret (*Ardea alba*)
Green Heron (*Butorides virescens*)
House Finch (*Haemorhous mexicanus*)
House Sparrow (*Passer domesticus*)
Little Blue Heron (*Egretta caerulea*)
Loggerhead Shrike (*Lanius ludovicianus*)
Mourning Doves (*Zenaida macroura*)
Northern Cardinal (*Cardinalis cardinalis*)
Northern Harrier (*Circus cyaneus*)
Northern Mockingbird (*Mimus polyglottos*)
Red-tailed Hawk (*Buteo jamaicensis*)
Sparrows
Swallows
Snowy Egret (*Egretta thula*)
Turkey Vulture (*Cathartes aura*)

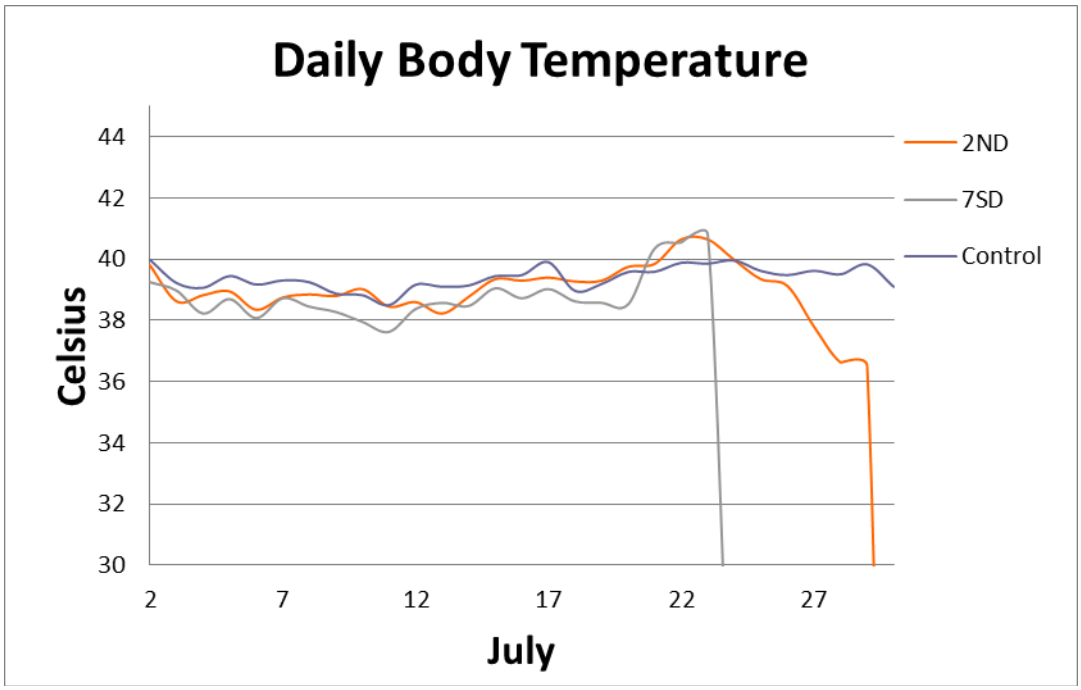

Figure 5.1 Daily body temperature for 2 West Nile Virus infected (2ND and 7SD) and non-infected (control n = 3) American White Pelicans *(Pelecanus erythrorhynchos*) from 1-30 July 2012, held at an outdoor research aviary at Mississippi State University.

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CHAPTER VI

IMPORTANCE AND APPLICATION OF AMERICAN WHITE PELICAN IMMUNOLOGY, ENERGETICS, AND DISEASE DATA

Summary chapter

Data obtained during this study on American White Pelicans (*Pelecanus erythrorhynchos*) up to fledging contributes to the basic ecological understanding of pelicans in addition to further detailing information on their foraging strategy, behavior, life-history, and evolution of the species. This research provides information to improve management practices and enhance conservation knowledge for captive and wild pelicans/avian species.

Previously, little data were available on morphology and growth, of the American White Pelicans from hatching to fledging and potential passive transfer of immunity from parent pelicans to chicks. Additional data is also supplied on nutritional contents of regurgitate, energetics, thermoregulation and disease (*Bolbophorus damnificus* and West Nile Virus) of American White Pelicans (hereafter, AWPE).

General pelican ecology is enhanced when previously unknown information regarding pelican behavior, energetics, metabolism and thermoregulation is further detailed. Behavioral notes were taken during the captive trial raising pelicans from hatching to fledging to record energetic requirements, somatic development, and disease susceptibility. Other developmental issues were discussed such as pelicans splaying of

legs under rapid body growth. Modeling and data pertaining to energetics such as caloric intake, body weight, growth of culmen/tarsus, and sub-cutaneous temperature data of pelicans and intake of parent pelicans (regurgitate) were examined. American White Pelican metabolism of fish (4 species), the effects of the trematode parasite *B. damnificus* and the clinical symptoms and progression of disease associated with natural West Nile virus (hereafter WNV) were also examined during the trial. Data obtained from examination of these factors could lead to a better understanding of AWPE ecology while also enhancing theories and management of pelican/avian species.

Optimal foraging theory is an extension of Darwin's theory of evolution, attempting to predict behavior of animals while they are foraging (Pyke 1984). Both energetics and food metabolism data could be used in optimal foraging models, which also help predict the diet of an organism (Carey 1996). Understanding what energetic costs are necessary for parent pelicans during chick rearing (feeding young) may give insight into pelican foraging strategy, further detailing what resources may be preferred. During this research the amount of fish required to raise pelicans from hatching to fledging was documented. These data allow researchers to create models to predict consumption by age, or the ability to further evaluate energetic requirements for other pelican species whether wild or captive. Knowing a pelican's energy requirements may allow us to further understand what prey items a parent pelican might choose, how often, and why (more digestible/less digestible). Because young pelicans may metabolize fish species differently than adults, or perhaps have different nutrient requirements, the data obtained in this study are valuable for predicting how parent pelicans choose to forage,

and further, help researchers determine how pelicans maximize their fitness during breeding/early stages of growth.

Determining what factors influence pelican foraging behavior (energetic requirements) or behavior during chick rearing is necessary to better refine both optimal foraging and central place foraging models (Orians and Pearson 1979). Use of these theories to predict how the animal forages using patches of resources may be valuable on an ecological basis as well as for management purposes. Pelicans over the last few decades have begun to consume farmed fish species, causing great economic losses whether associated with consumption itself or by perpetuating the spread of the trematode *B. damnificus* (Wise et al. 2008). The central place foraging theory characterizes the optimal rate of delivery of energy to a central place (Orians and Pearson 1979) and may be used to predict optimal locations for pelicans to feed and breed. Application of the optimal foraging theory and central foraging theory in connection with energetic data supplied during this study may allow researchers to identify optimal breeding habitat and regions or factors that affect conservation of the species (Carey 1996).

Gaining a better understanding of how pelicans (along with other birds) have developed strategies to maximize their fitness over their lifetimes requires an examination of several factors such as further detailing life history (Rogers and Smith 1993). Aside from the ecological importance of knowing the concentrations of Immunoglobulin Y and A (IgY and IgA) passively transferred to pelicans chicks in regurgitate, there are additional implications for life-history. Knowing at what concentrations and how immunity is transferred/acquired by pelican chicks helps shape immune function in a range of ecological contexts (McDade 2003). Data on

immunological function along with data on pelican developmental processes examined during growth, taken in relation to Darwinian fitness such as that done by David Lack (1968), provides researchers enhanced understanding of life-history attributes in an evolutionary context (Farner et al. 1983). Understanding the energetic cost of raising young may also be informative about the evolution of nesting; better equipping researchers to calculate tradeoff costs of parent pelicans protecting eggs/young from predation rather than abandoning young (Martin 1995). American White Pelican energy requirements and immunology could also be used to help determine age-specific mortalities or adult fecundity, also major drivers of life-history strategy (Charlesworth 1980, McDade 2003). To understand life-history/evolution requires evaluating what adaptations (Dykstra and Karasoy 1993) to morphology and behavior may influence pelican foraging success (Losos 1990, Rickelts and Miles 1994). Adaptations that could be evaluated include maximum rates of assimilation and digestion/metabolism. Regurgitate and metabolism data obtained during this experiment and in others (Brugger 1993, Ferguson et al. 2011) may help determine how such adaptations may develop (Dykstra and Karasov 1993).

Natural selection influences which adaptations may be inherited to ensure the greatest chance of survival of an animal. Phenotypic or genetic patterns can be identified (Kendeigh et al. 1977, Schreiber 1976) and interpreted as optimized by natural selection (Carey 1996). By analyzing the process of natural selection over time information can be gained on why and how certain phenotypes have prevailed over others (Carey 1996). Data collected on the aforementioned factors may allow evolutionary ecologists to determine what attributes of form and function result in increased reproductive success

(Smith 1978, Sterns and Schmid-Hempel 1987). Developmental processes occurring during growth can be applied to the theory of quantitative genetics in reference to the evolution of growth processes (Lerner 1937, Cock 1966).

To better understand pelican survival strategy, assessment of a chick's daily activities during growth (Burger 1980, Farner et al. 1983) is essential. There is a tradeoff between energy used for maintenance versus reproduction (Sheldon and Verhust 1996) as well as for growth versus immune function (McDade 2003). Insight provided by analyzing regurgitate content and diet metabolism allows researchers to determine what nutrients along with quantity of feed (in addition to immunoglobulin transfer) may give a chick its best chance of success as an adult. Since pelican chicks were growing at such a rapid rate, as demonstrated by data collected on the tarsus and culmen lengths and body weight of pelican chicks, there may have been little energy available for development of immune functions (McDade 2003). This may help explain why wild pelicans (similar to captive held) are most susceptible to WNV during weeks 6-8 (M. Sovada personal communication) as growth is very rapid and any passive transfer of immunity from parent pelicans (through regurgitate) may have dissipated. Pelicans in the trial were not supplemented with IgA or IgY when fed, perhaps making them more susceptible for pathogens (Nishanian et al. 1998). Examining factors such as passive immunity, disease and energetics also provides insight into success in terms of survival and recruitment into adult populations. There are likely several other factors attributing to differences in pelican susceptibility to various pathogens such as nutrition, frequency of feeding, strength of parent pelicans immunoglobulins, pelican feather development in addition to rapid growth.

While data obtained during this research study should enhance knowledge regarding foraging theories, behavior theories, basic ecology, life-history, and evolution of pelican species there are also implications for captive and wild management of pelican species, and perhaps other waterbird species.

Previously, AWPE had never been reported to have been raised from hatching to fledging in a captive setting, and in such large numbers. Methodology used during this experiment proved very successful, and could easy be replicated for other pelican species. Knowing how to raise pelicans in a captive setting would be very useful if the species became endangered and captive breeding was necessary to supplement wild populations. Captive management can also be improved when examining nutrient metabolism by the pelicans, allowing nutritionists in zoo settings to better gauge what items pelicans may consume in the wild, how well they metabolize various species of fish, and what nutrients (and even immunoglobulins) may be required during rearing of young. Examination of diet, passive immunity, and behavior may give better insight into handling various factors such as disease, abnormal behavior, and preference of fish species (McDade 2003).

Other benefits of this research include identifying the clinical signs of disease associated with WNV, allowing researchers to better manage outbreaks if they occur by both advising nearby human populations to control mosquito populations, and by exterminating mosquito vectors. In reference to both wild and captive pelicans, knowing the early signs of WNV infection may allow researcher to better handle potential outbreaks by actively managing nearby mosquito populations. Baseline data collected on immunoglobulin concentration in regurgitate shows that there is passive immunity from parent pelicans to chicks is possible, and when combined with information regarding diet,

both can be analyzed to determine how these factors affect susceptibility to disease (McDade 2003). Management of WNV may decrease juvenile/adult pelican mortality, enhancing conservation of the species (Owens and Bennet 2000).

It is also useful to know when pelicans can first become infected with the trematode *B.* damnificus, in addition to what the effects of a low level infection are. Low level infection meaning infection is typically non-lethal and not of concern for pelicans themselves, even though aquaculture may still be affected. Aquaculture and natural resource managers affected by predation of fish by AWPE may find data related to consumption and preference of fish species to be useful for managing wild pelican species, allowing them to better access and manage damages caused by pelican consumption (Wise et al. 2008). Information on preference and diet will improve conservation efforts of wild pelicans as researchers could identify specific breeding/foraging areas in need of protection.

This study was the first to accomplish raising pelicans from hatching to fledging while examining the impacts of various factors on growth; specifically the effects of immunology, nutrient metabolism, and both *B. damnificus* and WNV infection. While previously several bird species have been artificially infected with WNV, this is the first report of a natural WNV infection in captive pelicans. Although this data has multiple implications for AWPE, it can also be used to help manage and understand other pelican/avian species, more specifically piscivorous birds. These data could be used to identify growth patterns across species, and increase the understanding of daily energy expenditure. There is still more to be understood about the amount of energy birds expend for certain activities or periods of time, perhaps across a species natural

distribution over a range of seasons (Eduardo et al. 2010). These data incorporated with other data could be used to examine evolution of short versus long life spans and altitudinal versus latitudinal effects on a species daily energy needs (Eduardo et al. 2010). Research on energy expenditure of pelicans (or similar birds), may also help identify what parameters promote evolution of compensatory growth, which can help predict mortality (Mangel and Munch 2005). Combining studies of metabolism and thermoregulation with biochemical approaches in a range of avian taxa could also provide new information on avian evolution and how certain environments may have influenced avian developmental strategies (Eduardo et al. 2010). Further research is required in areas such as metabolism, disease, growth and energetics to enhance conservation and management of pelican species.

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