DIET AND EFFECTS OF ENVIRONMENTAL STRESSORS ON THE ALTRICIAL NESTLINGS OF DOUBLE-CRESTED CORMORANTS (PHALACROCORAX AURITUS)

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Jacob Gregory Randa

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Biological Sciences

September 2012

Fargo, North Dakota

North Dakota State University

Graduate School

Title

DIET AND EFFECTS OF ENVIRONMENTAL STRESSORS ON THE ALTRICIAL NESTLINGS OF DOUBLE-CRESTED CORMORANTS (PHALACROCORAX AURITUS)

By

Jacob Gregory Randa

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Wendy Reed
Chair

Dr. Mark Clark

Dr. John McEvoy

Dr. Steve Windels

Approved:

April 14, 2014	Dr. Wendy Reed
Date	Department Chair

ABSTRACT

Double-crested cormorants (*Phalacrocorax auritus*) are a common species of altricial waterbird found across much of North America. As a piscivorous colonial waterbird, cormorants are often persecuted due to perceived impacts on fisheries. In this study I examined the diet of cormorant nestlings at five cormorant colonies in central North America to answer two questions: 1) Is nestling diet reflective of opportunistic feeding behavior, thus diminishing the likelihood of negative impacts to the fishery? and 2) How do diet and environmental stressors effect the development of cormorant nestlings? By analyzing the caloric content of nestling diet and quantifying environmental stressors such as endoparasite and ectoparasite loads, I found diet was a significant contributor to structural long bone growth in both the wing and tarsus. Diet analysis also corroborated the long held belief that cormorants have highly variable diets reflective of local fish communities and may vary annually as fish assemblages change.

ACKNOWLEDGEMENTS

I owe a great deal of gratitude to my advisor, Dr. Wendy Reed, for all of the help, support, guidance, and opportunities she has given me. It is a rare thing to have an advisor so willing to make time for questions and to have so much trust in someone else's abilities. She allowed me to discover and learn on my own, but was always there when I needed a little push in the right direction. Wendy not only helped me mold this project into something that was both personally interesting as well as ecologically important, but she has also molded me into a more thoughtful and efficient ecologist.

I would also like to thank the other members of my committee: Dr. John McEvoy, Dr. Mark Clark, and Dr. Steve Windels. They were all essential in the early development of the project, and throughout the study as well. Mark, as well as fellow graduate student Shawn Weissenfluh, gathered the data from the cormorant colony at J. Clark Salyer NWR. Knowing how unglamorous this type of data collection can be, I will always be indebted to them.

I would also really like to thank Voyageurs National Park, the Ontario Ministry of Natural Resources, and Dr. Steve Windels for the funding and opportunity to work in such an amazing place. There was never a shortage of assistants, and never a shortage of memories made. There are too many to name, but just a few special thanks go to: Cameron Trembath, Lee Grimm, Scott Taylor, Jim Hummel, William Severud, Bryce Olson, Jerry Warmbold, Charles Eckman, Sam Smyrk, Claire Kissane, and Bridget Henning.

The US Fish and Wildlife Service has also been a very important participant in this project. Not only was I allowed to perform research at two National Wildlife Refuges, but I received a great deal of assistance as well. I would like to give a special thanks to everyone at Rice Lake NWR, not only did they give me my start in the US FWS, but particularly Michelle

McDowell, Mary Stefanski, and Walt Ford went out of their way to help me in this project, and in furthering my career.

Finally, I owe a huge thanks to my family and Bridget Henning. Their love, guidance, and encouragement is responsible for where I am today. Without them, this would not have been possible. Bridget, thank you so much for your assistance, friendship, love, edits, and the constant reminder to keep it “short and sweet”.

TABLE OF CONTENTS

ABSTRACT……iii

ACKNOWLEDGEMENTS……iv

LIST OF TABLES……...vii

LIST OF FIGURES……. viii

CHAPTER 1: A REVIEW OF THE DIET OF AND EFFECT OF ENVIRONMENTAL STRESSORS ON THE ALTRICIAL NESTLINGS OF DOUBLE-CRESTED CORMORANTS (*PHALACROCORAX AURITUS*)…… 1

CHAPTER 2: ENVIRONMENTAL STRESSORS AND THEIR EFFECTS ON THE ALTRICIAL NESTLINGS OF DOUBLE-CRESTED CORMORANTS (*PHALACROCORAX AURITUS*)…… 20

CHAPTER 3: THE DIET OF NESTLING DOUBLE-CRESTED CORMORANTS (*PHALACROCORAX* AURITUS) AT FIVE CENTRAL NORTH AMERICAN COLONIES……..50

CHAPTER 4: CONCLUSIONS…….. 80

LIST OF TABLES

Table		Page
2.1.	Length-weight regressions used to calculate biomass from bolus specimens	41
2.2.	Mean caloric content of one gram (wet mass) of fish for each of eight taxa	41
2.3.	Linear fit model evaluating the effect of environmental stressors on stress (H:L) levels with colony and individual nestling ID held as random variables	42
2.4.	Linear fit model evaluating the effect of environmental stressors and their H:L crosses on mass SGR (g/g/day) with colony held as a random variable	42
2.5.	Linear fit model evaluating the effect of environmental stressors and their H:L crosses on wing SGR (mm/mm/day) with colony held as a random variable	43
2.6.	Linear fit model evaluating the effect of environmental stressors and their H:L crosses on tarsus SGR (mm/mm/day) with colony held as a random variable	43
3.1.	Length-weight regressions used to calculate biomass from bolus specimens	68
3.2.	2008 diet of nestling double-crested cormorants at the NEP colony	69
3.3.	2009 diet of double-crested cormorants at the NEP colony	70
3.4.	2008 diet of double-crested cormorants at the 7SIS colony	71
3.5.	2009 diet of double-crested cormorants at the 7SIS colony	71
3.6.	2009 diet of double-crested cormorants at the NOD colony	72
3.7.	2009 diet of double-crested cormorants at the ML colony	72
3.8.	2009 diet of double-crested cormorants at the JCS colony	73

LIST OF FIGURES

Figure		Page
2.1.	Locations of study sites	44
2.2.	Fish per bolus by colony, 2009 (SE)	45
2.3.	Diet (calories) by colony, 2009 (SE)	46
2.4.	Endoparasites by colony, 2009 (SE)	47
2.5.	Ectoparasites by colony, 2009 (SE)	48
2.6.	Stress levels (H:L) by colony, 2009 (SE)	49
3.1.	NEP diet by raw mass, 2008	73
3.2.	NEP diet by biomass, 2009	74
3.3.	NEP diet composition by individual prey item, 2009	74
3.4.	7SIS diet by raw mass, 2008	75
3.5.	7SIS diet by biomass, 2009	75
3.6.	7SIS diet composition by individual prey item, 2009	76
3.7.	NOD diet by biomass, 2009	76
3.8.	NOD diet composition by individual prey item, 2009	77
3.9.	ML diet by biomass, 2009	77
3.10.	ML diet composition by individual prey item, 2009	78
3.11.	JCS diet by biomass, 2009	78
3.12.	JCS diet composition by individual prey item, 2009	79

CHAPTER 1: A REVIEW OF THE DIET AND IMPACTS OF ENVIRONMENTAL STRESSORS ON THE ALTRICIAL NESTLINGS OF DOUBLE-CRESTED CORMORANTS (*PHALACROCORAX AURITUS*)

Introduction

All species endure a great variety of environmental, ecological, and psychological stressors including, but not limited to: inter- and conspecific competition, harassment (including both human and interspecific sources), weather, poor diet, disease, parasites, pollution, degraded habitat, and predation. As biologists, we strive to better understand the effects of these stressors and the implications they have on population dynamics and species persistence. A complete understanding of the effects of stressors requires a broad diversity of research; from behavior to physiology and endocrinology to developmental biology. Double-crested cormorants (*Phalacrocorax auritus*; herein referred to as cormorants) are common, altricial waterbirds which present an opportunity to expand our understanding of the ecological factors that act as stressors on successful growth and reproduction in altricial species.

Stress Response

Stress is defined as an individual's perception to threats, which initiates a focus of energy on coping with these short-term threats to survival, and curtails long-term investments in functions such as courtship, territorial defense, reproduction, growth and/or immune defense (Busch and Hayward, 2009). Unavoidable as stress is, animals have evolved stress response mechanisms, and recently, scientists have been examining the actions and effects of glucocorticoids (GCs) on reproductive success (McEwen and Wingfield, 2003). Glucorticoids (e.g., cortisol and corticosterone), although commonly referred to as stress hormones, are

essential for basic life functions and embryonic development, in addition to aiding in the stress response.

At adult basal levels, GCs primary roles are energy regulation and maintaining salt regulation (acquisition, mobilization, and deposition) in response and in conjunction with mineralocorticoids (McEwen and Wingfield, 2003). When a threat is perceived, GC levels increase via the control of the hypothalamic-pituitary-adrenal (HPA) axis, consisting of three endocrine tissues: the adrenal gland, hypothalamus, and pituitary gland. The increase of GCs enables the animal to increase functioning in the short term (i.e., "fight or flight"). Both behavior and physiology are affected by GCs. Noted changes include: an increase in cardiac tone, the creation of glucose from energy stores, increased cerebral blood flow and glucose utilization, increased regulation of the immune system, enhanced cognition and memory, and increased restfulness by lowering basal metabolic rate (Wingfield and Ramenofsky, 1997; Wingfield *et al*., 1998; Sapolsky *et al*., 2000). One hypothesis is that more energy required for the stress response results in lower energy stores for other functions, which provides the nexus between conservation and the effects of GCs.

If animals are chronically stressed (e.g., through injury, illness, poor diet, etc.), GCs possess the potential for negative additive effects on health and fitness (McEwen and Wingfield, 2003). When additional stressors arise during a period of extended stress, supplementary GCs will be released, which can compound existing issues. Persistent elevation of GCs has numerous deleterious effects, which are documented in a number of species (Blas *et al*., 2006; Bortolotti *et al*., 2008; Cyr *et al*., 2007; Rich and Romero, 2005). Documented negative effects due to chronically elevate levels of GCs include: suppressed immune function, decreased growth, protein loss, hypertension, neuronal cell death, inhibition of reproductive behavior, decreased

performance at memory-related skills, and depression (Wingfield and Ramenofsky, 1997; Wingfield *et al.*, 1998; Sapolsky *et al.*, 2000; and McEwen and Wingfield, 2003).

In birds, corticosterone (CORT), the primary glucocorticoid, has been implicated in decreasing fitness, increasing mortality, and suppressing immune function. Furthermore, maternal glucocorticoids can be transmitted through the yolk of their eggs and negatively affect offspring (Bonier *et al.*, 2007; Rubolini *et al.*, 2005). Recent research has also indicated the possibility of high maternal stress creating uneven brood hierarchies (Bonier *et al.*, 2007; Kozlowski and Ricklefs, 2010) and an increase in stress response as adults (Hayward and Wingfield, 2003).

Diet as a Stressor

Diet is often examined as a potential source of environmental stress. Many migratory birds return to their breeding grounds when little food is available, or the food that is available is difficult to access. When combined with the energy expenditure required to complete migration, this often results in an overly stressed adult (high levels of CORT) (Schwabl *et al*., 1991). These elevated CORT levels are passed to offspring increasing the possibility of compounding stress effects on the nestlings (Bonier *et al*., 2007). Even in the absence of any preexisting physiological stressors, poor diet can negatively impact nestling growth rates through reduced calories or nutrition (Costantini, 2010; Boag, 1987; Johnson 1971). Independent of these direct impacts of diet on growth, an increase of baseline CORT levels in circulation has also been found in response to low quality diets (Honarmand *et al.*, 2010) raising the likelihood of correlations among diet, CORT, and growth. Slowed development during the nestling phase can result in catch-up growth, which is linked to long-term changes in phenotype as an adult, such as lower fecundity and shorter lifespan (Metcalfe and Monaghan, 2001; Criscuolo *et al.*, 2008).

Measuring Stress

Researchers have used CORT levels circulating in plasma to estimate stress levels in birds, but there are inherent difficulties with this approach (Wingfield and Ramenofsky, 1997). Both acclimation and sensitization to stressors can result in blurred variation between maximum and baseline CORT levels that may prevent researchers from accurately depicting the presence or absence of stressors. Corticosterone is biologically inactive when bound to carrier proteins (Breuner and Orchinik, 2002). This can be problematic as typical measurement of CORT using radioimmunoassays measures only the unbound fraction of CORT, thus the inability to account for bound and unbound CORT levels can result in an incomplete understanding of stress. Furthermore, even seasonal changes can result in CORT fluctuations largely unrelated to major stress events (Romero *et al.*, 1997; Romero *et al.*, 2000; Romero *et al.*, 2005).

One of the largest challenges of using circulating CORT levels to assess stress is when conducting field studies. Circulating CORT levels increase rapidly in response to a stressor, thus the act of capturing and handling, or even the simple appearance of a researcher/predator approaching can cause CORT levels to begin rising (Cockrem and Silverin, 2002*a*, *b*). Because this increase is both intense and unpredictable (Romero and Romero, 2002), measuring circulating CORT levels in colonial species is difficult at best, where target individuals may be alerted to the presence of researchers well before the researcher is even visible.

One method researchers have discovered to bypass the issues of using CORT in field studies of animals is to instead use a blood smear and examine the ratio between heterophils and lymphocytes (HL ratio), two common leukocytes. Heterophils, the primary phagocytic leukocyte, proliferate in response to infection, inflammation, and stress (Jain 1993; Campbell 1995; Rupley 1997; Harmon 1998), whereas lymphocytes are involved in a number of

immunological functions, including immunoglobulin production and modulation of immune defense (Campbell 1996). A comparison of the relative abundance of these two leukocytes is correlated with the level of CORT circulating in the blood (McFarlane and Curtis, 1989; Gross and Siegel, 1983). Increasing CORT levels can stimulate an influx of heterophils from the bone marrow (Bishop *et. al* 1968) into circulation, while concomitantly promoting the redistribution of lymphocytes into other compartments such as the lymph nodes, spleen, bone marrow, or skin (Dhabhar 2002), ultimately resulting in a higher HL ratio (McFarlane and Curtis, 1989). This technique has particular advantages when used in the field , as the speed at which these cellular changes happen are much slower than the hormonal (i.e., CORT) changes which limits the accuracy of basal measurements. Slower cellular changes allow researchers to handle birds, especially colonial species, without concern of skewing data.

Altricial Development

Poultry scientists have long concerned themselves with the impacts of environmental stressors in captive settings. Now, as we begin to understand these negative effects, researchers have begun to evaluate the impact of ecological stressors under field conditions in wild populations of birds. To date, this research has covered a range of species and developmental modes, but has greatly neglected colonial species with altricial young. Altricial species exhibit a unique developmental strategy by being seemingly underdeveloped at hatch. Altricial bird species are typically born without down, have closed eyes, are immobile, and are completely reliant on parental care for food, heat, and protection (Starck and Ricklefs, 1998).

The impact of stressors on offspring development may be influenced by the mode of development. Ricklefs and Starck (1998) describe three hypotheses for developmental plans that result in altricial and precocial states at hatching. The first states that precocial bird species

simply remain in the egg longer than their altricial counterparts, thus resulting in a longer maturation period and a higher degree of development at hatch (Portmann, 1955). The second hypothesis proposes the existence of different rates of maturation throughout embryonic development, resulting in precocial species developing faster. The third hypothesis involves divergent developmental rates just prior to hatch, when precocial species undergo a more rapid phase of maturation than altricial species (Starck and Ricklefs, 1998). Ricklefs and Starck (1998) found little support for the first hypothesis because the lengths of incubation periods (i.e., developmental time) among species are not associated with developmental modes. In regards to the second and third hypotheses, a similar embryonic growth curve was found throughout much of the developmental periods in both precocial and altricial species. This finding indicates that differentiation between the altricial and precocial modes of development must occur shortly before hatching, after much of the embryonic growth has been completed (Starck and Ricklefs, 1998).

The timing of developmental events (i.e. HPA development and stimulation) could have a large impact on the ability of offspring to handle stressors. If an altricial hatchling has a fully developed HPA axis, it will physiologically respond to stressors by releasing CORT, but will be incapable of a physical response (e.g., increased begging in response to malnutrition, competitive behavior with siblings, escape from predators and harsh conditions, etc.). Lacking a physical response, the bird is not only experiencing the original stressor, but the additional negative effects of elevated CORT levels. At the nestling stage, the effects of CORT are likely displayed in slower growth and increased disease and parasite loads due to a depressed immune response. Fluctuating CORT levels may also interrupt normal feather growth, which is critical for survival (Romero *et al.*, 2005). There are simultaneous advantages of a fully developed HPA axis at

hatch, which can also be very important for nestling survival. Corticosterone does initiate physiological and endocrine changes, possibly helping even a nest-bound bird overcome stressors. Although protection from adverse conditions may be provided by parents, nestlings yet face variation in food availability, sibling competition, parasites, illness, and predators (Blas *et al.*, 2005). Corticosterone is responsible for promoting begging (Kitaysky *et al.*, 2001a; Kitaysky *et al.*, 2001b) and aggressive behaviors (Kitaysky *et al.*, 2003), both of which are important survival tools.

At the other end of the developmental spectrum, birds with precocial young hatch with down, eyes open, and are often self-sufficient soon after hatch (Starck and Ricklefs, 1998). This mode of development provides an obvious advantage over the altricial mode in regards to environmental stressors; precocial young are not nest bound, allowing them to escape detrimental or hazardous conditions. In these circumstances, the "fight or flight" reaction initiated through hormonal changes is indeed beneficial. Although not reliant on parental resources and protection, most precocial species still receive parental care, save very select species (e.g., moundbuilders; (Starck and Ricklefs, 1998)). It is in this sense the altricial-precocial spectrum becomes obvious. The super-precocial moundbuilders, fully developed at hatch, have high survival rates, but low hatching rates due to the difficulty in laying and incubating such an egg. The majority of precocial species bridge the gap between the super-precocial moundbuilders and the typical altricial species, with young capable of a stress response but still moderately reliant on parental care.

The questions to be asked at this point are: 1) how does altricial development benefit birds in terms of stress response, and 2) if this bird, as a young nestling, is initially incapable of responding to environmental stressors (i.e., not physically capable of fight or flight response),

would the suppression of the HPA axis/stress response pathway be beneficial during this initial period? With the second question paying special consideration to the deleterious effects of chronically elevated levels of CORT.

Analyzing the Stress Response

To thoroughly examine the stress response in natural settings, one must be able to evaluate the effects across multiple levels, from the individual, to the brood, to the population. Blas et al. (2005) utilized a four-tier approach to study altricial White Storks (*Ciconia ciconia*): at the individual level, within brood, among broods of different sizes, and individuals in different local environments. By using this technique, the researchers were able to tease apart individual and overarching factors that may lead to stress responses. The findings of this study showed an effect of age and location on CORT levels, indicating a shift in HPA activity as birds mature, but it did not examine the initial development of HPA activity by investigating birds in their first three weeks of development. Researchers were therefore unable to attribute age differences in HPA activity to either a period of HPA inactivity or suppression. The location effect also indicated the presence of other environmental stressors having additive effects on CORT levels, but markedly different habitat types were noted in the study.

Cormorants as a Study Species

Double-crested cormorants present an excellent opportunity for studying the effects of environmental stressors. Cormorants are a common piscivorous waterbird with altricial young whose populations have increased dramatically since the 1970's (Johnsgard, 1993; Wires *et al.*, 2001). In the years preceding the 1970s, populations were at very low levels due to reproductive failure associated with toxic contaminant exposure (PCBs, DDE and DDT) and human persecution (Wires *et al*., 2001). Since the 1970's, population numbers have risen to colonies

that include 1000's of breeding pairs (Hatch and Weseloh, 1999a). In the Great Lakes alone, cormorant numbers doubled from 1991 to 1997 (Tyson *et al.*, 1997), and the Breeding Bird Survey indicates a 7.1% annual mean increase in the United States and a 12% annual mean increase in Canada from 1999-2009 (Sauer et al.2011). With these large populations, and Minnesota hosting a 2004 population of 16,000 pairs in 38 colonies (Wires *et al.*, 2005), it is easy for researchers to access multiple colonies with a variety of potential environmental stressors, including but not limited to: diet, weather, competition, harassment, and parasite loads.

Cormorants are opportunistic feeders and prey on a wide variety of fish, invertebrate, and amphibian species (Hatch and Weseloh, 1999b). Although the general public often faults these birds for sport fish declines, a large body of scientific research exists demonstrating the positive correlation between prey species availability and the abundance of those species in cormorant diets (i.e., a wetland with an abundant minnow population will result in a large minnow component of the cormorants' diet). This issue (feeding on what is available), and the fact cormorants make only short feeding flights (often less than two kilometers [Custer and Bunck, 1992]), during the breeding season, results in highly varied diets between colonies on adjacent bodies of water, or even the same body of water. The limited feeding range of cormorants allows researchers to study colonies close to one another, with minimal concern of diet and resource overlap.

Because diet composition varies so widely among colonies, diet quality may vary greatly as well. Fish and energetic research has shown great differences in caloric value, percent fat, and percent protein among different species of prey. For example, a cormorant consuming 100 grams of frog receives 73 calories (0.3% fat and 16.4% protein), while a cormorant consuming 100 grams of American eel receives 233 calories (18.3% fat and 15.9% protein) (University of

Wisconsin Sea Grant Institute, 2001). Given the high potential for variation in prey quality and the influence of diet and stress, diet quality and composition may play a large role in the development of cormorant nestlings.

Cormorant Diet

In addition to the effects of environmental stressors on altricial nestlings, there is a great deal of interest in cormorant diet. This is due to the growing concern from various user groups indicating the negative impacts of cormorants on recreational fisheries. The possible effects on sport and commercial fisheries have created a large body of cormorant diet research (Dalton *et al.*, 2009; Mortensen *et al.*, 2007; Rudstam *et al.*, 2004; Stickley *et al.*, 2002; Glahn and Brugger, 1995; Custer and Bunck, 1992; Hobson *et al.*, 1989; Craven and Lev, 1985). Most studies suggest little or no impact on commercial or sport fisheries but the highly varied nature of cormorant diet still influences concerned groups to inquire about the diet of local colonies.

Although rare, one of the few examples of notable impact on a fishery was observed at Oneida Lake, New York, where it was estimated that 57-77% of the cormorant diet consisted of walleye (*Sander vitreus*)(Rudstam *et al*., 2004). Cormorant foraging appears to have a greater impact on commercial fishing than sport fishing. A study in Wisconsin's Apostle Islands in the early 1980's found a weak correlation between increasing cormorant numbers and a decrease in commercial lake whitefish (*Coregonus clupeaformis*) catch (Craven and Lev, 1985).

Perhaps the most conclusive evidence of cormorant impact was seen in the American southeast, where cormorants were found to consume between 5 and 28 catfish per cormorant-hour in closed-system catfish rearing ponds, resulting in significant losses to some aquaculture facilities (Stickley *et al.*, 1992). These losses increased social awareness and distrust in cormorants, as cormorant consumption ultimately cost the industry 4% of its total stocks and an

estimated two million dollars per year (Glahn and Brugger, 1995, Glahn and Stickley, 1995). These losses induced legal actions in 1998 resulting in depredation orders allowing the legal harassment of cormorants at roosting sites and the culling of cormorants doing damage to private or public resources in 24 states, Minnesota included (Trapp, 1998).

Although impacts of cormorant foraging to sport fisheries are rarely found in well-performed studies, there can be significant local pressure to blame or remove cormorants when fisheries start to decline. At Leech Lake, MN the number of nesting cormorants increased 35-fold between 1998 and 2004. Diet studies performed in 2005-2006 on Leech Lake did not support the hypothesis that cormorants were directly responsible for the decline in walleye abundance and recruitment that started in 2001(Mortensen *et al.*, 2007), yet an aggressive culling program was adopted by managers to reduce cormorant numbers due to pressure from local fishermen and businesses. In an analogous case, the anadromous alewife (*Alosa pseudoharengus*) was also seeing population declines in Connecticut, and cormorants were believed to play a key role in the decline. However research indicated that cormorants played no role in the alewife decrease and were not an immediate threat to the existing population of alewife (Dalton *et al.*, 2009). A 1987 study on Lake Winnipegosis, Manitoba, revealed a diet consisting primarily of white sucker (*Catostomus commersoni*), which made up nearly half of the biomass, where walleye and sauger (*Stizostedion canadense*), sought commercially, made up only 0.1% and 0.2% of the prey biomass respectively (Hobson *et al.*, 1989). The researchers of this study hypothesized the dramatic increases in cormorant populations seen in the surrounding areas in the 1980's was a result of overfishing the predatory game fish. This allowed smaller bait fish (e.g. sucker, perch, minnow spp.) populations to increase, in fact creating more suitable forage for cormorants and their nestlings.

Cormorants in Central North America

As the cormorant populations continue to recover, areas are re-colonized and/or colonized. Voyageurs National Park, located on the border of Minnesota and Ontario, saw the colonization of Northeast Pine Island in 1999 on Lake Kabetogama, a popular recreational fishing destination. Several colonies exist on Rainy Lake in southern Ontario, where there is concern over the potential impact on that fishery as well, in great part due to the local economy's dependence on fishing and ecotourism. The existence of a cormorant colony on Lake Mille Lacs, in central Minnesota, has also raised the interest of fishermen and local officials who are concerned about the potential impact on the well-known walleye fishery. The movement of the cormorant westward into the prairie pothole regions of western Minnesota and North Dakota has also raised questions regarding the potential impact to these areas (Wires et al., 2001).

Conclusions

Environmental stressors present challenges to all living organisms, and developmental mode (e.g., altricial or precocial) likely affects how organisms cope with these stressors. Young altricial nestlings, though seemingly underdeveloped, must react to stressors with either a physiological response (i.e. CORT) and perhaps cope with the negative ramifications of this action, or conversely, mount no response (i.e. HPA inactivity or suppression) and undergo the stressor directly but continue to develop without CORT interference. The nexus of avian physiology and wildlife management therefore requires an examination of ecological processes as well as environmental stressors and their impact on growth, development and ultimately fitness.

Although cormorant diet continues to be of great interest to wildlife managers and the public, we have an opportunity to advance our understanding of developmental physiology and

ecology by looking beyond diet alone. Cormorants offer an opportunity to explore the link between larger ecological processes (i.e., both real and perceived impacts to fisheries) while simultaneously informing us about the effects of environmental stressors on the development of altricial nestlings. By examining cormorant diet alone in hopes of assessing the effects on a particular fishery, we fail to draw conclusions regarding an array of topics including: energetic requirements, prey selection, parasite and disease transmission, environmental stress, and growth and developmental factors. If we use this information in conjunction with hematology and stress tests, diet may also reveal important conclusions regarding altricial ontogeny, which may prove useful for developing management strategies concerning cormorants and other altricial species. Finally, by studying the effects of environmental stressors we may reveal ways to mitigate environmental and anthropogenic issues (e.g., pollutants, harassment, disease, predation, etc.) facing native wildlife populations.

Literature Cited

Blas, J., R. Baos, G. R. Bortolotti, T. Marchant, and F. Hiraldo. 2005. A multi-tier approach to identifying environmental stress in altricial nestling birds. Functional Ecology 19:315-322.

Boag, P.T. 1987. Effects of nestling diet on growth and adult size of zebra finches (Poephila guttata). The Auk. 104:155-166.

Bonier, F., P. R. Martin, and J. C. Wingfield. 2007. Maternal corticosteroids influence primary offspring sex ratio in a free-ranging passerine bird. Behavioral Ecology 18:1045-1050.

Bortolotti, G. R., T. A. Marchant, J. Blas, and T. German. 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Functional Ecology 22:494-500.

Breuner, C.W., and M. Orchinik. 2002. Plasma binding proteins as mediators of corticosteroid action in vertebrates. Journal of Endocrinology 175:99-112.

Busch, D. S., and L. S. Hayward. 2009. Stress in a conservation context: A discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biological Conservation 142:2844-2853.

Cockrem, J. F., and B. Silverin. 2002a. Sight of a predator can stimulate a corticosterone response in the great tit (Parus major). General and Comparative Endocrinology 125:248-255.

Cockrem, J. F., and B. Silverin. 2002b. Variation within and between birds in corticosterone responses of great tits (Parus major). General and Comparative Endocrinology 125:197-206.

Costantini, D. 2010. Effects of diet quality on growth pattern, serum oxidative status, and corticosterone in Pigeons (Columba livia). Canadian Journal of Zoology-Revue Canadienne De Zoologie 88:795-802.

Craven, S. R., and E. Lev. 1985. Double-crested Cormorant Damage to a Commercial Fishery in the Apostle Islands, Wisconsin. in Eastern Wildlife Damage Control Conferences, University of Nebraska, Lincoln.

Criscuolo, F., P. Monaghan, L. Nasir, and N. B. Metcalfe. 2008. Early nutrition and phenotypic development: 'catch-up' growth leads to elevated metabolic rate in adulthood. Proceedings of the Royal Society B-Biological Sciences 275:1565-1570.

Custer, T. W., and C. Bunck. 1992. Feeding flights of breeding double-crested cormorants at two Wisconsin colonies. Journal of Field Ornithology 63:203-211.

Cyr, N. E., K. Earle, C. Tam, and L. M. Romero. 2007. The effect of chronic psychological stress on corticosterone, plasma metabolites, and immune responsiveness in European starlings. General and Comparative Endocrinology 154:59-66.

Dalton, C. M., D. Ellis, and D. M. Post. 2009. The impact of double-crested cormorant (Phalacrocorax auritus) predation on anadromous alewife (Alosa pseudoharengus) in south-central Connecticut, USA. Canadian Journal of Fisheries and Aquatic Sciences 66:177-186.

Glahn, J. F., and K. E. Brugger. 1995. The impact of double-crested Cormorants on the Mississippi Delta catfish industry: A bioenergetics model. Colonial Waterbirds 18:168-175.

Glahn, J. F., and A. R. Stickley. 1995. Wintering Double-crested Cormorants in the Delta region of Mississippi: Population levels and their impact on the catfish industry. Colonial Waterbirds 18:137-142.

Gross, W. B., and H. S. Siegel. 1983. Evaluation of the heterophil lymphocyte ratio as a measure of stress in chickens. Avian Diseases 27:972-979.

Hatch, J. J., and D. V. Weseloh. 1999a. Double-crested cormorant (Phalacrocorax auritus). in In The Birds of North America The Birds of North America Inc., Philadelphia, PA.

Hayward, L. S., and J. C. Wingfield. 2003. Maternal corticosterone slows chick growth and increases adult stress response. Integrative and Comparative Biology 43:925-925.

Hobson, K. A., R. W. Knapton, and W. Lysack. 1989. Population, diet, and reproductive success of double-crested cormorants breeding on Lake Winnipegosis, Manitoba. 1987. Colonial Waterbirds 12:191-197.

Honarmand, M., W. Goymann, and M. Naguib. 2010. Stressful Dieting: Nutritional Conditions but Not Compensatory Growth Elevate Corticosterone Levels in Zebra Finch Nestlings and Fledglings. Plos One 5:7.

Johnsgard, P. A. 1993. Cormorants, darters, and pelicans of the world. Smithsonian Institute.

Johnson, N.F. 1971. Effects of dietary protein on Wood Duck growth. Journal of Wildlife Management. 35:798-802.

Kitaysky, A. S., E. Kitaiskaia, J. Piatt, and J. C. Wingfield. 2003. Benefits and costs of increased levels of corticosterone in seabird chicks. Hormones and Behavior 43:140-149.

Kitaysky, A. S., E. V. Kitaiskaia, J. C. Wingfield, and J. F. Piatt. 2001a. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 171:701-709.

Kitaysky, A. S., J. C. Wingfield, and J. F. Piatt. 2001b. Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. Behavioral Ecology 12:619-625.

Kozlowski, C. P., and R. E. Ricklefs. 2010. Egg size and yolk steroids vary across the laying order in cockatiel clutches: A strategy for reinforcing brood hierarchies? General and Comparative Endocrinology 168:460-465.

Metcalfe, N.B., and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends in Ecology & Evolution 16:254-260.

McEwen, B. S., and J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. Hormones and Behavior 43:2-15.

McFarlane, J.M., and S.E. Curtis. 1989. Multiple Concurrent Stressors in Chicks. Poult Sci 68:522-527.

Mortensen, S., J. Ringle, P. Hundt, and O. Goktepe. 2007. Double-crested cormorant food habits and predation effects on selected fish species in Leech Lake - Final Report. in Project Number: W-3-04-017, MN-U-9-NA Tribal Wildlife Grant Program.

Portmann, A. 1955. Die postembryonale Entwicklung der Vogel als Evolutionsproblem. 138-151 Acta XI Congress of International Ornithologists, Basel.

Rich, E. L., and L. M. Romero. 2005. Exposure to chronic stress downregulates corticosterone responses to acute stressors. Regulatory, Integrative and Comparative Physiology 288:R1628-R1636.

Romero, L. M., M. Ramenofsky, and J. C. Wingfield. 1997. Season and migration alters the corticosterone response to capture and handling in an Arctic migrant, the white-crowned sparrow (Zonotrichia leucophrys gambelii). Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology 116:171-177.

Romero, L. M., J. M. Reed, and J. C. Wingfield. 2000. Effects of weather on corticosterone responses in wild free-living passerine birds. General and Comparative Endocrinology 118:113-122.

Romero, L. M., and R. C. Romero. 2002. Corticosterone responses in wild birds: The importance of rapid initial sampling. Condor 104:129-135.

Romero, L. M., D. Strochlic, and J. C. Wingfield. 2005. Corticosterone inhibits feather growth: Potential mechanism explaining seasonal down regulation of corticosterone during molt. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 142:65-73.

Rubolini, D., M. Romano, G. Boncoraglio, R. P. Ferrari, R. Martinelli, P. Galeotti, M. Fasola, and N. Saino. 2005. Effects of elevated egg corticosterone levels on behavior, growth,

and immunity of yellow-legged gull (Larus michahellis) chicks. Hormones and Behavior 47:592-605.

Rudstam, L. G., A. J. VanDeValk, C. M. Adams, J. T. H. Coleman, J. L. Forney, and M. E. Richmond. 2004. Cormorant predation and the population dynamics of walleye and yellow perch in Oneida Lake. Ecological Applications 14:149-163.

Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21:55-89.

Sauer, J. R., J. E. Hines, J. E. Fallon, K. L. Pardieck, D. J. Ziolkowski, Jr., and W. A. Link. 2011. The North American Breeding Bird Survey, Results and Analysis 1966 - 2009. Version 3.23.2011 USGS Patuxent Wildlife Research Center, Laurel, MD

University of Wisconsin Sea Grant Institute. 2001. Calorie, Fat, and Protein Content of Fish and Seafoods.. http://seagrant.wisc.edu/greatlakesfish/ctable.html

Schwabl, Hubert, Franz Bairlein, and Eberhard Gwinner. 1991. Basal and stress-induced corticosterone levels of garden warblers, Sylvia borin, during migration. Journal of Comparative Physiology B 161.6: 576-580.

Starck, J., and R. Ricklefs. 1998. Avian Growth and Development: Evolution within the Altricial-Precocial Spectrum. Oxford University Press, New York, New York.

Stickley, A. R., G. L. Warrick, and J. F. Glahn. 1992. Impact of Double-Crested Cormorant Depredations on Channel Catfish Farms. Journal of the World Aquaculture Society 23:192-198.

Trapp, J.L. 1998. Migratory Bird Permits; Establishment of a Depredation Order for the double-crested cormorant. Federal Register 63, no. 42, 10550-10561, March 4, 1998.

Tyson, L. A., J. L. Belant, F. J. Cuthbert, and D. V. Weseloh. 1997. Populations of Double-crested Cormorants in the United States and Canada. in Symposium on Double-crested Cormorant population status and management issues in the Midwest., WI Technical Bulletin, US Dept of Agriculture.

Wingfield, J. C., D. L. Maney, C. W. Breuner, J. D. Jacobs, S. Lynn, M. Ramenofsky, and R. D. Richardson. 1998. Ecological bases of hormone-behavior interactions: The "emergency life history stage". American Zoologist 38:191-206.

Wingfield, J. C., and M. Ramenofsky. 1997. Corticosterone and facultative dispersal in response to unpredictable events. Ardea 85:155-166.

Wires, L. R., F. J. Cuthbert, D. R. Trexel, and A. R. Joshi. 2001. Status of the Double-crested Cormorant (Phalacrocorax auritus) in North America. in Final Report to USFWS.

Wires, L. R., K. V. Haws, and F. J. Cuthbert. 2005. The double-crested cormorant and American white pelican in Minnesota: a statewide assessment. in Final report submitted to the Nongame Wildlife Program State Wildlife Grants Program Report.

CHAPTER 2: ENVIRONMENTAL STRESSORS AND THEIR EFFECTS ON THE ALTRICIAL NESTLINGS OF DOUBLE-CRESTED CORMORANTS (*PHALACROCORAX AURITUS*)

Abstract

Environmental and ecological stressors are ever present in natural settings. Poor diet, competition, disease, parasites and weather are just several on the long list of stressors animals experience daily. Past research examining the effect of these stressors has largely neglected wild populations of colonial and altricial species. In this study I examine the effect of ectoparasite load, endoparasite load, and diet quality on three specific growth rate metrics (mass, wing digit, and tarsus) in the altricial nestlings of double-crested cormorants (*Phalacrocorax auritus*) at five natural colonies in the upper Midwest. I also examined the utility of a correlative approach for measuring stress in colonial birds. I identified a change in growth rates in response to environmental stressors in altricial nestlings. Endoparasites and diet quality were significantly correlated to growth and development of double-crested cormorant nestlings. This study illustrates the possible additive effects of multiple environmental stressors and the potential for mitigation of these stressors when management is required.

Introduction

In recent years there has been a growing interest regarding the effects of stress on avian species in natural settings. However, it has been questioned whether altricial nestlings, in particular, perceive and respond to ecological stressors (Blas *et al.*, 2005). A period of physiological hypo-responsiveness in altricial nestlings is believed to assist in avoiding any negative effects of the stress response (Kitaysky *et al.*, 2003). Environmental stressors, labeled as biological, chemical, or physical (Jobling, 1994), can include: weather, competition (inter- and

conspecific), harassment, parasites (ecto- and endoparasites), contaminants, and poor diet. Of these, only competition with siblings and ectoparasite loads may be regulated by nest-bound birds, and any adrenocortical response may impair the bird's ability to overcome the immediate stressor (Sims and Holberton, 2000). The existing hypothesis states that nest-bound birds are unable to overcome many environmental stressors and develop their stress response later in development to avoid difficulties associated with stress during early development.

Stressors elicit a suite of physiological and behavioral changes and it is generally assumed when animals respond to a stressor there is an energetic trade-off (Wingfield and Ramenofsky, 1997). In short, at a critical point the ability of an animal to respond to stress becomes maladaptive. The energy used to overcome the stressor surpasses the amount of energy needed for other functions (e.g., growth, reproduction, immune response, etc.), which results in negative effects on fitness.

Birds respond to adverse stimuli (stressors) by releasing corticosterone (CORT), a glucocorticoid, from the adrenal gland. The adrenal gland is centrally controlled by the hypothalamus and pituitary glands in the brain and the three endocrine tissues form the hypothalamic-pituitary-adrenal (HPA) axis. Changes in weather, diet, drought, or risk of depredation activates the HPA axis, releasing CORT (Blas *et al.*, 2005, Romero *et al.*, 2000; Astheimer *et al.*, 1995,), which triggers behavioral and physiological changes to suspend activities not necessary for immediate survival (Silverin, 1998). This facultative response allows birds to deal with the immediate presence of a stressor (Wingfield and Ramenofsky, 1997;Wingfield *et al.*, 1998;Sapolsky *et al.*, 2000), which is highly beneficial in younger nestlings, as it may increase activity and begging behavior in subordinate chicks and increase nestling survival (Vallarino *et al.*, 2006).

In this study I used a two-tiered approach to identify environmental variables associated with stress in the altricial nestlings of double-crested cormorants: (1) within individuals, and (2) among different colonies. Over a three-week period in 2009, during early nestling development, I examined the effects of environmental stressors on growth and development by taking weekly measurements of mass and long bone growth. Stress was evaluated using heterophil to lymphocyte ratios (HL ratio), as this has been found to directly relate to the level of circulating CORT in avian species, but with slower fluctuations than CORT in response to an immediate stressor like handling (Gross and Siegel, 1983). Under normal circumstances, this leukocyte ratio will increase (i.e., heterophils will increase) as CORT levels increase. HL ratios have been used in a number of studies examining avian stress along the developmental and age spectrum. One study examining the altricial nestlings of pied flycatchers (*Ficedula hypoleuca*) revealed an inverse relationship between tarsus length and HL ratios, indicating increased HL ratios are likely correlated with decreased growth rates (Moreno 2002). Yet another study on nestling pied flycatchers documented increased HL ratios in artificially enlarged broods, perhaps linking nutritional stress and increased HL ratios (Ilmonen et al. 2003).

Methods

Study Species and Study Area

Double-crested cormorants are a common, moderately-sized (1.2-2.5 kg), colonial waterbird that feeds on fish, amphibians and crustaceans. Heavily persecuted in North America since European settlement, double-crested cormorants were facing potential extirpation as recently as the 1970's. Following litigation restricting the use of DDT and the rise in popularity of aquaculture, populations of cormorants rebounded rapidly, and are now found across much of North America. There is substantial regional variation in size, with birds becoming larger to the

north and west. Islands and cliffs are the most common habitat in the northern ranges found to support active colonies. Cormorant chicks hatch after approximately 30 days of incubation and remain in the nest for three to four weeks. At this point, they form crèches and roam the ground, and if accessible, will take to water if threatened. At six to seven weeks young are able to begin making short flights and are nearly independent (Mendall, 1936; Hatch and Weseloh, 1999).

I examined five cormorant colonies, three of which are found in the border-lakes region of Minnesota and Ontario, and the other two are found in central Minnesota and north-central North Dakota. The border-lakes region of Minnesota and Ontario is strewn with lakes of various depth, size, and productivity. Lake Kabetogama, fully contained within Voyageurs National Park, is a roughly 9,700 hectare lake, with most of the acreage under 11 meters deep. It is a highly productive lake and is well known for its walleye (*Sander vitreus*) fishery. A cormorant colony was established on Northeast Pine Island (NEP) in 1999, after which the population quickly grew. To the north, Rainy Lake, much larger at upwards of 220,000 acres is also considered to be a quality sport fishery. Rainy supports several different sport fish species than Kabetogama, including ciscoes, muskellunge (*Esox masquinongy*), and rainbow smelt (*Osmerus mordax*). The lake is separated into two arms, the north arm and south arm. The south arm, also partially contained within Voyageurs National Park, is home to the Seven Sisters Islands (7SIS), an archipelago with a number of islands hosting nesting cormorants. The north arm, stretching well into Ontario, has seen the colonization and rapid expansion of a cormorant colony on an island near the Noden Causeway (NOD).

In central Minnesota, Minnesota's second largest inland lake, Lake Mille Lacs (ML), has gained national fame for its sport fishery, particularly walleye and muskellunge. This relatively shallow, 128,224 acre lake possesses two boulder islands near its southeastern and southwestern

shores. These two islands, Spirit and Hennepin, compose the Mille Lacs National Wildlife Refuge (NWR), at just over 1/2 acre, it is the country's smallest NWR. Spirit Island, the larger island of the two, remains unmanaged save yearly cormorant nest counts. The cormorant population at Spirit Island has remained relatively steady for many years, yet there is local concern surrounding its existence.

In contrast to the previous lacustrine colonies, North Dakota's J. Clark Salyer National Wildlife Refuge (JCS) is found on the northern end of the Souris River in the north-central part of the state. Although not a popular fishing destination, this federally protected land is dominated by river, shallow backwaters, and impoundments, all with greatly fluctuating seasonal water levels. This cormorant colony, completely surrounded by nesting gulls (mostly Franklin's gulls (*Leucophaeus pipixcan*)), is unlike the aforementioned colonies in other respects as well. Boat traffic is nearly nonexistent, whereas the habitats with developed sport fisheries experience significant activity from recreational boats near cormorant colonies. The riparian habitat found surrounding this colony likely supports excellent spawning grounds for common river game fish, such as northern pike. Other common river species are likely present as well (e.g. cyprinids, catostomids, hiodontids, and ictalurids). Bullheads (*Ameiurus spp.*) and channel catfish (*Ictalurus punctatus*) round out the list of species most likely to be found in this habitat.

Field Procedures

We visited each colony three times at approximately one week intervals. Sampling was not initiated until there was a population of approximately 30 hatchlings one week of age or less, as this was the target sample number. The initial sampling dates varied from June 19 to July 29, as initiation of egg laying is highly variable even among adjacent colonies (older colonies are generally two or three weeks ahead of younger colonies) and even within colonies (Hatch and

Weseloh, 1999). All colonies sampled were found on islands, and access was only possible with watercraft. We attempted to visit colonies in the morning (7-11a.m.) to avoid diel effects and overheating young hatchlings, as cormorant nestlings are unable to thermoregulate for 14-15 days (Dunn, 1976). We actively monitored nestling condition during sampling visits and left the colony if daytime temperatures exceeded 27°C.

Upon reaching the colony, a processing area was located in an area to minimize stress on nestlings (out of sight, if possible). We then formed two working groups, with one group consisting of three people to process birds while the alternate group protected the remaining colony from predators (e.g., gulls: *Larus spp.* and *Leucophaeus spp.*) and collected diet samples. Before removing chicks from a nest, a numbered biodegradable tag was attached to the nest to assure the chick was returned to the correct nest. Chicks were then weighed to the nearest 1.0 gram using either a 30 or 100 g spring balance (Pesola®) and measured (tarsus and wing digit) to the nearest 0.1 mm with digital calipers (Tool Shop®). The chick received an individually identifiable color coded nape tag during the first capture. This nape tag allowed weekly recapture and consecutive measurements of the same individual over the three week period. A 15-second search for ectoparasites (i.e., Order Phthiraptera) was performed, focusing on the warm areas created in the junctions between the wings and body, and legs and body. The tally of ectoparasites was ultimately used to categorize colonies into groups of high, medium, and low levels of ectoparasitism. Finally, using a 25-gauge hypodermic needle, I elicited a drop of blood from the ulnar vein and created a blood smear on a 75×25mm glass microscope slide. The slide was labeled with the bird's identification, date, and colony then placed in a hard-sided slide box for later laboratory analysis of HL ratio.

We collected regurgitated boluses from nestlings and preserved them in formalin following the procedures set in *Fish Collection Methods and Standards Version 4.0 (1997)* by the Canadian Resources Information Standards Committee. Boluses are easy and inexpensive to analyze as well as do no harm to the birds (Wires *et al.* 2001). Cormorants will often regurgitate food (i.e., fish) when they are threatened (Duffy and Jackson, 1986). By visiting colonies in the morning, we maximized the likelihood of chicks having full stomachs and minimized the level of digestion of the boluses, making prey identification possible. Boluses are often found in or around the nest, and single boluses often consist of multiple fish. When boluses consisted of multiple fish, they were all placed in a single specimen jar or bag. As soon as possible, the specimens were placed in a 10% formalin solution for fixation. The specimens were placed in a refrigerator at 1 °C to minimize decomposition before fixation was complete. Large prey items (>20cm) were injected with formalin directly into the body cavity through the vent to reduce any tissue loss or decomposition. Endoparasites (e.g., phyla Platyhelminthes and Nematoda) are also commonly present in regurgitated boluses. The parasites, dislodged from the digestive tract during regurgitation, can be used to assess parasitism loads at the colony level. Individual endoparasite loads cannot be assumed unless specific boluses can be attributed to a given nestling, which was not possible in this study. Endoparasites were counted during laboratory analyses of boluses.

Laboratory Procedures

Diet specimens were fixed in a 10% formalin solution for two weeks. Specimens were then rinsed and soaked in water for a 24 hour period. When possible, prey items were identified to species, standard lengths were measured, and mass was recorded. Endoparasites were also enumerated at this time, establishing an endoparasite per bolus value for each colony. A standard

length was estimated for partial prey items using like-sized representative samples trapped or seined from local waters. Standard lengths were used to calculate biomass using regressions found in published literature (Table 2.1). I estimated energy content as a measure of diet quality by calculating caloric content of fish from published regressions relating fish body mass to caloric content (Table 2.2). Closely related species (e.g., cyprinids) were grouped during calculations due to their similar caloric content.

Blood smears were stained in the laboratory using Hemacolor® Hematology Stain. This product uses a methanol fixative with eosin and methylene blue stains for differential staining of peripheral blood cells. Slides were then randomized by colony, and heterophil and lymphocytes were counted using oil immersion microscopy by two examiners to assure accuracy and minimize observer bias. Using *Avian Hematology and Cytology* (Campbell, 1988) and *Atlas to Avian Hematology* (Lucas and Jamroz, 1961) as references, the first 100 leukocytes identified as either heterophils or lymphocytes were recorded and a ratio extrapolated. When 100 leukocytes could not be found across the entire smear, which was rare (< 1%), the ratio was still extrapolated as long as the total number of leukocytes exceeded 50.

Age class was assigned to nestlings based on published tarsus length to age ratios. I used the tarsus length from the initial capture and assigned an age at capture. The length between successive captures was then added to determine age at recapture. Age classes were then assigned to each nestling at each capture as: Age Class 0 (0-6 days), Age Class 1 (7-13 days), Age Class 2 (14-20 days), Age Class 3 (21-27 days), Age Class 4 (28+ days).

Statistical Analyses

Statistical analyses were performed in either JMP 9 by SAS or program R v 2.12.1. *SAS for Mixed Models* was used as a reference for modeling design (Littell *et al.*, 2006). Boluses

(containing fish and endoparasites) were not uniformly attributable to particular nestlings, thus diet samples and endoparasite loads were analyzed at a colony level. Boluses were deemed statistical outliers and removed from follow up analyses if their total mass exceeded three standard deviations above the mean. To establish colony level patterns, an assessment of diet quality (calories • $bolus^{-1}$) and endoparasite load (endoparasites • $bolus^{-1}$) were conducted using an ANOVA and student's t-test to determine mean values and disparities among colonies. Mean colony values were then assigned to all individual birds originating in that colony.

Growth, HL ratio, and ectoparasite parameters were attributable to individual nestlings and did not require colony level averaging. Mass and length specific growth rates (SGR) were determined for all growth parameters. Specific growth rates, a measure of growth per unit time, were calculated by [ln(final condition)-ln(initial condition)*100]/time. Long bone growth (i.e., wing digit and tarsus) was measured and calculated in mm/mm/day, and mass growth calculated in g/g/day. Nestlings observed in each of the three observation periods received two SGR's; the first from week one through week two, and the second from week two through week three. When both SGR's were available for an individual, they were ultimately averaged for a mean SGR for that particular nestling. Stress levels, using HL, were similarly averaged for each nestling while modeling stress effects on SGR's. Because cormorants are altricial birds and hatch with no down, they support very few ectoparasites at early ages. As down becomes present, ectoparasites become more numerous and are easily counted. As plumage continues to grow, however, ectoparasites become much harder to see, though they are present. This limited window of accurate evaluation necessitated the use of maximum ectoparasite load as the modeling parameter.

To model the effects of environmental stressors on HL, maximum ectoparasite load, endoparasite load, age class, and diet quality were chosen as independent fixed variables. To assess population level effects, colony and individual nestling identification were added as random effects due to repeated measures. These parameters were applied to a linear mixed model, which used a restricted/residual maximum likelihood (REML) methodology. This method computes marginal likelihoods based on error contrasts and is useful in estimating variance and covariance functions. The REML method estimates are less biased than maximum likelihood estimates, and are useful for smaller datasets (Proust, 2010). This model allowed us to test the hypothesis of hypo-responsiveness among altricial nestlings, as well as testing for physiological changes as a result of environmental stressors.

When modeling effects on SGR's, the effect parameters included in the analyses were: maximum ectoparasite load, endoparasite load, diet quality, average H:L (stress), and all effect crosses with H:L due to the assumed effect of stressors on H:L. Colony was held as a random effect to test stress effects on the total population of double-crested cormorants. These parameters were similarly used in a linear mixed model utilizing a restricted/residual maximum likelihood methodology.

Results

Diet and Endoparasites

In total, 213 boluses were examined for diet quality (n=3 at 7SIS, 8 at JCS, 96 at ML, 86 at NEP, 20 at NOD). An analysis of variance (ANOVA) rejected the $H_{o,}$ revealing differing diet quality among colonies ($F_{4,208} = 2.5901$ $P<0.05$). A post hoc power test revealed a low power (0.227) to detect differences among the three colonies with the lowest sample sizes, and approximately 55 more samples would be required to detect significant differences. Therefore,

we excluded these colonies and compared diet quality between ML and NEP using a student's t-test. ML significantly differed ($\bar{x}$ = 82.86 calories•bolus^{-1}, *SD*=68.56) from NEP ($\bar{x}$=57.15 calories•bolus^{-1}, *SD*=50.57; t_{208}=1.971, *P* = <0.05). Large numbers of gulls were present at the three remaining colonies and may have consumed cormorant boluses opportunistically, greatly reducing our ability to collect samples. Mean diet quality calculated for these colonies were 88.54 calories•bolus^{-1} (*SD*= 49.54) at 7SIS, 49.74 calories•bolus^{-1} (*SD*=66.38) at JCS, and 77.50 calories•bolus^{-1} (*SD*=38.89) at NOD.

An examination of endoparasite loads (n=3 at 7SIS, 8 at JCS, 96 at ML, 86 at NEP, 20 at NOD) by colony using an ANOVA exposed differences at significant levels ($F_{4, 208}$ = 11.51, *P* < 0.05; Figure 2.4). Noden Causeway ($\bar{x}$ = 4.85 endoparasites•bolus^{-1}, *SD*=7.12) was found to deviate significantly from NEP ($\bar{x}$ = 0.50 endoparasites•bolus^{-1}, *SD*=1.30; t_{208}=6.48, *P* <0.05), ML ($\bar{x}$=0.67 endoparasites•bolus^{-1}, *SD*=1.75; t_{208}=6.29, *P*<0.05) and JCS ($\bar{x}$ = 1.88 endoparasites•bolus^{-1}, *SD*=3.83; t_{208}=2.63, *P* <0.05) by means of a student's t-test. NOD was not significantly different from 7SIS colony ($\bar{x}$ = 2.00 endoparasites•bolus^{-1}, *SD*= 3.46).

Ectoparasites

In total, we examined 210 nestlings for ectoparasites (n=30 at 7SIS, 61 at ML, 61 at NEP, and 58 at NOD). An examination of ectoparasites using an ANOVA rejected the H_o, exposing significant differences in ectoparasite loads among colonies ($F_{3,206}$=19.72, *P* <0.05). An analysis of the number of ectoparasites, using a student's t-test, revealed several significant differences among colonies. The Noden Causeway (NOD) colony was found to have a much higher level of ectoparasitism ($\bar{x}$ =39.48 ectoparasites) than 7SIS ($\bar{x}$ = 16.567 ectoparasites, t_{206}=3.26, *P*<0.05), NEP ($\bar{x}$ = 1.705 ectoparasites, t_{206}=6.59, *P*<0.05, and ML ($\bar{x}$ = 0.705 ectoparasites, t_{206}=6.76,

$P<0.05$). Mean ectoparasite load at 7SIS colony was significantly higher than both NEP and ML (t_{206}=-2.13, $P<0.05$; t_{206}=-2.27, $P<0.05$ respectively; Figure 2.5).

Stress Levels

In total, 173 blood smears were analyzed using a fit model to assess the effect of environmental factors on HL ratios. The model explained 19% of the variation in stress levels (HL ratios) with $P<0.05$. The fixed effect parameters used in the model include: diet quality, maximum ectoparasites, endoparasites, and age class. Colony and individual nestling identification were added to the model as random effects due to repeated measures and to allow us to extrapolate findings to the total cormorant population. All modeling parameters were found to have *P*-values >0.05, with age class being the only factor approaching a moderately significant level (P=0.107; Table 2.3).

An ANOVA revealed significant differences in HL among the varying age classes (n=14 age class 0, 76 age class 1, 47 age class 2, 31 age class 3, 5 age class 4; $F_{4,168}$=4.36, $P<0.05$). Age effect was then analyzed using a student's t-test. It was found stress levels were highest in the two youngest age classes, age 0 and 1 ($\bar{x}$=1.098, 1.271 respectively) and lowest at age class 4 ($\bar{x}$ = 0.649). HL at age class 1 was found to be significantly higher than age classes 2 ($\bar{x}$ = 0.961, t_{168}=-3.231, $P<0.05$), 3 ($\bar{x}$ = 0.971, t_{168}=-2.73, $P<0.05$), and 4 ($\bar{x}$ = 0.6493, t_{168}=-2.607, $P<0.05$), but lacked significance over age class 0 ($t_{168=1.153,}$ P = 0.25). A post hoc linear fit model was run to test how much variation in HL could be accounted for by age alone (14.2%, RMSE=0.499, $P<0.05$).

In the examination of stress (HL) variation among colonies, an ANOVA revealed significant differences among the colonies (n=28 at 7SIS, 13 at JCS, 25 at ML, 24 at NEP, and 12 at NOD; $F_{4,97}$=6.997, $P<0.05$). Using average HL, a comparison of each colony pair using a

student's t-test revealed ML nestlings to have a significantly lower H:L ($\bar{x}$ = 0.817) than 7SIS ($\bar{x}$ =1.445, t_{97}=-5.269, P<0.05), JCS ($\bar{x}$ =1.185, t_{97}=-2.653, P=0.01), NOD ($\bar{x}$ =1.195, t_{97}=2.254, P=0.03), and NEP ($\bar{x}$ =1.127, t_{97}=2.658, P=0.01) nestlings (Figure 2.5). 7SIS ($\bar{x}$ = 1.445) was found to differ from NEP at a significant level as well ($\bar{x}$ = 1.146, t_{97}=-2.481, P=0.01), although this significance is questionable, as the effects were biased high due to only age classes 0 and 1 being analyzed. The model indicates younger birds should be expected to have higher stress levels.

Growth

Mass and length specific growth rate modeling exposed similarities across all three metrics (i.e., mass, wing digit, and tarsus). All three models found diet quality to produce consistently lower *P*-values, although not significant in the mass SGR model ($F_{1,41}$=2.205, P=0.145). The fit model examining mass SGR (Table 2.4) resulted in a significant model explaining 14.2% of the variation (n=42, RMSE=0.042, P<0.05), yet no individual modeling effects were found to be statistically significant. Continuing on, diet quality was found to be a significant parameter for both wing digit ($F_{1,41}$=6.142, P=0.02; Table 2.5) and tarsus SGR's ($F_{1,41}$=7.358, P=0.01; Table 2.6). The modeling of wing digit SGR resulted in a significant model explaining 31.3% of the variation detected with both diet quality and endoparasite load ($F_{1,41}$=6.846, P=0.01) being significant modeling parameters. (n = 46, RMSE=0.023, P<0.05; Table 2.5). The tarsus SGR model was also found to explain a significant amount of the variation, at 23.4% (n= 46, RMSE=0.017, P < 0.05; Table 2.6).

Discussion

While trying to determine how environmental stressors affect the stress response, I additionally tested for age effects to examine the emergence of the stress response in altricial

nestlings. The fit model results provided several interesting insights. I found the environmental stressors that were tested had little impact on HL levels, with none producing significant values. The age class of the nestling was the only parameter found to approach a significant *P*-value (*P*=0.145). This effect of age, if it does exist, may diminish the credence to the theory of delayed development and hypo-responsiveness of the HPA axis during the nestling phase. It was believed stress levels would be diminished in the first two sampling periods if nestlings were unresponsive to stressors. Müllner and colleagues (2003) found stress levels to be lower in juvenile hoatzins while early in the nestling stage; while just prior to fledging, stress levels increased resulting in higher mortality rates. Sims and Holberton (2000) also document suppressed stress levels in nestling mockingbirds, but concurrently demonstrated the ability of adrenocortical tissues to manage a hormonal response to stressors. In effect, the HPA axis of nestling mockingbirds was developed and functional, but the activity of the stress response developed in concert with the developing ability to mitigate potential stressors. Interestingly, the opposite was observed in this study. Stress levels in double-crested cormorant nestlings, as measured by HL ratios, were highest during the first two weeks after hatch, and the lowest at age class 4 (i.e. 3-4 week old chicks). These findings corroborate those observed in the European White Stork (*Ciconia ciconia*) (Blas et al. 2005), which suggests that altricial nestlings are not completely reliant on parental mitigation of environmental stressors, but do have the physiological ability to mount a stress response.

Changes in sensitivity of the HPA axis during the nestling phase is one hypothesis to explain the trend found in this study. Measurements of HL ratios are not a direct measure of HPA activity, and alternative hypotheses for elevated HL levels in the early nestling stage include maternal effects transmitted through eggs, as well as changes in the developing immune

system as both heterophils and lymphocytes are integral parts of the immune system. If these hypotheses hold true, it is still possible for these birds to be in a hypo-responsive period early in development, albeit with elevated stress levels. It is also possible that high initial stress levels may be the result of, or in preparation for, the stress associated with the process of hatching.

Environmental stressors appear to affect growth in structural size similarly, with diet quality affecting both wing digit and tarsus SGR's. This finding indicates increased diet quality is positively correlated with increased structural specific growth rates. Wing growth was also found to be affected by endoparasite load, with increased endoparaisite loads correlated to a reduced SGR. This finding is interesting due to the ability of cormorants to "self-medicate" for digestive endoparasites by consuming small rocks that dislodge the parasites from the digestive lining, leaving the bird theoretically unaffected by the presence of the parasite (Robinson et al. 2008). The indicator of this behavior is the presence of numerous small rocks in and around the nest, which was witnessed at the colonies involved in this study, but the findings of this study may indicate nestling cormorants do not partake in this behavior (Randa, personal observation).

Diet, again a significant modeling parameter, was found to marginally account for variation in HL, and concomitantly growth rates. This finding agrees with, and confirms, previous studies documenting a negative correlation between stress and diet quality, as well as a positive correlation between diet quality and growth rates (Costantini, 2010; Boag, 1987; Johnson 1971). Also noteworthy is the lack of significance of HL as a fit model parameter for SGR's, as well as the crosses between environmental stressors and HL. It appears that if HL is a suitable method for testing CORT and stress levels in nestlings, our samples were not stressed enough to see significant changes in SGR's. It is also possible, as noted before, that lack of significant findings in regards to HL ratios, may be due to the inactivity or changes in HPA

sensitivity, as well as a developing immune function. Furthermore, the presence of endoparasites as significant SGR modeling parameters corroborates with the theory correlating environmental stressors and differing developmental rates.

Regarding the use of HL as a correlative approach to estimating CORT and stress levels, the lack of significant correlations between environmental stressors and HL may invalidate this methodology as a determinant of stress in field research. This technique ultimately proved to be only marginally valuable. This being stated, this technique did reveal significant variation in HL at various age classes, at least indicating changes in immune response.

In conclusion, it was found that HL analyses are likely an uacceptable method for assessing stress levels in nest-bound birds, but may prove to be a preferred methodology for examining age effects on the development of the immune response. It was also discovered that environmental stressors, such as diet and endoparasites do impact the growth and development of nest-bound altricial species. Linear fit models examining SGR's only described 14-31% of the variation, leading one to believe in the likelihood of other significant environmental "ghost" factors effecting nestling development. As ANOVA tests demonstrated, these "ghost" factors may be attributable to specific colony-level factors such as: harassment, predation, weather, exposure, nest density, age of colony, etc. Perhaps future research should utilize the inclusion of these other qualitative stress factors as well as a maternal stress evaluation. The discovery of an age effect on stress levels successfully demonstrates a change in immune response, but does not conclusively indicate a period of HPA inactivity or hypo-responsiveness. An examination of hormonal titers in the first two weeks post-hatch, as well as a prolonged sampling period examining birds post-fledge would be required to completely capture the physiological changes occurring within these birds to ready them for short and long-term environmental perturbations.

Literature Cited

Astheimer, L. B., W. A. Buttemer, and J. C. Wingfield. 1995. Seasonal and acute changes in adrenocortical responsiveness in an arctic-breeding bird. Hormones and Behavior 29:442-457.

Blas, J., R. Baos, G. R. Bortolotti, T. Marchant, and F. Hiraldo. 2005. A multi-tier approach to identifying environmental stress in altricial nestling birds. Functional Ecology 19:315-322.

Brugger, K. E. 1992. Differential digestibilities of Channel Catfish, Bluegill, and Gizzard Shad - invitro standards for gastric digestion by seabirds. Colonial Waterbirds 15:257-260.

Brugger, K. E. 1993. Digestibility of 3 fish species by Double-Crested Cormorants. Condor 95:25-32.

Bryan, S. D. 1995. Bioenergetics of Walleye in Lake Oahe, South Dakota, South Dakota State University.

Campbell, T. W. 1988. Avian Hematology and Cytology. Iowa State Press.

Carlander, K. D. 1969. Handbook of freshwater fishery biology. Iowa State University Press, Ames.

Carlander, K. D. 1977. Handbook of freshwater fishery biology. Iowa State University Press, Ames.

Carlander, K. D. 1997. Handbook of freshwater fishery biology. Iowa State University Press, Ames.

Committee, R. I. S. 1997. Fish collection methods and standards. http://archive.ilmb.gov.bc.ca/risc/pubs/aquatic/fishcol/index.htm Province of British Columbia.

Duffy, D. C., and S. Jackson. 1986. Diet studies of seabirds: a review of methods. Colonial Waterbirds 9:1-17.

Dunn, E. H. 1975. Growth, body components and energy content of nestling double-crested cormorants. The Condor 77:431-438.

Dunn, E. H. 1976. Development of endothermy and existence energy expenditure of nestling double-crested cormorants. Condor 78:350-356.

Gross, W. B., and H. S. Siegel. 1983. Evaluation of the heterophil lymphocyte ratio as a measure of stress in chickens. Avian Diseases 27:972-979.

Hatch, J. J., and D. V. Weseloh. 1999. Double-crested cormorant (Phalacrocorax auritus). In The Birds of North America The Birds of North America Inc., Philadelphia, PA.

Hundt, P. J. 2009. The diet of Double-crested Cormorants (Phalacrocorax auritus) of Leech Lake, Minnesota, University of Minnesota.

Ilmonen, P., Hasselquist, D., Langefors, A., 2003. Stress, immunocompetence and leukocyte profiles of pied flycatchers in relation to brood size manipulation. Oecologia. 136:148-154.

Jobling, M. 1994. Fish Bioenergetics. Chapman & Hall, London.

Kitaysky, A. S., E. Kitaiskaia, J. Piatt, and J. C. Wingfield. 2003. Benefits and costs of increased levels of corticosterone in seabird chicks. Hormones and Behavior 43:140-149.

Kushalan, J. A., and L. C. McEwan. 1982. Nesting phenology of the double-crested cormorant. Wilson Bulletin 94:201-206.

Littell, R., G. Milliken, W. Stroup, R. Wolfinger, and O. Schabenberger. 2006. SAS for Mixed Models. SAS Press.

Love, O. P., E. H. Chin, K. E. Wynne-Edwards, and T. D. Williams. 2005. Stress hormones: A link between maternal condition and sex-biased reproductive investment. American Naturalist 166:751-766.

Love, O. P., and T. D. Williams. 2008a. Plasticity in the adrenocortical response of a free-living vertebrate: The role of pre- and post-natal developmental stress. Hormones and Behavior 54:496-505.

Love, O. P., and T. D. Williams. 2008b. The adaptive value of stress-induced phenotypes: effects of maternally-derived corticosterone on sex-biased investment, cost of reproduction and maternal fitness. American Naturalist 172:135-149.

Lucas, A. M., and C. Jamroz. 1961. Atlas of Avian Hematology. Washington D.C.: U.S. Dept. of Agriculture.

Mayo, K. R., J. H. Selgeby, and M. E. McDonald. 1998. A Bioenergetics Modeling Evaluation of Top-down Control of Ruffe in the St. Louis River, Western Lake superior. Internat. Assoc. Great Lakes Res. 24:329-342.

Meakins, R. H. 1976. Variations in the energy content of freshwater fish. Journal of Fish Biology 8:221-224.

Mendall, H. L. 1936. The home-life and economic status of the double-crested cormorant, Phalacrocorax auritus auritus, (Lesson). Univ. Maine Stud., Second Ser., no. 38. Maine Bulletin 39:Univ. Press, Orono.

Moreno, J., Merino, S., Martinez, J., Sanz, JJ., Arriero, E., 2002. Heterophil/lymphocyte ratios and heat-shock protein levels are related to growth in nestling birds. Ecoscience. Vol. 9, no. 4, pp. 434-439. 2002.

Mullner, A., Linsenmair, K.E. & Wikelski, M. 2004. Exposure to ecotourism reduces survival and affects stress response in hoatzin chicks (Opisthocomus hoazin). Biological Conservation. 118: 549–558.

Proust, M. 2010. JMP 9 Modeling and Multivariate Methods. SAS.

Robinson, J. M., K. J. Jirka, and J. A. Chiotti. 2010. Age and growth analysis of the centrall mudminnow, Umbra limi (Kirtland, 1840) 18. Journal of Applied Icthyology 26:89-94.

Robinson, S.A., M.R. Forbes, and C.E. Hebert. 2008. Is the ingestion of small stones by double-crested cormorants a self-medication behavior? The Condor. 110: 782-785.

Romero, L. M., J. M. Reed, and J. C. Wingfield. 2000. Effects of weather on corticosterone responses in wild free-living passerine birds. General and Comparative Endocrinology 118:113-122.

Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21:55-89.

Schreckenbach, K., R. Knosche, and K. Ebert. 2001. Nutrient and energy content of freshwater fishes. Journal of Applied Ichthyology 17:142-144.

Silverin, B. 1998. Stress responses in birds. Poultry and Avian Biology Reviews 9:153-168.

Sims, C., and R. Holberton. 2000. Development of the corticosterone stress response in young northern mockingbirds (Mimus polyglottos). General and Comparative Endocrinology 119:193-201.

Vallarino, A., J. C. Wingfield, and H. Drummond. 2006. Does extra corticosterone elicit increased begging and submissiveness in subordinate booby (Sula nebouxii) chicks? General and Comparative Endocrinology 147:297-303.

Vallée, M., S. Maccari, F. Dellu, H. Simon, M. Le Moal, and W. May. 1999. Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: a longitudinal study in the rat. European Journal of Neuroscience 11:2906-2916.

Wingfield, J. C., D. L. Maney, C. W. Breuner, J. D. Jacobs, S. Lynn, M. Ramenofsky, and R. D. Richardson. 1998. Ecological bases of hormone-behavior interactions: The "emergency life history stage". American Zoologist 38:191-206.

Wingfield, J. C., and M. Ramenofsky. 1997. Corticosterone and facultative dispersal in response to unpredictable events. Ardea 85:155-166.

Wires, L. R., F. J. Cuthbert, D. R. Trexel, and A. R. Joshi. 2001. Status of the Double-crested Cormorant (Phalacrocorax auritus) in North America. P.^Pp. in Final Report to USFWS.

Table 2.1. Length-weight regressions used to calculate biomass from bolus specimens (Carlander, 1969, 1977, 1997, Hundt, 2009, Robinson *et al.*, 2010)

Species	Equation	Base	base(W) = a + b Base SL	
			a	b
Amblopites rupestris	logW=-4.574+3.057 log SL	log	-4.574	3.057
Ameiurus melas	logW=-4.049+2.801 log SL	log	-4.049	2.801
Centrarchid sp.	logW=-4.770+3.152 log SL	log	-4.77	3.152
Coregonus sp.	logW=-5.056+3.168 log SL	log	-5.056	3.168
Culaea inconstans	lnW=-11.873+3.248 ln SL	ln	-11.873	3.248
Etheostoma sp.	logW=-4.6576+2.8983 log SL	log	-4.6576	2.8983
Esox lucius	logW=-5.622+3.223 log SL	log	-5.622	3.223
Lepomis sp.	logW=-4.770+3.152 log SL	log	-4.77	3.152
Lota lota	logW=-5.203+3.065 log SL	log	-5.203	3.065
Luxilus sp.	lnW=-11.873+3.248 ln SL	ln	-11.873	3.248
Micropterus salmoides	logW=-4.777+3.058 log SL	log	-4.777	3.058
Notropis sp.	lnW=-11.873+3.248 ln SL	ln	-11.873	3.248
Perca flavescens	lnW=-11.038+3.062 ln SL	ln	-11.038	3.062
Percina sp.	logW=-4.6576+2.8983 log SL	log	-4.6576	2.8983
Phoxinus sp.	lnW=-11.873+3.248 ln SL	ln	-11.873	3.248
Pimephales sp.	lnW=-11.873+3.248 ln SL	ln	-11.873	3.248
Pungitius pungitius	lnW=-11.873+3.248 ln SL	ln	-11.873	3.248
Sander canadensis	lnW=-12.251+3.182 ln SL	ln	-12.251	3.182
Sander vitreus	lnW=-12.251+3.182 ln SL	ln	-12.251	3.182
Umbra limi	lnW=-10.238+2.829 ln SL	ln	-10.238	2.829

Table 2.2. Mean caloric content of one gram (wet mass) of fish for each of eight taxa

Species	***Mean cal/g***
Clupeids	1964.78
Ictalurids	1332.76
Gasterosteids	1272.83
Sander vitreus	1247.50
Esocids	1189.45
*Cyprinids**	1119.82
*Percids***	1111.67
Centrarchids	976.62

*Includes *Umbra limi*

**Excludes *Sander vitreus*

(Brugger, 1992, 1993, Bryan, 1995, Jobling, 1994, Meakins, 1976, Schreckenbach *et al.*, 2001).

Table 2.3. Linear fit model evaluating the effect of environmental stressors on stress (H:L) levels with colony and individual nestling ID held as random variables

H:L Model: R^2 = .190 p = < 0.0001
RMSE = 0.5007 n = 151

Parameter	Estimate	Std Error	DF	*F* - Value	*P*
Intercept	1.1580	1.2153	1	0.9500[a]	0.5164[a]
Endoparasites	0.0434	0.01158	1, 1.193	0.1403	0.7637
Diet Quality	1.592E-5	0.0162	1, 0.982	0.0000	0.9994
Ectoparasites	0.0009	0.0018	1, 89.15	0.2318	0.6314
Age Class	-0.0781	0.0481	1, 145.4	2.6379	0.1065

[a] = *t*-test

Table 2.4. Linear fit model evaluating the effect of environmental stressors and their H:L crosses on mass SGR (g/g/day) with colony held as a random variable

Mass SGR Model: R^2 = 0.142 p = < 0.0001
RMSE = 0.042 n= 49

Parameter	Estimate	Std Error	DF	*F* - Value	*P*
Intercept	0.0494	0.0542	1, 41	0.9100[a]	0.3673[a]
Ectoparasites	-0.0002	0.0002	1, 41	0.9705	0.3303
Diet Quality	0.0009	0.0006	1, 41	2.2051	0.1452
H:L	-0.0016	0.0204	1, 41	0.0060	0.9387
Ectoparasites × H:L	5.271E-5	0.0006	1, 41	0.0072	0.9330
Endoparasites × H:L	-0.0033	0.0115	1, 41	0.0842	0.7732
Diet Quality × H:L	-0.0004	0.0019	1, 41	0.0373	0.8478
Endoparasites	-0.0028	0.0054	1, 41	0.2572	0.6148

[a] = *t*-test

Table 2.5. Linear fit model evaluating the effect of environmental stressors and their H:L crosses on wing SGR (mm/mm/day) with colony held as a random variable

Wing SGR Model: $R^2 = 0.313$ $p = < 0.0001$
RMSE = 0.0234 df = 49

Parameter	Estimate	Std Error	DF	*F*-Value	*P*
Intercept	0.0175	0.0303	1,41	0.5800[a]	0.5682[a]
Ectoparasites	3.06E-05	0.0001	1,41	0.0595	0.8085
Diet Quality	0.0008	0.0003	1,41	6.1416	0.0174
H:L	-0.0012	0.0114	1,41	0.0113	0.9158
Ectoparasites × H:L	0.0004	0.0003	1,41	1.3485	0.2523
Endoparasites × H:L	-0.0058	0.0065	1,41	0.8113	0.3730
Diet × H:L	-0.0003	0.0011	1,41	0.0628	0.8034
Endoparasites	-0.0079	0.0030	1,41	6.8459	0.0124

[a] = *t*-test

Table 2.6. Linear fit model evaluating the effect of environmental stressors and their H:L crosses on tarsus SGR (mm/mm/day) with colony held as a random variable.

Tarsus SGR Model: $R^2 = 0.2342$ $p = < 0.0001$
RMSE = 0.0172 df = 49

Parameter	Estimate	Std Error	DF	*F* - Value	*P*
Intercept	-0.0085	0.0223	1, 41	-0.3800[a]	0.7062[a]
Ectoparasites	-6.075E-5	9.227E-5	1, 41	0.4335	0.5140
Diet Quality	0.0007	0.0002	1, 41	7.3576	0.0097
H:L	0.0035	0.0084	1, 41	0.1740	0.6787
Ectoparasites × H:L	9.541E-5	0.0003	1, 41	0.1383	0.7119
Endoparasites × H:L	-0.0026	0.0047	1, 41	0.3049	0.5838
Diet × H:L	-0.0002	0.0008	1, 41	0.0377	0.8470
Endoparasites	-0.0029	0.0022	1, 41	1.6285	0.2091

[a] = *t*-test

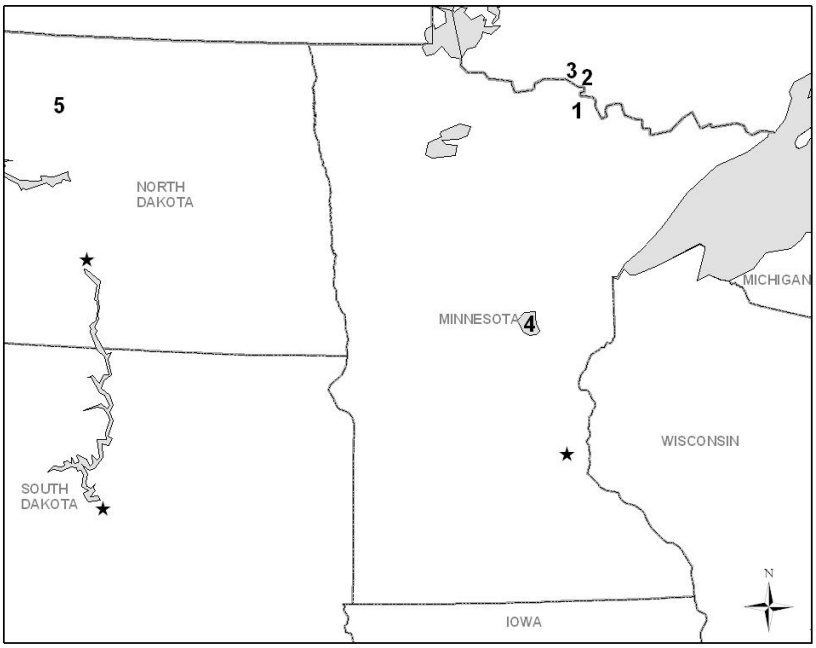

Figure 2.1. Locations of study sites
(1-North East Pine Island, Lake Kabetogama (NEP), 2- Seven Sisters Islands, Rainy Lake (7SIS), 3- Noden Causeway, Rainy Lake (NOD), 4- Mille Lacs NWR (ML), 5- J. Clark Salyer NWR (JCS))

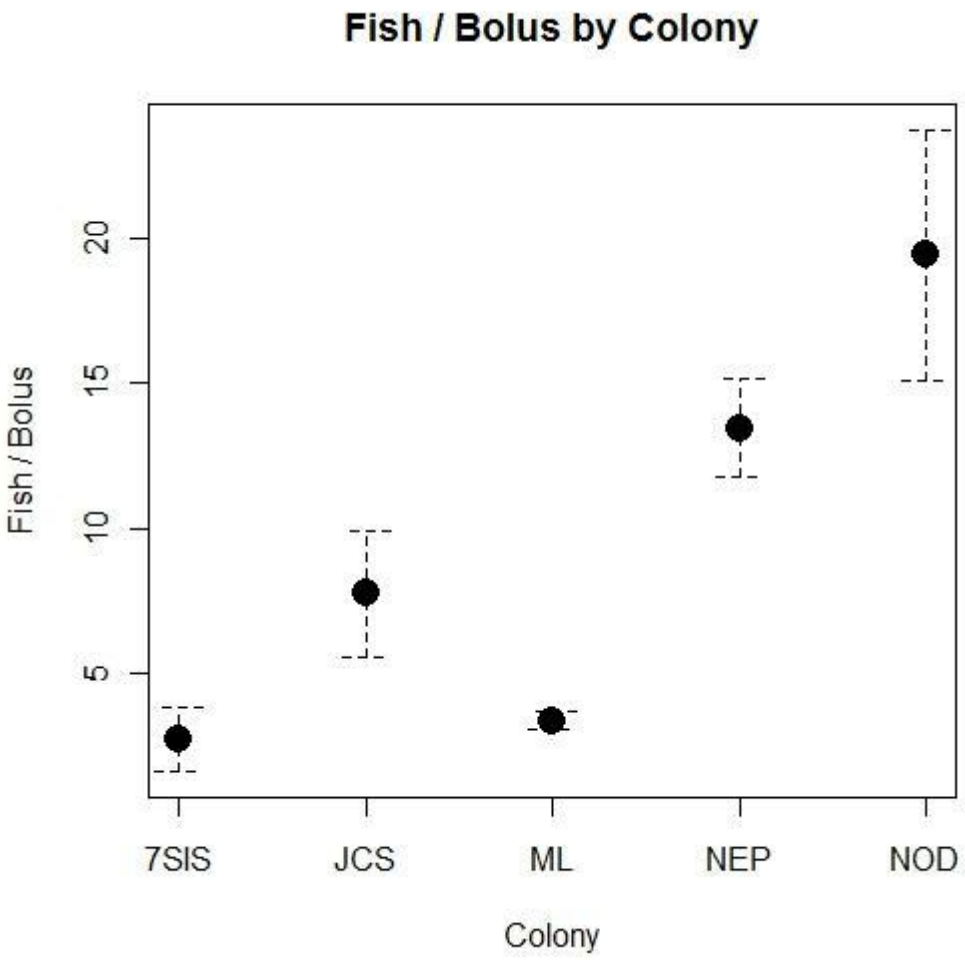

Figure 2.2. Fish per bolus by colony, 2009 (SE)

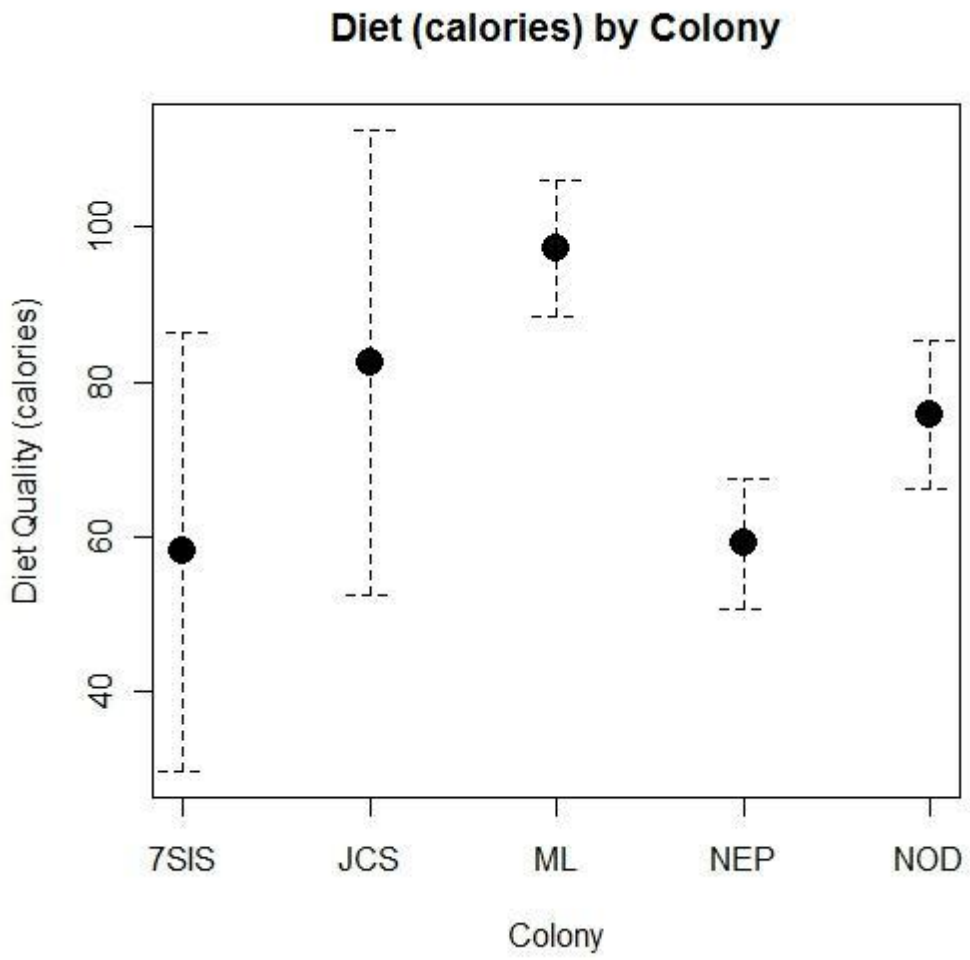

Figure 2.3. Diet (calories) by colony, 2009 (SE)

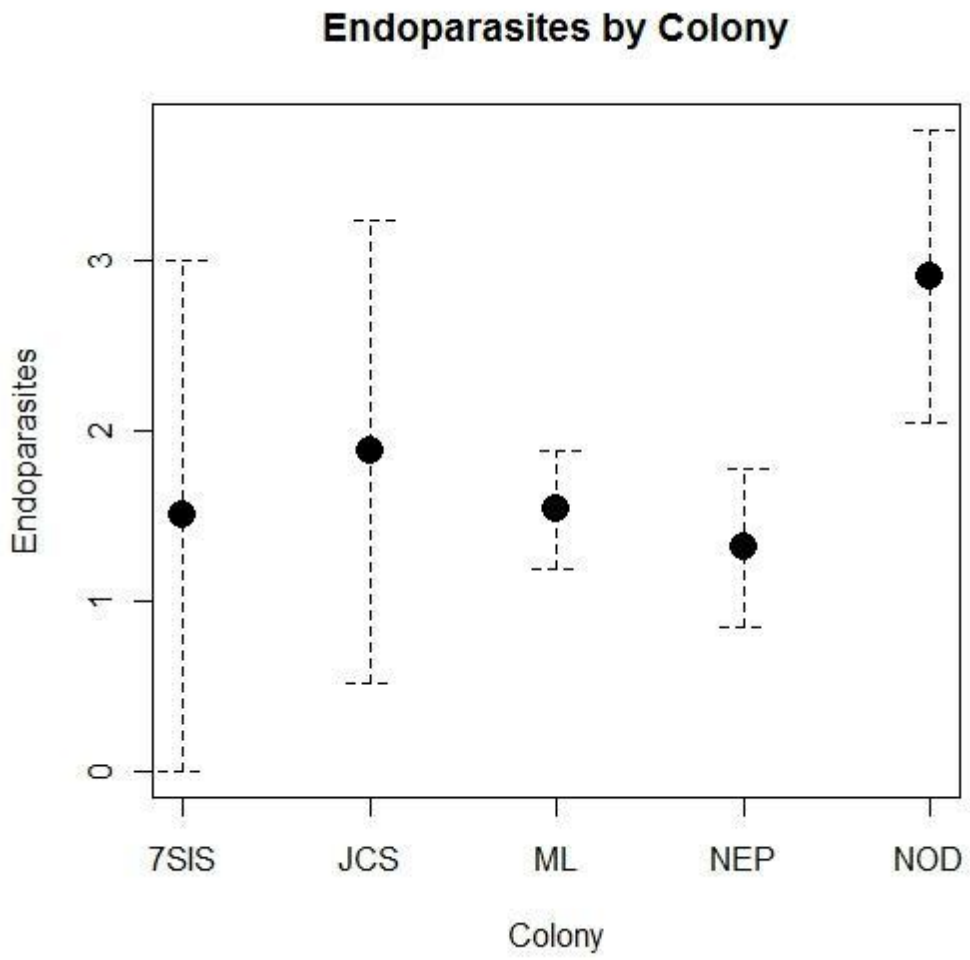

Figure 2.4. Endoparasites by colony, 2009 (SE)

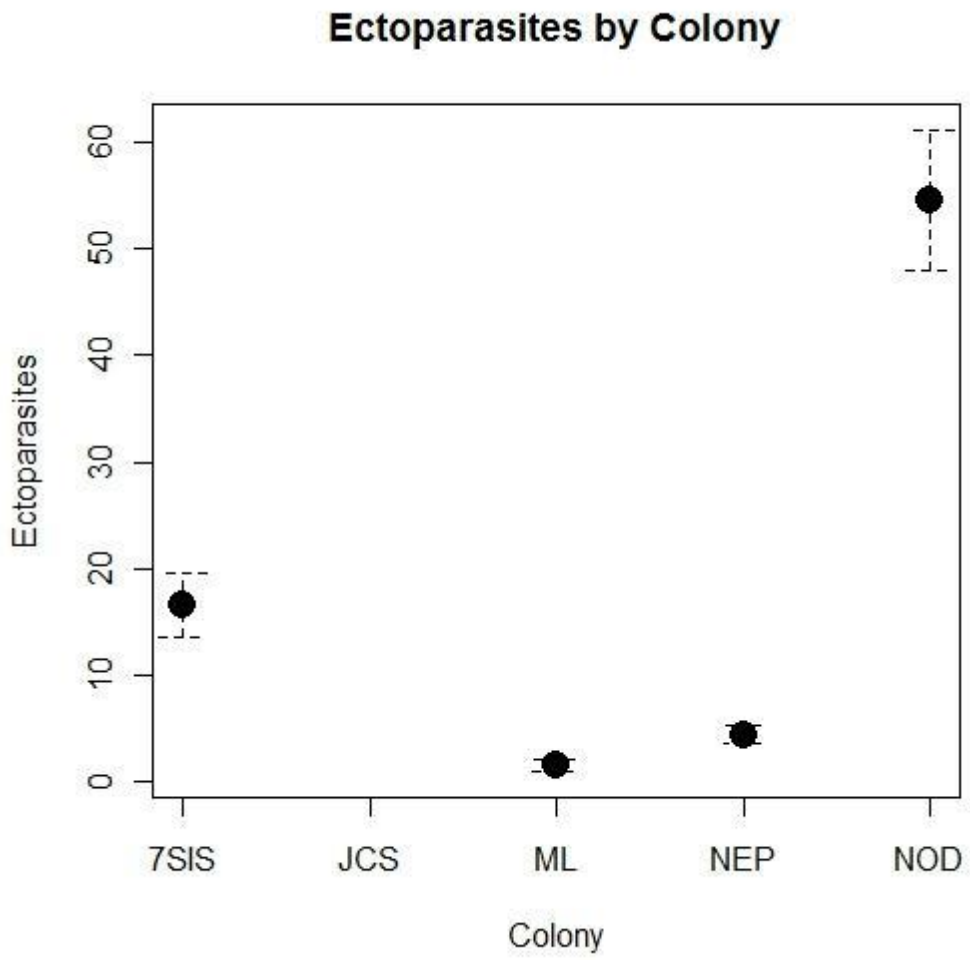

Figure 2.5. Ectoparasites by colony, 2009 (SE)

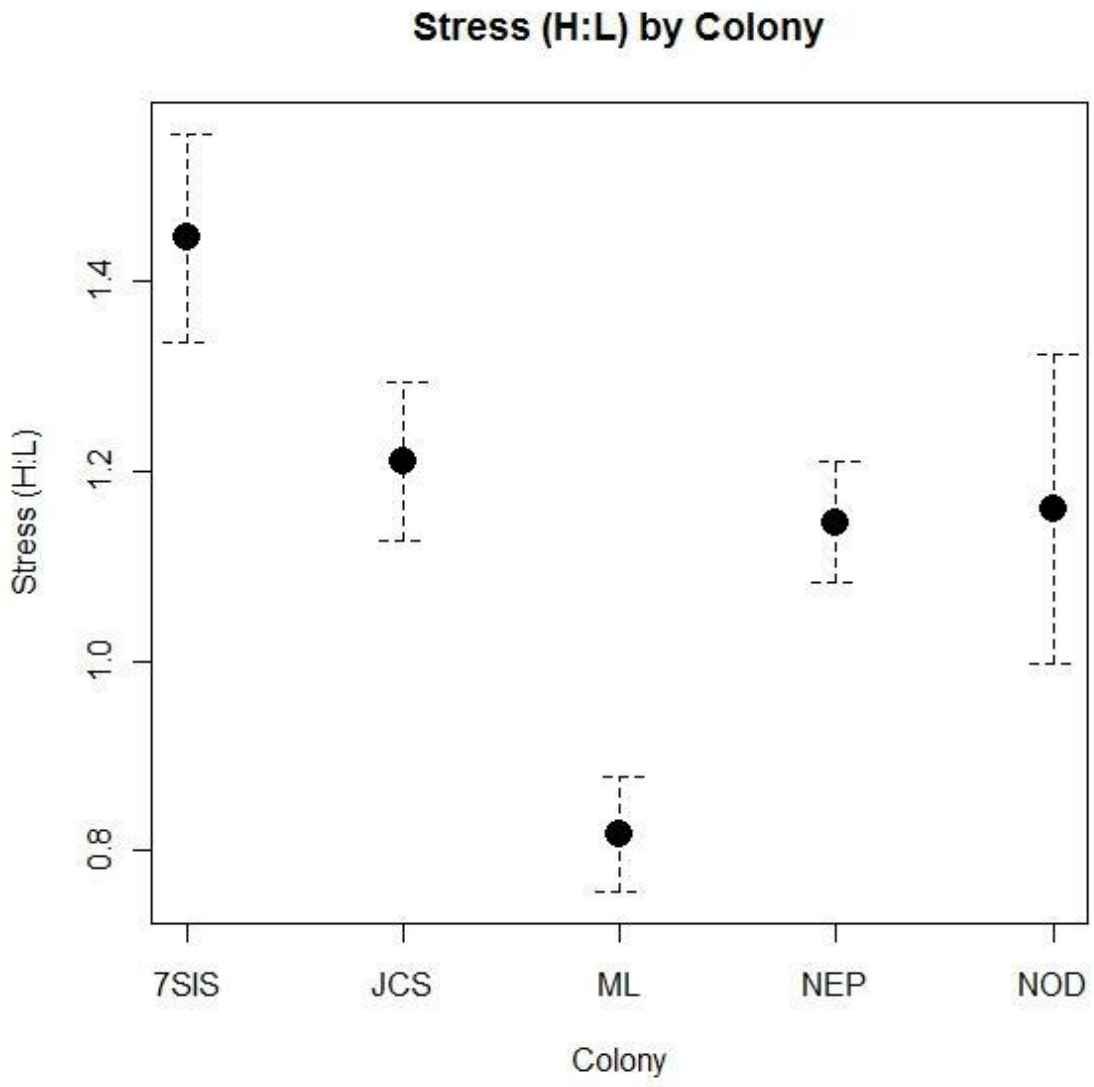

Figure 2.6. Stress levels (H:L) by colony, 2009 (SE)

CHAPTER 3: THE DIET OF NESTLING DOUBLE-CRESTED CORMORANTS (*PHALACROCORAX AURITUS*) AT FIVE CENTRAL NORTH AMERICAN COLONIES

Abstract

The rise in double-crested cormorant (*Phalacrocorax auritus*) populations beginning in the 1970's has spurred interest among user groups concerned with the impact of cormorant diet on sport fisheries. Community reliance on these fisheries has prompted an examination of cormorant diet composition across North America, but few studies have examined the energetic content of cormorant diet. In the summers of 2008 and 2009 I examined five separate cormorant colonies, four colonies being found at popular lacustrine sport fisheries in Minnesota and Ontario: Lake Kabetogama, Rainy Lake, and Lake Mille Lacs. The fifth colony, found at North Dakota's J. Clark Salyer National Wildlife refuge (NWR), is on the Souris River and does not support a large sport fishery. In this study, diet was determined using a nestling bolus analysis, and length-weight regressions were used to determine prey biomass and caloric contents were calculated for nestling cormorant diets. Diets were highly varied among colonies on the same lake (i.e., Rainy Lake) as well as among sites. Overall lacustrine diets consisted primarily of non-game fish, with yellow perch (*Perca flavescens*), shiners (*Notropis spp.*), central mudminnows (*Umbra limi*), sticklebacks (family *Gasterosteidae*), and ciscoes (*Coregonus artedi*) comprising the majority. In contrast, the riparian J. Clark Salyer NWR colony diet was dominated by young Northern Pike (*Esox lucius*) and ictalurids, common riverine species.

Introduction

Double-crested cormorants (hereafter referred to as cormorants), have a long history of human persecution (Hatch and Weseloh, 1999). Champlain in 1604 noted vast numbers of cormorants in North America (Grant and Jameson, 1907) and by 1634 the first written

implication of negative impacts as a result of cormorants was found in Wood's publication regarding New England. Here, Wood stated cormorant populations are capable of "destroying abundant small fish populations" (Wood, 1634). For this reason, there is a long history of cormorant colony destruction in North America (Duffy, 1995). Although nest destruction and harassment may be responsible for the initial decrease in cormorant populations in the last several hundred years, the most dramatic decline followed World War II. This population decline has been documented and studied and the commonly accepted explanation is the detrimental effects of dichlorodiphenyltrichloroethane (DDT) on breeding populations (Bishop *et al.*, 1992; Elliott *et al.*, 1989). The use of DDT, a pesticide, interfered with nesting success by ultimately thinning egg shells and raising the likelihood of broken eggs during incubation (Anderson and Hickey, 1972, Postupalsky, 1978). Increases in adult (Greichus and Hannon, 1973) and embryonic mortality (Weseloh *et al.*, 1983), as well as increases in embryonic malformations were seen as a result of increased DDT and DDE levels in eggs (Ludwig *et al.*, 1996).

In 1972, DDT was banned and concurrently cormorants were added to the United States Migratory Bird Treaty Act and to the Audubon Society's Blue List (Wires et al. 2001). Along with these regulatory changes, significant changes in the birds wintering grounds were occurring. Aquaculture facilities became more prevalent features on the landscape, and more than doubled fish production between 1990 and 2001 (Naylor et al. 2001). Catfish farms in the southeastern United States, which provided a large volume of easily accessible prey for cormorants proved to be particularly important (Glahn *et al.*, 1995; Glahn and Stickley, 1995; Schramm *et al.*, 1984; Stickley *et al.*, 1992). The rise of fish stocking in the north and the growing popularity of fish farming provided the environmental factors needed to allow a rapid population expansion of

cormorants. One unforeseen shortcoming of this rapid expansion was a renewed interest in regulating and extirpating cormorant populations as well as interest in the impact of their foraging and diets on sport and commercial fisheries.

In 1998, after years of concern, legislation was undertaken to pass a federal aquaculture depredation order. This order allowed the harassment of cormorants while on their winter roosts and lethal control of cormorants doing damage to private resources in 13 states (50 CFR, RIN 1018-AE11). In 2003, a public resource depredation order was enacted in twenty-four states allowing harassment, and even lethal control of cormorants damaging either public or private resources. This depredation order has again reinforced the need for research pertaining to the diet of cormorants and the possible negative effects they may have on sport fisheries.

Three methods of determining cormorant diet exist: from pellets, boluses, and gut or stomach contents (Wires *et al.*, 2001). Pellet analyses allows researchers to examine the diet of adult and sub-adult cormorants non-lethally. Cormorants cast a pellet of indigestible materials, often the otoliths of consumed fishes. These otoliths can be paired, often identified to species, and estimates of prey length can be obtained. This method is labor intensive and the least accurate approach, because the level of digestion in pellets can vary greatly among specimens and the possibility of secondary consumption also exists (Blackwell and Sinclair, 1995; Craven and Lev, 1987; Duffy and Laurenson, 1983; Johnson *et al.*, 1997; Johnstone *et al.*, 1990). The second method, a gut analysis, can be done either lethally or non-lethally. The non-lethal technique involves a stomach pump or emetics, which will likely remove all material from the upper digestive tract, including the proventriculus. This can be done on any age bird, but capture of adult and sub-adult birds can be difficult. In addition to difficulty catching birds, flushing the stomach of cormorants is particularly stressful and may cause irreversible damage, thus is no

longer recommended (Duffy and Jackson, 1986; Harris and Wanless, 1993; Wires *et al.*, 2001). A lethal gut analysis most often includes shooting of birds and surgically removing prey from all parts of the digestive tract (including gizzard). This is the most accurate method of diet analysis, as it can be done on birds of any age, and if removed promptly, or preserved rapidly, results in the least digested specimens (Wires *et al.*, 2001). The third method, the most favored due to its low impact and relatively high level of accuracy is a bolus/regurgitant analysis. Drawbacks of this method include a bias towards chick diet because of the higher affinity of chicks to regurgitate when confronted, along with the inability to be certain of a singular source for each bolus (Hatch and Weseloh, 1999; Wires *et al.*, 2001). There is also a wider range of digestive states when examining boluses due to the contents being both partially digested by the adult and nestling, but often boluses are minimally digested.

The effect of cormorants on a fishery cannot be calculated from diet composition alone (Lewis, 1929; Mendall, 1936; Wires *et al.*, 2001), but it is an essential first step. By examining prey composition and biomass, we begin to suggest how many prey items (i.e., fishes) must be consumed to support the existing cormorant colony on a given body of water. Thus to fully comprehend cormorant effects, a thorough assessment of the current state of the fishery is required at all trophic levels, which is rarely available. The objectives of this study were to: (1) Determine the species present in nestling cormorant diet at five different breeding colonies, (2) calculate the percent biomass composition of each species represented, and (3) determine average bolus biomass at each colony (i.e. mass of prey per feeding). For this study a non-lethal method of diet analysis (regurgitants) was required, because three of the five colonies were on federally protected lands (Voyageurs National Park, Mille Lacs NWR, and J. Clark Salyer

NWR). These data can be used in the future when specific fishery data becomes available to assess potential cormorant effects on the fishery.

Methods

Study Species and Study Areas

Double-crested cormorants (*Phalacrocorax auritus*) are a common, moderately-sized (1.2-2.5 kg), colonial water bird that feeds on fish, amphibians and crustaceans. Heavily persecuted in North America since European settlement, double-crested cormorants were facing potential extirpation as recently as the 1970's. Following litigation restricting the use of DDT and the rise in popularity of aquaculture, populations of cormorants rebounded rapidly, and are now found across much of North America. There is substantial regional variation in size, with birds becoming larger to the north and west. Islands and cliffs are the most common habitat in the northern ranges found to support active colonies. Cormorant chicks hatch after approximately 30 days of incubation and remain in the nest for three to four weeks. At this point, they form crèches and roam the ground, and if accessible, will take to water if threatened. At six to seven weeks young are able to begin making short flights and are nearly independent (Mendall, 1936; Hatch and Weseloh, 1999).

I examined five cormorant colonies, three of which are found in the border-lakes region of Minnesota and Ontario, and the other two are found in central Minnesota and north-central North Dakota. The border-lakes region of Minnesota and Ontario is strewn with lakes of various depth, size, and productivity. Lake Kabetogama, fully contained within Voyageurs National Park, is a roughly 9,700 hectare lake, with most of the acreage under 11 meters deep. It is a highly productive lake and is well known for its walleye (*Sander vitreus*) fishery. A cormorant colony was established on Northeast Pine Island (NEP) in 1999, after which the population

quickly grew. To the north, Rainy Lake, much larger at upwards of 220,000 acres is also considered to be a quality sport fishery. Rainy supports several different sport fish species than Kabetogama, including ciscoes, muskellunge (*Esox masquinongy*), and rainbow smelt (*Osmerus mordax*). The lake is separated into two arms, the north arm and south arm. The south arm, also partially contained within Voyageurs National Park, is home to the Seven Sisters Islands (7SIS), an archipelago with a number of islands hosting nesting cormorants. The north arm, stretching well into Ontario, has seen the colonization and rapid expansion of a cormorant colony on an island near the Noden Causeway (NOD).

In central Minnesota, Minnesota's second largest inland lake, Lake Mille Lacs (ML), has gained national fame for its sport fishery, particularly walleye and muskellunge. This relatively shallow, 128,224 acre lake possesses two boulder islands near its southeastern and southwestern shores. These two islands, Spirit and Hennepin, compose the Mille Lacs National Wildlife Refuge (NWR), at just over 1/2 acre, it is the country's smallest NWR. Spirit Island, the larger island of the two, remains unmanaged save yearly cormorant nest counts. The cormorant population at Spirit Island has remained relatively steady for many years, yet there is local concern surrounding its existence.

In contrast to the previous lacustrine colonies, North Dakota's J. Clark Salyer National Wildlife Refuge (JCS) is found on the northern end of the Souris River in the north-central part of the state. Although not a popular fishing destination, this federally protected land is dominated by river, shallow backwaters, and impoundments, all with greatly fluctuating seasonal water levels. This cormorant colony, completely surrounded by nesting gulls (mostly Franklin's gulls (*Leucophaeus pipixcan*)), is unlike the aforementioned colonies in other respects as well. Boat traffic is nearly nonexistent, whereas the habitats with developed sport fisheries experience

significant activity from recreational boats near cormorant colonies. The riparian habitat found surrounding this colony likely supports excellent spawning grounds for common river game fish, such as northern pike. Other common river species are likely present as well (e.g. cyprinids, catostomids, hiodontids, and ictalurids). Bullheads (*Ameiurus spp.*) and channel catfish (*Ictalurus punctatus*) round out the list of species most likely to be found in this habitat.

Field Procedures

Regurgitants were collected using methods adapted from Blackwell et al. (1995). A minimum of one weekly visit during the nesting season was conducted at the Northeast Pine Island colony and the Seven Sisters colony during the summers of 2008 and 2009. Samples were collected weekly at the Mille Lacs, Noden Causeway, and J. Clark Salyer colonies for three weeks during the summer of 2009 exclusively. Samples were collected during colony visits on fair weather days, occurring between 07:00 and 11:00. This timing ensured sufficient time for parents to have returned from a feeding flight to feed their young. Attempting to collect boluses later than this time often resulted with samples in a much greater digestive state, creating problems with prey identification. Collection was done rapidly, as young cormorants are unable to thermoregulate for nearly two weeks (Dunn 1976), and are easily preyed upon by gulls.

The samples collected in 2008 from the Northeast Pine Island and Seven Sisters Islands colonies were placed in a freezer after collection. Freezing rapidly stopped any decomposition, but resulted in difficulties during later analyses. Specimens remained frozen until the fall, where they were examined in a laboratory setting. Difficulties arose when thawing specimens. Freezing and thawing fish, which have week intramuscular connection, causes myomeres and myosepta to easily break apart (denature proteins) (Wisconsin Seagrant Institute, 2001), which makes identification of specimens difficult. Analyses were limited to separating each species

within a bolus, counting individuals, and weighing a total (raw) mass of prey species (mostly fish) within each bolus.

During the 2009 field season boluses were preserved in a 10% formalin solution to avoid the difficulties with measurement and identification associated with freezing. Single boluses were placed in either sealable plastic bags or directly into plastic specimen jars for fixation and preservation. The collection goal was 30 specimens per colony visit, but this was often difficult to achieve at all colonies, save Mille Lacs. Possible reasons for this include: high numbers of resident gulls consuming regurgitants, colony size, and uneven distribution of cormorant nestling ages.

Laboratory Procedures (2009 Only)

Immediately following sample collection, the individually packaged boluses were placed in specimen jars and fixed with a 10% formalin solution. Prey items over 20 cm were injected through the vent with the formalin solution directly into their body cavity to promote rapid fixation and prevent tissue loss. Specimens were then placed in a refrigerator at 4 C for two weeks. Refrigeration ensured minimal decomposition before fixation occurred. After two weeks, the formalin was removed from the jars and the specimens were rinsed and allowed to soak in water for 24 hours, removing the majority of formalin. Boluses were then examined for content.

Prey items, dominated by fish species, were identified to species, except for minnows (Cyprinidae), which were identified to genus. I measured standard length of whole fish to the nearest 0.1 mm using a digital caliper (Tool Shop®) and weighed to the nearest 0.01 g using a digital scale (Ohaus®). Partial fish were compared to like-size specimens of the same species captured in minnow traps or by seining in local bodies of water in the same time frame as diet

sampling occurred. I compared the partial fish with whole specimens and used the standard length of the like-sized sample, to the nearest millimeter, as a replacement value. The part of the fish that was present was also recorded as either: head (H), body (B), tail (T), or whole (W). This was used to ensure the same fish was not counted multiple times (e.g. if a head and body were separated, they may be counted as two separate fish). An estimation of digestive state was also recorded on a scale of 0% to 100%. After diet samples were analyzed, all samples were archived by preserving in 70% ethanol.

Statistical Analyses

Diet composition analyses were performed for each of the five colonies examined. Prey biomass was calculated using species specific length-weight regressions that were chosen from gray and published literature in which the region and lake-type matched the habitats found in the present study (Carlander, 1969, 1977, 1997; Hundt, 2009; Robinson *et al.*, 2010) (Table 3.1). These length-weight regressions were applied to all specimens with an associated standard length, producing a biomass in grams to remove digestion induced variability. Biomass for each species was then summed for a total biomass at the colony level. Species biomass was then divided by total biomass to determine the percent composition of that species within the diet. The total number of individual specimens was also summed for each species at the colony level. To determine the composition of diet by the number of individual prey items, the number or individuals within a species was divided by the total number of specimens. Samples collected in 2008 could not be analyzed using length-weight regressions, thus analyses utilized a raw bolus mass rather than biomass. Diet composition by species mass was performed by dividing raw species mass by raw total mass.

Results

Eighty-two (82) samples were collected at Northeast Pine Island (NEP) in the summer of 2008, and ninety-eight (98) collected in 2009. Twenty-one fish species and one invertebrate species were identified. In 2008, of identifiable remains, yellow perch (36%), lake whitefish (10%), and central mudminnows (10%) were the most prominent diet components by mass (Figure 3.1, Table 3.2). By individuals, central mudminnow (27%), walleye (22%), brook stickleback (12%), fathead minnow (11%) and yellow perch (10%) made up the majority of diet (Table 3.2). Unknown or unidentifiable fish remains made up 12% of the samples by mass, but just over 1% by individuals, indicating these to be remains of larger fish. One bolus contained the remains of a herring gull (*Larus smithsonianus*) chick. In 2009, lake whitefish (23%), walleye (20%), and central mudminnows (17%) represented the largest portions of diet by biomass (Figure 3.2, Table 3.3). By individuals, central mudminnow (38%), brook stickleback (23%), and walleye (11%) represented the largest portions of diet (Figure 3.3). Mean bolus biomass could not be assessed for the first year of the study, but changes in procedure allowed for corrections in response to digestive state of prey items (i.e. length-weight regressions) in the second year of the study, resulting in a 2009 mean bolus biomass of 70.9 grams.

In 2008, 54 boluses were collected from the Seven Sisters Islands colony (7SIS). From these samples, 12 species of fish were detected. By raw mass, 66% of cormorant diet consisted of cisco. Central mudminnow (7%), walleye (6%), and yellow perch (3%) made up the next highest proportions of diet (Figure 3.4, Table 3.4). During the 2009 field season the 7SIS colony was a total loss due to bald eagle (*Haliaeetus leucocephalus*) predation before most chicks reached 3 weeks of age. Only 4 boluses were collected in 2009, containing only 10 individual fish. Of these 10 specimens, one was a northern pike which represented 59% of the total

biomass. Five unknown whitefish, likely ciscoes, were summed to represent 24% of total biomass and 2 confirmed ciscoes equaled 11%, resulting in 35% of total biomass from Coregonid species. An individual central mudminnow and walleye were also present, collectively representing the remaining 6% of biomass (Figure 3.5, 3.6, Table 3.5).

Twenty-two boluses were used from the Noden Causeway colony (NOD) for analysis in 2009. Eleven fish species, one invertebrate species, and one vegetative species were detected. With 548 individual fish specimens collected, the mean number of fish per bolus, just over 25, was much higher than other colonies. Ninespine and brook stickleback made up 36% and 26% of the biomass consumed, respectively. Burbot (16%), central mudminnow (8%), and rock bass (7%) followed as the next largest proportions of diet by biomass (Figure 3.7, Table 3.6). The percent of diet by individuals was topped by ninespine stickleback at 51% and brook stickleback at 38%. Central mudminnow, at 8%, was the only other species to represent over 1% of the total number of individual fish consumed (Figure 3.8, Table 3.6). Two separate boluses, collected on the same day, were found to contain kernels of corn (*Zea mays*), 18 kernels in total. The source of this corn is unknown, but corn is a popular fishing bait commonly used for panfish and is also commonly found in wildlife feeding stations near the lake.

During the 2009 season 97 boluses were collected at the Lake Mille Lacs (ML) colony and contained 320 individual fish. Only five species of fish, in addition to two unidentifiable remains, were identified: cisco, yellow perch, walleye, rock bass, and one shiner species. Cisco (41%), yellow perch (26%), shiners (17%), and walleye (14%) were the largest contributors to biomass consumed (Figure 3.9, Table 3.7). Diet composition by number of individuals, resulted in shiners (44%), yellow perch (29%), cisco (18%), and walleye (8%) being the largest contributors. One rock bass was identified, resulting in a biomass component of just over 1%

and an individual component of around 0.3%. As stated, two unidentifiable fish were found, but represented only 0.26% of the total biomass (Figure 3.10, Table 3.7).

During the 2009 season 8 boluses were collected from the J. Clark Salyer NWR colony (JCS) and contained 61 individual fish. Of the 61 fish, 54 were found to be northern pike, representing 88% of the individual composition and 51% of the biomass composition. Six ictalurids represented nearly all the remaining biomass (49%), save one unidentified specimen, which was likely a cyprinid based on its small size (.2% of biomass) (Figure 3.11, 3.12, Table 3.8).

Discussion

This study revealed a great deal of spatial heterogeneity in regards to diet. Considerable variation exists among cormorant colonies, both those in close proximity to one another, and colonies separated by great distances. As opportunistic feeders, spatial heterogeneity is to be expected and has been documented in a number of studies in North America (Neuman *et al* 1997; Blackwell *et al* 1995). An examination of previous studies of cormorant diet have revealed a wide range of taxa including over 250 different species of fish in over 60 families (Hatch and Weseloh, 1999). This study in discovered over 23 species of fish, one species of invertebrate (O*rconectes* sp.), one vegetative species (corn), and one incident of gull chick consumption. The finding of the corn and gull chick is, at the time of this writing, the only record of this behavior.

With regards to the presence of popular sport fish (e.g., northern pike, walleye, and smallmouth bass) in the cormorant diet, the relative insignificance of these fish in the diet samples, with the exception of the JCS colony, indicates sport fish are not a major prey item for the cormorant. At the riverine site, 88.5% of the individual prey items were found to be northern

pike. This resulted in northern pike representing approximately 51% of the biomass consumed at this colony. These findings, though limited, likely represent the prevalence of northern pike in this riverine ecosystem. The presence of YOY walleye at NEP, NOD, 7SIS, and ML, though never above 20% of cormorant diet (by biomass), requires further examination to determine long term effects on the sport fishery. Although a regional comparison can be made using Hundt's 2009 assessment of cormorant diet at Leech Lake, MN, where 2-8% of the total biomass consumed consisted of walleye. With the exception of 2009 at NEP (19.6%) and ML (13.7%), levels of walleye consumption fell within or below Hundt's 2-8% range

The diets of cormorants at the lacustrine colonies revealed a trend towards slow swimming and schooling species. Central mudminnows, yellow perch, sticklebacks, shiners, cisco, and young-of-the-year (YOY) walleye were all common prey items. In 2009 at NEP, central mudminnow increased to 38% of the total diet composition up from 27% in 2008 (by individuals). At ML, the 2009 diet consisted of cisco representing 41% of the biomass consumed and *Notropis* species representing 44% of the total number of fish consumed. At NOD in 2009, it was found that 88% of the individual fish consumed were sticklebacks, ultimately representing 62% of the total biomass consumed. Panfish (e.g., yellow perch, bluegill, and rockbass) did contribute 43% of the diet by biomass in 2008 at NEP, with yellow perch making up nearly 36% of the total diet. This large proportion of yellow perch is in line with studies done at Little Galloo Island in Lake Ontario, where 37% of the cormorant diet consisted of this species (Johnson et al. 2002), and well below Hundt's highest level of 78% at Leech Lake (2009). In contrast to the 2008 season however, yellow perch contributed to only 7% of the NEP diet in 2009. Differences in diet are typically consistent with changes in fish behavior during the breeding season, thus temporal heterogeneity in cormorant diet is to be

expected annually as well as seasonally (Neuman et al. 1997). The presence of cisco in the NOD, 7SIS, and ML colony diets and absence at NEP (Lake Kabetogama contains no cisco), again reinforces the concept of cormorants as short ranged opportunistic feeders.

In conclusion, cormorant diets are spatially variable, which is reflective of the local fish communities. To determine the effect of these diets on a fishery, a complete assessment of fish assemblages and fish diet is needed to evaluate the impact of cormorant diets on trophic level dynamics. Without these data, all findings between increasing cormorant populations and fish declines are only correlative and lead to unnecessary culling and harassment programs. A well-documented study at Lake Oneida, one of the few studies to find cormorants to have a detrimental effect on sport fish, saw walleye and yellow perch comprise 40-82% of cormorant diet from 1995-2000 (Rudstam *et al.*, 2004). We did not see consumption of game fish approach these levels at the lacustrine colonies, but cormorant diet is far from the only determining factor of fish populations. Natural fish reproduction, fish stocking actions, sport fishermen catch, seasonal variation, and commercial fishing are just a few possible factors that can affect fish populations from year to year. It is perhaps in our fishery's best interest to continue to monitor the cormorant population and its diet as an indicator of fishery health, rather than as a potential threat to it, as changes in cormorant diet were found to correlate to changes in the assemblages of benthic fishes in Penobscot Bay, Maine (Blackwell et al. 1995). It is in this light that cormorant diets studies should be regarded, as a monitoring tool to be used by fishery managers as a method for assessing change while still considering the contribution of cormorant diet to fish population fluctuations.

Literature Cited

Anderson, D. W., and J. J. Hickey. 1972. Eggshell changes in certain North American birds. Proc. Int. Ornithol. Congr. 15:514-540.

Bishop, C. A., D. V. Weseloh, N. M. S. Burgess, J., and R. J. Norstrom. 1992. An atlas of contaminants in eggs of fish-eating birds of the Great Lakes (1970-1988). Can. Wildl. Serv. Tech. Rep. Ser. no. 152 and 153, Ontario Region 1 and 2.

Blackwell, B. F., and J. A. Sinclair. 1995. Evidence of secondary consumption of fish by Double-crested Cormorants. Marine Ecology 123:1-4.

Blackwell, B.F., W.B. Krohn, and R. Bradford Allen. 1995. Foods of Nestling Double-crested Cormorants in Penobscot Bay, Maine, USA: Temporal and Spatial Comparisons. Colonial Waterbirds. 18:199-208.

Carlander, K. D. 1969. Handbook of freshwater fishery biology. Iowa State University Press, Ames.

Carlander, K. D. 1977. Handbook of freshwater fishery biology. Iowa State University Press, Ames.

Carlander, K. D. 1997. Handbook of freshwater fishery biology. Iowa State University Press, Ames.

Craven, S. R., and E. Lev. 1987. Double-crested Cormorants in the Apostle Islands, Wisconsin, USA: population trends, food habits and fishery depredations. Colonial Waterbirds 10:64-71.

Grant, W.L., Jameson, J.F., 1907 Voyages of Samuel de Champlain 1604-1618. Charles Scribner's Sons, New York.

Dow, D. 1977. Indiscriminate interspecific aggression leading to almost sole occupancy of space by a single species of bird. Emu 77:115-121.

Duffy, D.C. 1995. Why is the Double-crested Cormorant a problem? Insights from cormorant ecology and human sociology. Colonial Waterbirds 18 (Spec. Publ 1):25-32.

Duffy, D. C., and S. Jackson. 1986. Diet studies of seabirds: a review of methods. Colonial Waterbirds 9:1-17.

Duffy, D. C., and L. J. B. Laurenson. 1983. Pellets of Cape Cormorants as indicators of diet. Condor 85:305-307.

Elliott, J. E., D. G. Noble, R. J. Norstrom, and P. E. Whitehead. 1989. Organochlorine contaminants in seabird eggs from the Pacific Coast of Canada 1971-1986. Environ. Monit. Assess.:67-82.

Glahn, J. F., P. J. Dixon, G. A. Littauer, and R. B. McCoy. 1995. Food habits of Double-crested Cormorants wintering in the Delta region of Mississippi. Colonial Waterbirds 18 (Spec. publ 1):158-167.

Glahn, J. F., and A. R. Stickley. 1995. Wintering Double-crested Cormorants in the Delta region of Mississippi: Population levels and their impact on the catfish industry. Colonial Waterbirds 18:137-142.

Greichus, Y. A., and M. R. Hannon. 1973. Distribution and biochemical effects of DDT, DDD and DDE in penned Double-crested Cormorants. Toxicol. Appl. Pharmacol. 26:483-494.

Harris, M. P., and S. Wanless. 1993. The diet of Shags during the chick rearing period assessed by three methods. Bird Study 40:135-139.

Hatch, J. J., and D. V. Weseloh. 1999. Double-crested cormorant (Phalacrocorax auritus). in In The Birds of North America The Birds of North America Inc., Philadelphia, PA.

Hundt, P. J. 2009. The diet of Double-crested Cormorants (Phalacrocorax auritus) of Leech Lake, Minnesota, University of Minnesota.

Johnson, J. H., R. M. Ross, and D. R. Smith. 1997. Evidence of secondary consumption of invertebrate prey by Double-crested Cormorants. Colonial Waterbirds 20:547-551.

Johnstone, I. G., M. P. Harris, S. Wanless, and J. A. Graves. 1990. The usefulness of pellets for assessing the diet of adult Shags Phalacrocorax aristotelis. Bird Study 37:5-11.

Lewis, H. F. 1929. The natural history of the Double-crested Cormorant. Ru-Mi-Lou Books, Ottawa, Ontario.

Ludwig, J. P., H. Kurita-Matsuba, H. J. Auman, M. E. Ludwig, and C. L. Summer. 1996. Deformities, PCBs, and TCDD-equivalents in Double-crested Cormorants and Caspian Terns of the upper Great Lakes 1986-1991: Testing a cause-effect hypothesis. Journal of Great Lakes Research 22:172-197.

Mendall, H. L. 1936. The home-life and economic status of the Double-crested Cormorant. University Press, Orono.

Naylor, R.L., Williams, S.L., Strong, D.R., 2001 Aquaculture - a gateway for exotic species. Science. 294:1655-1656.

Postupalsky, S. 1978. Toxic chemicals and cormorant populations in the Great Lakes. Canadian Wildlife Services Wildlife Toxicology Division, Ottawa.

Robinson, J. M., K. J. Jirka, and J. A. Chiotti. 2010. Age and growth analysis of the centrall mudminnow, Umbra limi (Kirtland, 1840) 18. Journal of Applied Icthyology 26:89-94.

Rudstam, L. G., A. J. VanDeValk, C. M. Adams, J. T. H. Coleman, J. L. Forney, and M. E. Richmond. 2004. Cormorant predation and the population dynamics of walleye and yellow perch in Oneida lake. Ecological Applications 14:149-163.

Schramm, H. L., Jr., B. French, and M. Ednoff. 1984. Depredation of channel catfish by Florida Double-crested Cormorants. Progr. Fish-Cult. 46:41-43.

University of Wisconsin Sea Grant Institute. Calorie, Fat, and Protein Content of Fish and Seafoods. Seagrant. 5 February 2002. 10 October 2011.<http://seagrant.wisc.edu/greatlakesfish/ctable.html>.

Stickley, A. R., G. L. Warrick, and J. F. Glahn. 1992. Impact of Double-Crested Cormorant Depredations on Channel Catfish Farms. Journal of the World Aquaculture Society 23:192-198.

Weseloh, D. V., S. M. Teeple, and M. Gilbertson. 1983. Double-crested Cormorants of the Great Lakes: egg-laying parameters, reproductive failure and contaminant residues in eggs. Can. J. Zool. 61:427-436.

Wires, L. R., F. J. Cuthbert, D. R. Trexel, and A. R. Joshi. 2001. Status of the Double-crested Cormorant (Phalacrocorax auritus) in North America. in Final Report to USFWS.

Wood, W. 1634. New England Prospect. Tho. Cotes, for John Ballamie, London.

Table 3.1 Length-weight regressions used to calculate biomass from bolus specimens (Carlander, 1969, 1977, 1997, Hundt, 2009, Robinson *et al.*, 2010)

Species	Equation	Base	base(W) = a + b Base SL	
			a	b
Amblopites rupestris	logW=-4.574+3.057 log SL	log	-4.574	3.057
Ameiurus melas	logW=-4.049+2.801 log SL	log	-4.049	2.801
Centrarchid sp.	logW=-4.770+3.152 log SL	log	-4.77	3.152
Coregonus sp.	logW=-5.056+3.168 log SL	log	-5.056	3.168
Culaea inconstans	lnW=-11.873+3.248 ln SL	Ln	-11.873	3.248
Etheostoma sp.	logW=-4.6576+2.8983 log SL	log	-4.6576	2.8983
Esox lucius	logW=-5.622+3.223 log SL	log	-5.622	3.223
Lepomis sp.	logW=-4.770+3.152 log SL	log	-4.77	3.152
Lota lota	logW=-5.203+3.065 log SL	log	-5.203	3.065
Luxilus sp.	lnW=-11.873+3.248 ln SL	Ln	-11.873	3.248
Micropterus salmoides	logW=-4.777+3.058 log SL	log	-4.777	3.058
Notropis sp.	lnW=-11.873+3.248 ln SL	Ln	-11.873	3.248
Perca flavescens	lnW=-11.038+3.062 ln SL	Ln	-11.038	3.062
Percina sp.	logW=-4.6576+2.8983 log SL	log	-4.6576	2.8983
Phoxinus sp.	lnW=-11.873+3.248 ln SL	Ln	-11.873	3.248
Pimephales sp.	lnW=-11.873+3.248 ln SL	Ln	-11.873	3.248
Pungitius pungitius	lnW=-11.873+3.248 ln SL	Ln	-11.873	3.248
Sander canadensis	lnW=-12.251+3.182 ln SL	Ln	-12.251	3.182
Sander vitreus	lnW=-12.251+3.182 ln SL	Ln	-12.251	3.182
Umbra limi	lnW=-10.238+2.829 ln SL	Ln	-10.238	2.829

Table 3.2. 2008 diet of nestling double-crested cormorants at the NEP colony

NEP 2008					
Species	**Scientific Name**	**Individ.**	**% by Diet Individuals**	**Raw Mass**	**% Diet by Mass**
Bluegill	*Lepomis macrochirus*	2	0.1815	83.560	0.877
Brook Stickleback	*Culea inconstans*	130	11.797	154.42	1.621
Burbot	*Lota lota*	14	1.270	618.62	6.494
Central Mudminnow	*Umbra limi*	298	27.042	920.28	9.661
Crayfish	*Orconectes sp.*	20	1.815	135.24	1.420
Dace sp.	*Phoxinus sp.*	8	0.726	17.000	0.178
Darter spp.	*Etheostoma spp.*	6	0.5445	5.000	0.052
Fathead Minnow	*Pimepahales promelas*	126	11.434	242.340	2.544
Lake Whitefish	*Coregones clupeaformis*	18	1.633	907.580	9.527
Northern Pike	*Esox lucius*	6	0.545	276.900	2.907
Rock Bass	*Ambloplites rupestris*	8	0.726	593.100	6.226
Sauger	*Sander canadensis*	2	0.181	105.700	1.110
Shiner sp.	*Luxilus sp.*	6	0.5445	72.460	0.761
Shiner sp.	*Notropis sp.*	14	1.270	98.340	1.032
Unknown	Unknown Dace sp.	14	1.270	1146.400	12.034
Unknown Centrarchid	*Centrarchidae*	2	0.181	44.200	0.464
Unknown Cyprinid	*Cyprinidae*	16	1.452	24.960	0.262
Unknown Dace sp.	*Cyprinidae*	46	4.174	196.120	2.059
Walleye	*Sander vitreus*	252	22.868	468.800	4.921
Yellow Perch	*Perca flavescens*	114	10.3445	3414.980	35.849
Totals		1102		9526	

Table 3.3. 2009 diet of double-crested cormorants at the NEP colony

NEP 2009					
Species	**Scientific Name**	**Individ**	**% Diet by Individuals**	**Biomass**	**% Diet by Mass**
Brook Stickleback	*Culea inconstans*	253	22.979	336.801	4.844
Burbot	*Lota lota*	1	0.091	391.353	5.629
Central Mudminnow	*Umbra limi*	423	38.420	1192.988	17.159
Crayfish sp.	*Orconectes sp.*	5	0.454	NA	NA
Dace spp.	*Phoxinus spp. (eos, neogaeus)*	131	11.898	224.208	3.225
Darter sp.	*Etheostoma sp.*	1	0.091	1.641	0.024
Darter sp.	*Percina sp.*	1	0.091	0.750	0.011
Hornyhead Chub	*Nocomis biguttatus*	4	0.363	56.306	0.810
Lake Whitefish	*Coregonus clupeaformis*	7	0.636	1572.829	22.623
Minnow sp.	*Pimephales sp.*	9	0.817	32.343	0.465
Ninespine Stickleback	*Pungitius pungitius*	6	0.545	9.303	0.134
Northern Pike	*Esox lucius*	1	0.091	161.261	2.319
Pearl Dace	*Margariscus margarita*	5	0.454	27.826	0.400
Rock Bass	*Ambloplites rupestris*	7	0.636	523.822	7.534
Sauger	*Sander canadensis*	1	0.091	2.806	0.040
Shiner sp.	*Luxilus sp.*	2	0.182	21.952	0.316
Shiner sp.	*Notropis sp.*	53	4.814	268.317	3.859
Smallmouth Bass	*Micropterus dolomieu*	1	0.091	100.065	1.439
Sunfish sp.	*Lepomis sp.*	6	0.545	161.448	2.322
Unknown Minnow	*Unknown Cyprinidae*	1	0.091	3.726	0.054
Walleye	*Sander vitreus*	118	10.718	1361.560	19.584
Yellow Perch	*Perca flavescens*	65	5.904	493.657	7.101
Totals		1101		6944.961	

Table 3.4. 2008 diet of double-crested cormorants at the 7SIS colony

7SIS 2008					
Species	**Scientific Name**	**Individ**	**% Diet by Individuals**	**Raw Mass**	**% Diet by Mass**
Black Bullhead	*Ameiurus melas*	2	0.457	57.540	1.918
Brook Stickleback	*Culea inconstans*	14	3.196	11.110	0.370
Central Mudminnow	*Umbra limi*	178	40.639	195.930	6.531
Ciscoe	*Coregonus artedi*	168	38.356	1983.580	66.116
Dace sp.	*Phoxinus sp.*	6	1.370	11.940	0.398
Minnow sp.	*Cyprinid sp.*	1	0.228	3.570	0.119
Ninespine Stickleback	*Pungitius pungitius*	1	0.228	0.650	0.022
Northern Pike	*Esox lucius*	1	0.228	27.980	0.933
Shiner sp.	*Notropis sp.*	10	2.283	90.210	3.007
Smallmouth Bass	*Micropterus dolomieu*	1	0.228	35.730	1.191
Unknown	Unknown	5	1.142	296.010	9.867
Walleye	*Sander vitreus*	23	5.251	188.340	6.278
Yellow Perch	*Perca flavescens*	28	6.393	97.560	3.252
Totals		438		3000.150	

Table 3.5. 2009 diet of double-crested cormorants at the 7SIS colony

7SIS 2009					
Species	**Scientific Name**	**Individ**	**% Diet by Individuals**	**Biomass**	**% Diet by Mass**
Central Mudminnow	*Umbra limi*	1	10.000	9.942	5.451
Ciscoe	*Coregonus artedi*	2	20.000	20.417	11.195
Northern Pike	*Esox lucius*	1	10.000	107.600	58.999
Unknown Whitefish	*Unknown* (Coregonus sp.)*	5	50.000	43.200	23.687
Walleye	*Sander vitreus*	1	10.000	1.218	0.668
Totals		10		182.377	

Table 3.6. 2009 diet of double-crested cormorants at the NOD colony

NOD 2009					
Species	**Scientific Name**	**Individ**	**% Diet by Individuals**	**Biomass**	**% Diet by Mass**
Brook Stickleback	*Culea inconstans*	198	37.288	337.610	25.959
Burbot	*Lota lota*	2	0.377	207.823	15.980
Central Mudminnow	*Umbra limi*	44	8.286	97.841	7.523
Corn (Kernels)	*Zea mays*	18	NA	1.390	0.107
Crayfish	*Orconectes sp.*	3	0.565	NA	NA
Dace spp.	*Phoxinus spp.*	5	0.942	49.120	3.777
Darter sp.	*Percina sp.*	1	0.188	0.300	0.023
Ninespine Stickleback	*Pungitius pungitius*	270	50.847	469.652	36.112
Rock Bass	*Ambloplites rupestris*	2	0.377	87.836	6.754
Smallmouth Bass	*Micropterus dolomieu*	1	0.188	45.372	3.489
Unknown Minnow	*Unknown Cyprinid sp.*	1	0.188	1.882	0.145
Walleye	*Sander vitreus*	1	0.188	0.871	0.067
Yellow Perch	*Perca flavescens*	2	0.377	0.863	0.066
Totals		548		1300.560	

Table 3.7. 2009 diet of double-crested cormorants at the ML colony

ML 2009					
Species	**Scientific Name**	**Individ**	**% Diet by Individuals**	**Biomass**	**% Diet by Mass**
Ciscoe	*Coregonus artedi*	58	18.069	2338.050	41.407
Rock Bass	*Ambloplites rupestris*	1	0.312	68.589	1.215
Shiner sp.	*Notropis sp.*	140	43.614	967.255	17.130
Walleye	*Sander vitreus*	25	7.788	774.671	13.719
Yellow Perch	*Perca flavescens*	94	29.283	1482.985	26.264
Unknown	Unknown	2	0.623	14.985	0.265
Totals		320		5646.535	

Table 3.8. 2009 diet of double-crested cormorants at the JCS colony

JCS 2009					
Species	**Scientific Name**	**Individ**	**% Diet by Individuals**	**Biomass**	**% Diet by Mass**
Catfishes	*Ictalurid spp.*	6	9.836	159.816	49.186
Northern Pike	*Esox lucius*	54	88.525	165.108	50.814
Unknown	Unknown	1	1.639	0.597	0.184
Totals		61		325.520	

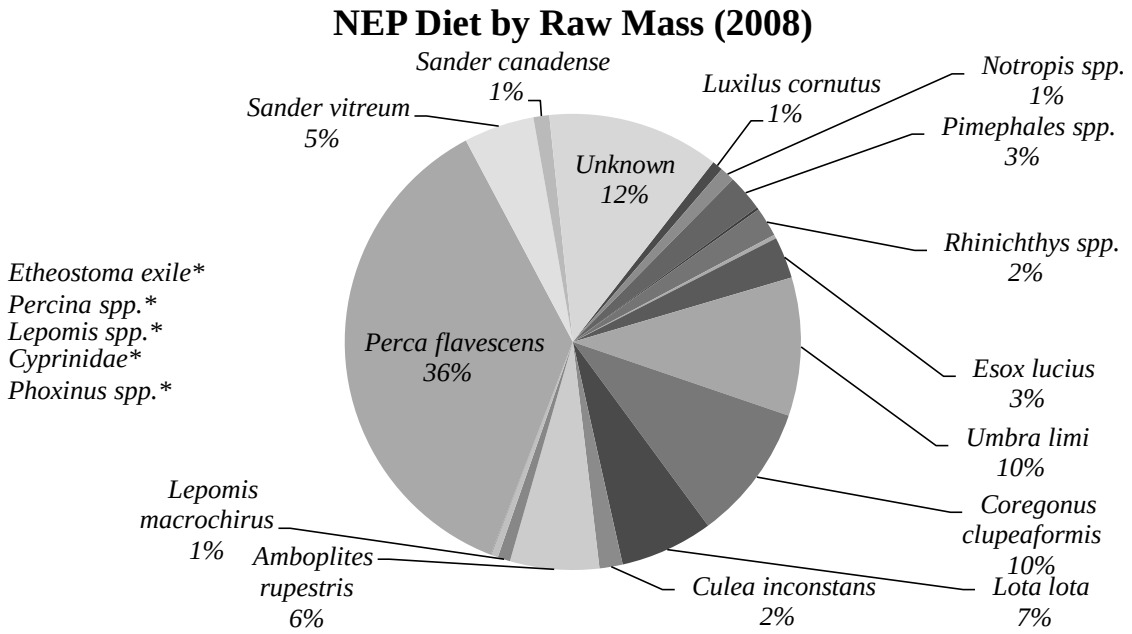

Fig 3.1. NEP diet by raw mass, 2008
(* Species making up < 1% of raw mass)

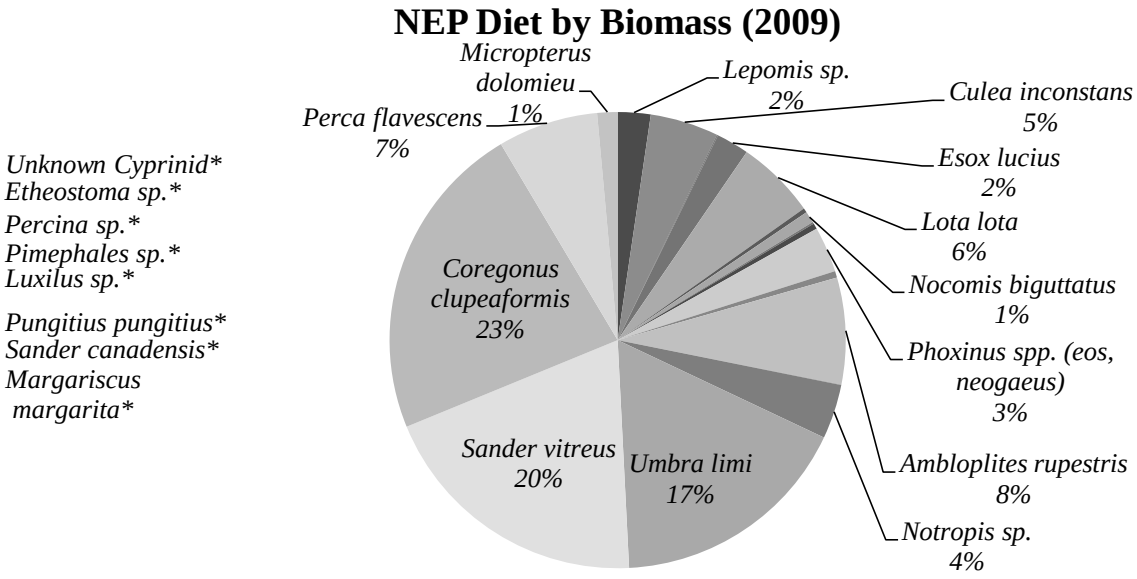

Fig 3.2. NEP diet by biomass, 2009
(* Species making up < 1% of total biomass)

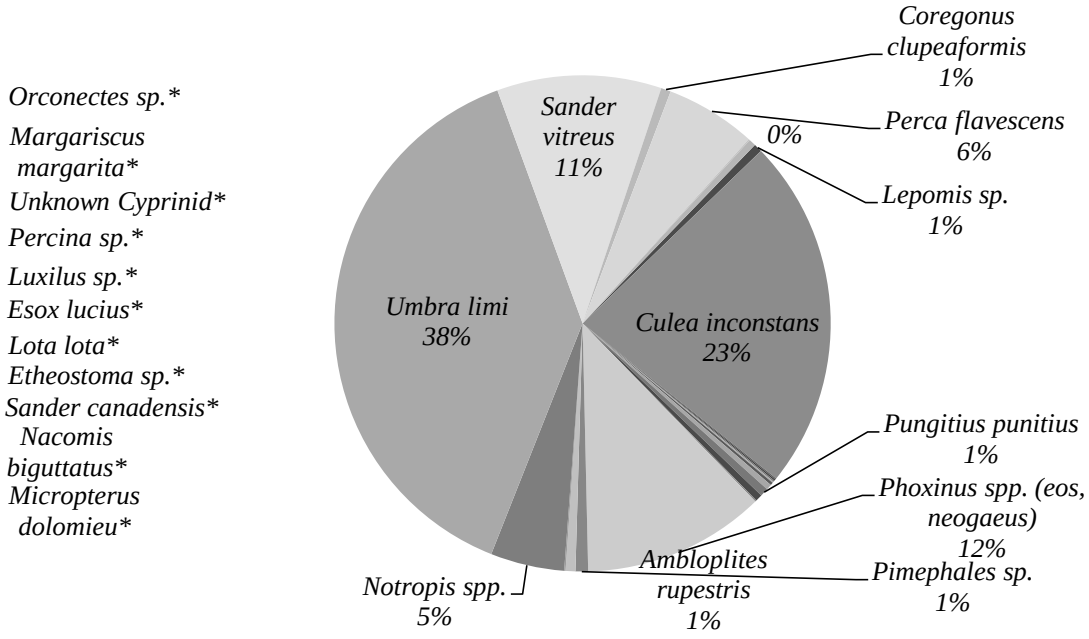

Fig 3.3. NEP diet composition by individual prey item, 2009
(* Species making up < 1% of total diet by number of individuals)

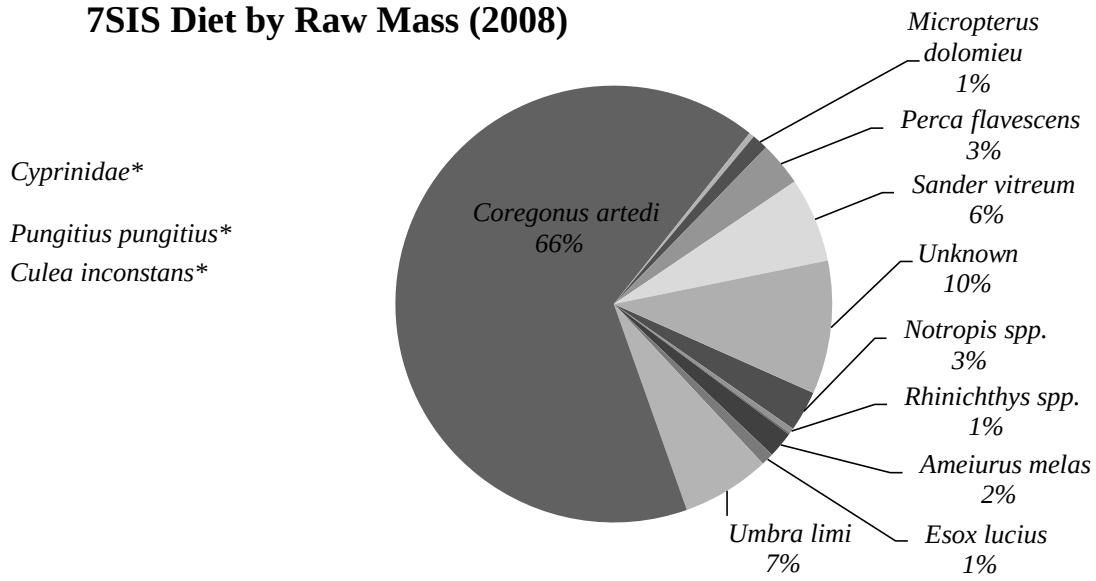

Fig 3.4. 7SIS diet by raw mass, 2008
(* Species making up < 1% of raw mass)

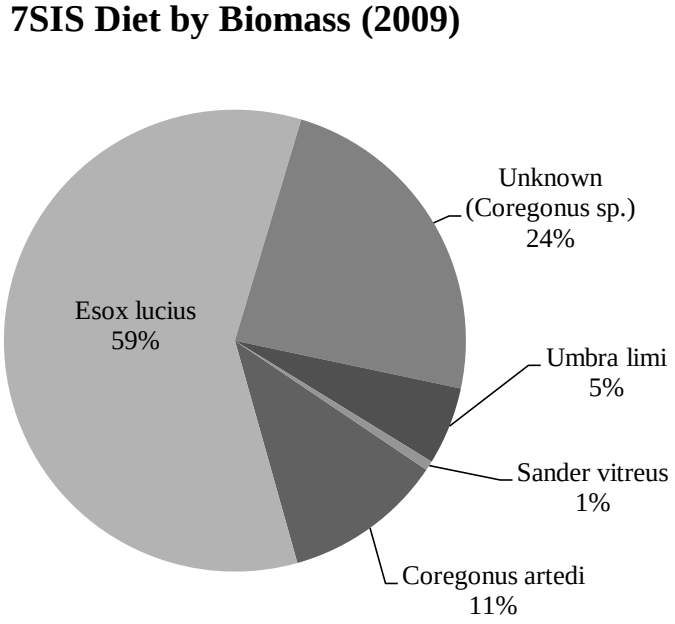

Fig 3.5. 7SIS diet by biomass, 2009

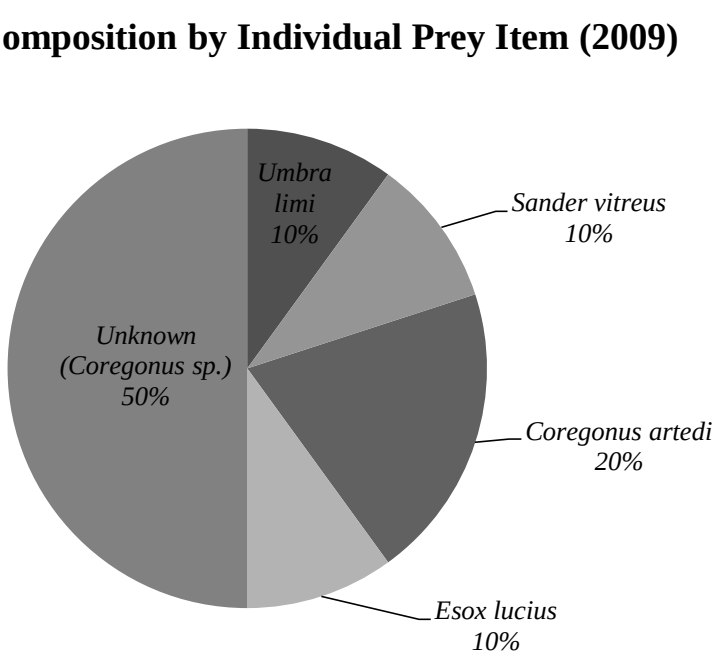

Fig 3.6. 7SIS diet composition by individual prey item, 2009

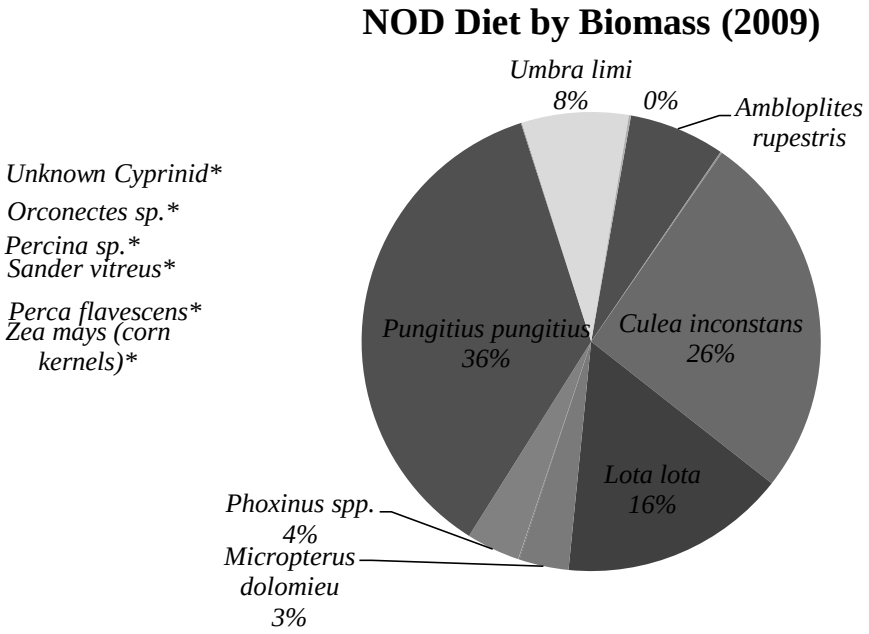

Fig 3.7. NOD diet by biomass, 2009
(* Species making up < 1% of total biomass)

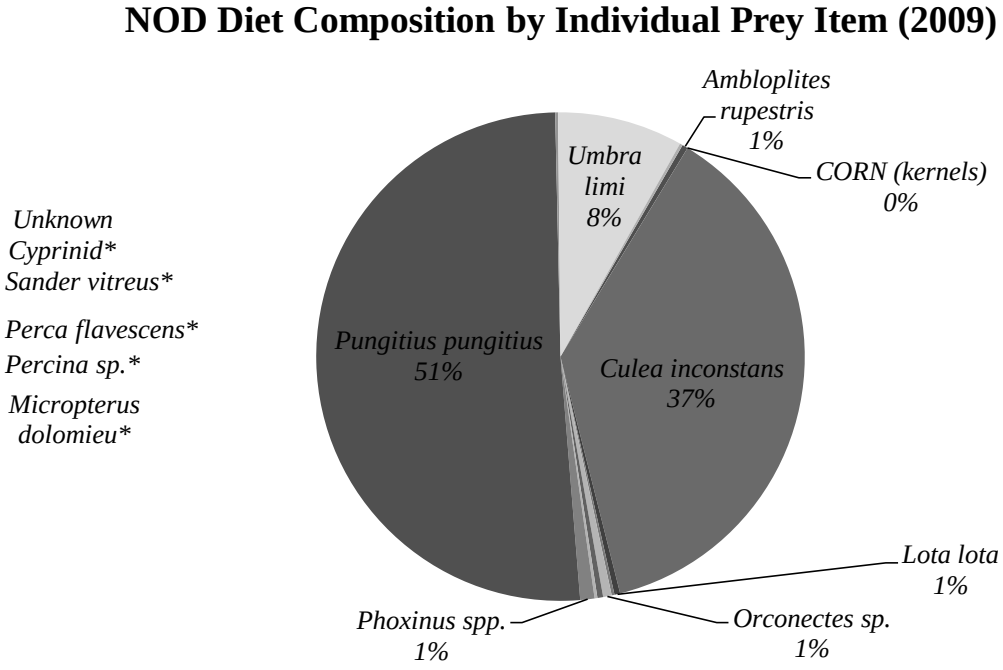

Fig 3.8. NOD diet composition by individual prey item, 2009
(* Species making up < 1% of total diet by number of individuals)

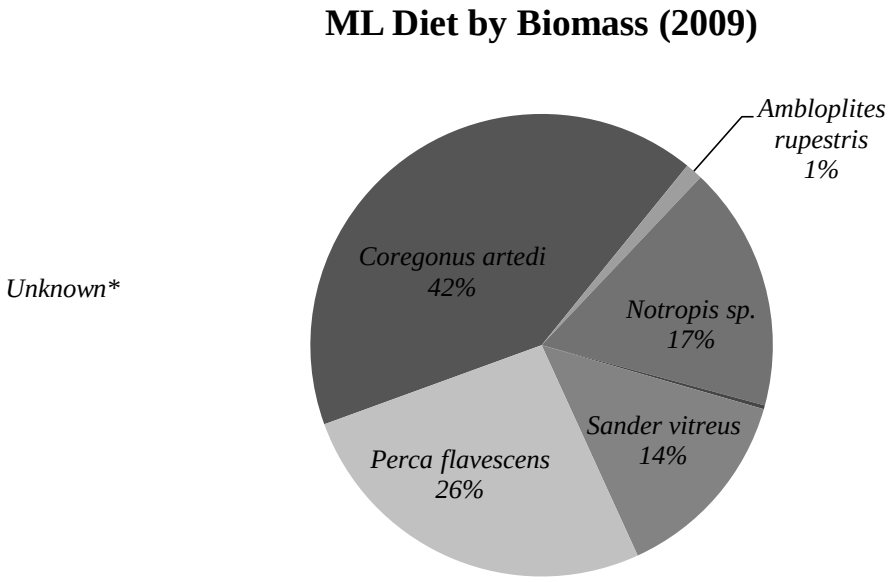

Fig 3.9. ML diet by biomass, 2009
(* Species making up < 1% of total biomass)

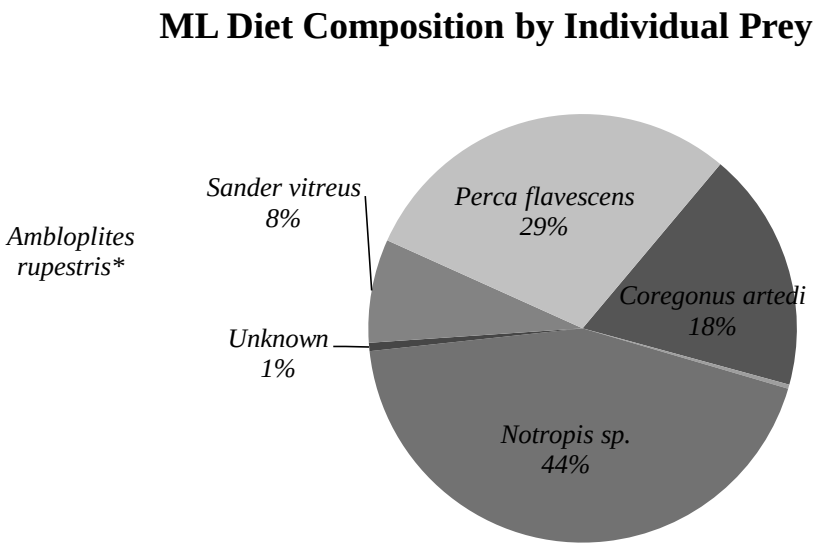

Fig 3.10. ML diet composition by individual prey item, 2009
(* Species making up < 1% of total diet by number of individuals)

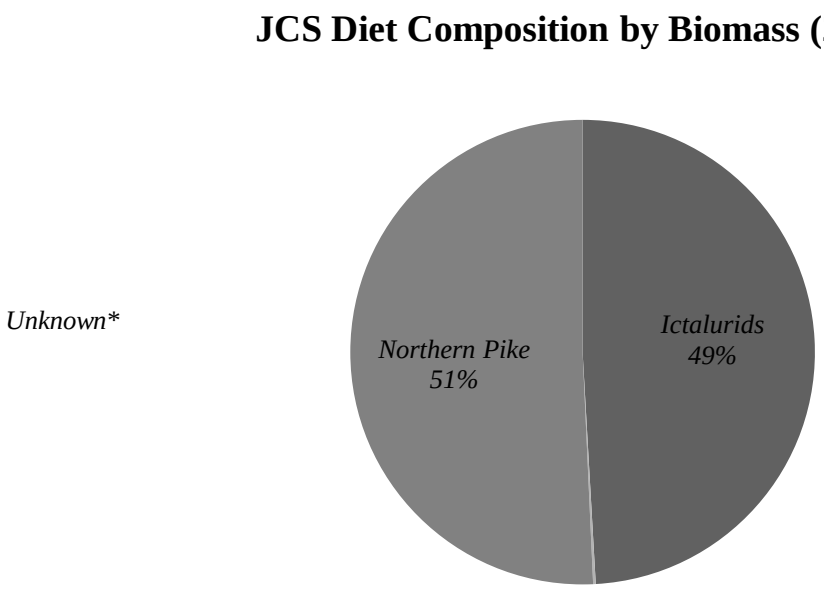

Fig 3.11. JCS diet by biomass, 2009
(* Species making up < 1% of total biomass)

JCS Diet Composition by Individual Prey Item (2009)

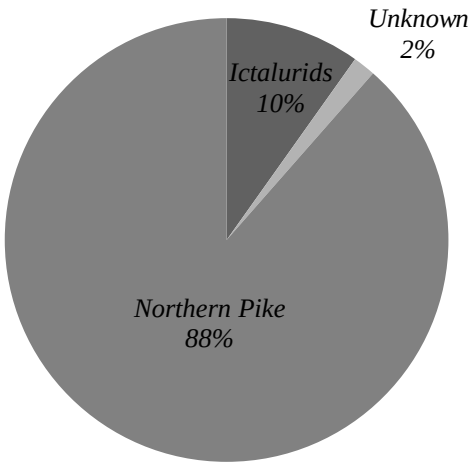

Fig 3.12. JCS diet composition by individual prey item, 2009
(* Species making up < 1% of total diet by number of individuals)

CHAPTER 4: CONCLUSIONS

Double-Crested Cormorants (*Phalacrocorax auritus*), a largely reviled species by some natural resource user groups, has long been condemned due to its perceived impacts to both sport and commercial fisheries. This study was able to reinforce the numerous existing studies demonstrating the highly opportunistic nature of cormorant foraging behavior, and hopefully alleviates some concern among resource user groups, notably sport fishermen, commercial fishermen and aquaculturists. This study also provided an opportunity to expand our understanding of the development of the immune and stress responses, as well as the impact of ecological factors on the growth of altricial nestlings.

Cormorants have been persecuted in North America for centuries, and it was not until their near extirpation in the 1970's did this species gain a modicum of legal protection. Largely due to congressional protection and the banning of the pesticide DDT, cormorant populations were able to recover and perhaps expand their range beyond pre-World War II levels. With this rapid population and range expansion came renewed interest and condemnation of this species. In 1999, Northeast Pine Island on Lake Kabetogama, MN saw the establishment of a cormorant colony. Lake Kabetogama, a popular sport fishery fully contained within Voyageurs National Park sees a large contingent of sport fishermen each year. As the cormorant colony continued to grow, concern from returning fishermen was voiced, explaining beliefs that growing cormorant numbers within the national park were resulting in lower catch rates among fishermen. This belief is far from rare, and is often the genesis for studies examining cormorant diet, this study being among them.

This study examined three facets of cormorant diet: species composition, biomass composition, and caloric content using a non-invasive bolus examination. Through this diet

analysis, I was able to accomplish two goals. Firstly, I was able to demonstrate a large degree of spatial and temporal heterogeneity in cormorant diet, and secondly I was able to indicate the importance of caloric content to developing nestlings. Spatial heterogeneity was evident, as colonies found in the boarder waters region of Minnesota and Ontario (Northeast Pine Island, Seven Sisters, and Noden Causeway) were found to possess unique diets in regards to both species composition and caloric content. These colonies, although in close proximity to one another, demonstrated diet compositions reflective of their own microhabitat, with the Noden Causeway colony showing a large proportion of gasterosteids, Seven Sisters showing a large proportion of coregonids (2008), and Northeast Pine Island showing a large diversity of prey species dominated by lake whitefish (*Coregonus clupeaformis*), walleye (*Sander vitreus*), and central mudminnows (*Umbra limi*). The lack of cisco (*Coregonus artedi*) as a diet component at Northeast Pine Island is notable, as this species is not commonly found in Lake Kabetogama, indicating adult birds were foraging primarily on this body of water. This assessment was also validated with a concurrent radio telemetry project. If this degree of diet diversity among colonies in close proximity to one another was seen, it is quite logical to expect to see this in the two remaining colonies separated by a greater distance (Mille Lacs and J. Clarke Salyer). This logic was confirmed, as again the diet of these two colonies greatly reflected their microhabitats, with the riverine J. Clarke Salyer colony providing diet samples consisting of nearly 100% ictalurids and northern pike (*Esox lucius*) and the Mille Lacs colony showing a diet consisting of a large number of individuals within the genus notropis, as well as a large biomass component of coregonids, yellow perch (*Perca flavescens*), and walleye.

Temporal heterogeneity in diet was most obvious when comparing the consecutive years of sampling at the Northeast Pine Island colony, with the stark discrepancies in yellow perch

consumption being immediately evident. A reduction of 29% in the total mass of yellow perch consumed in a single year likely denotes a change in fish community, possibly due to a low recruitment level of a particular age class, or a host of other biotic and abiotic factors limiting the cormorant's ability to prey on this species in the second year of the study.

Ultimately, one must be watchful when drawing conclusions from diet data alone. Without a thorough assessment (i.e. including all trophic levels) of the current status of the fishery in question, we are unable to directly test the impact of cormorant diet on said fishery. Conclusions we can draw however, based on the highly varied nature of the findings in this study, include the existence of short range feeding flights and opportunistic feeding behavior. If this was not the case we would expect to see similar diets among all colonies as the birds would be sharing from the same pool of resources. In a separate examination of sportfish predation, although cormorants were found to prey on a number of sportfish species, the rates of predation were not found to be comparable to those studies in which impacts to the fishery were the result of cormorant diet. So although this study was unable to directly test for deleterious effects on fishery health, I believe the combination of findings should help assuage concerns regarding the impact of this species and their associated colonies. I also believe cormorant diet assessments can be an effective way for fishery managers to monitor the health of their fishery, as sudden changes in diet composition may indicate important changes in the local ecosystem.

The second aspect of this study was two-fold. First, I examined the effect of environmental stressors on growth, and second I tested for delayed development and hyporesponsiveness in the stress response. The examination of growth and the effects of environmental stressors used three specific growth rates: wing digit, tarsus, and mass. I used a correlative approach to estimate stress levels by using a heterophil to lymphocyte ratio (HL), as

corticosterone measures possess inherent difficulties when used in field studies, especially among colonial species. Heterophil to lymphocyte ratios were then examined with a fit model using three environmental stressors as explanatory variables: endoparasite load, ectoparasite load, and diet quality (Calories•bolus^{-1}). These environmental factors were then combined with HL as modeling parameters to test their effects on the aforementioned growth metrics.

The fit model examining the effects of environmental stressors on HL resulted in a model explaining a significant amount of variation ($P<0.05$). Although no single modeling parameter was found to be statistically significant, it appears the interaction and combination of environmental stressors does affect the HL ratio. A subsequent evaluation of HL looked at the effect of age to test for delayed development of the stress response pathway. This test being important as it raises the issue of the development of the stress response pathway and immune response in regards to the differentiation between altricial and precocial species. This is significant, as it has been postulated that altricial species may delay the development of the stress response to help mitigate the negative effects associated with elevated levels of corticosterone, as they are unable to physically ameliorate their condition when stressed. An ANOVA did reveal significant variation in HL among different age classes, with the first two weeks showing the highest HL ratio. This is contrary to what I expected to find, if there is in fact a period of delayed development. Although contrary, it does not negate the possibility of a period of hyporesponsiveness or delayed development because heterophils and lymphocytes are ultimately important components of the immune system, and the HL levels witnessed may be the result of a developing immune system rather than a developing stress response pathway. There is also a possibility of maternal effects, passed through the egg, skewing early HL levels. These factors

being stated, I question the utility and accuracy of this method to assess stress levels in young altricial nestlings.

In modeling the effects of environmental stressors and HL on growth, it was revealed that a significant amount of variation was explained across all three growth metrics. Specifically, diet quality in conjunction with endoparasite load (endoparasite•bolus^{-1}) were found to be statistically significant modeling parameters when testing the correlation to wing digit growth, and diet quality alone was found to be a significant modeling parameter when examining the specific growth rate of the tarsus. Again it should be noted that the lack of significance from HL as a modeling parameter may be an indication of this methodologies shortcomings in terms of truly representing stress levels.

At the end of this study, I have accomplished several goals. I determined the diet composition of double-crested cormorant nestlings at five distinct colonies and concluded that diet is highly variable across both temporal and spatial scales. I have determined that Caloric content is important to developing nestlings and is positively correlated with both wing digit and tarsus growth rates. Caloric content in conjunction with other environmental stressors (e.g. endoparasite load) are also correlated to growth rates, with endoparasites in particular demonstrating an inverse correlation to wing digit SGR. I was able to detect significant changes in HL at various age classes (0-4 weeks of age), but I was unable to detect a delay in the development of the stress response pathway.

In conclusion, I believe it is important to question and examine the importance of these findings in terms of potential scientific and management implications. At a broad scale, I believe diet quality is of ultimate importance when it comes to the growth and development of young birds. This may be valuable information especially when it pertains to imperiled altricial species

dealing with short windows of time to mature. Low quality diets may not allow for sufficient growth necessary to be an effective self-sustaining adult, ultimately resulting in a lower level of fitness for the individual, and possibly the species. Also in terms of diet as an environmental stressor, we can conclude that ecological factors are indeed correlated to the development of nestlings. This information may allow managers to assist populations by mitigating environmental stressors, or may diminish nuisance populations by contributing stressors to the system. At a narrower scale, in reference to cormorants in particular, the examination of diet composition should diminish concerns among both sport and commercial fishermen. The predation of sportfish was consistently low at the popular fishing lakes, and it appears that adults in general forage in close proximity to their colony. I believe these findings should help prevent, or at least deter, aggressive culling programs that remove cormorants to help restore or maintain a fishery. The existence of cormorants at a fishery may in fact be beneficial for two reasons. Firstly, fishery biologists can analyze cormorant diet to monitor for abrupt changes in fish assemblages, and secondly cormorants may provide biological controls by diminishing rapid population expansions of certain fish species (e.g. exotic fishes). Due to the highly opportunistic nature of cormorant foraging behavior, any prey species that becomes more abundant will become proportionally more abundant in cormorant diet. Finally, it is in this light I believe we need to view this species. Not as a potential nuisance, but as a species with great potential to help monitor natural systems, maintain healthy fisheries, and further our understanding of ecological effects and the stresses they induce on wild populations of altricial birds.