ECOLOGY OF THE INTERIOR POPULATION OF DOUBLE-CRESTED CORMORANTS: PREVALENCE OF DISEASE AND COLONY ATTENDANCE

By

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ABSTRACT

ECOLOGY OF THE INTERIOR POPULATION OF DOUBLE-CRESTED CORMORANTS: PREVALENCE OF DISEASE AND COLONY ATTENDANCE

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The interior population of double-crested cormorants (*Phalacrocorax auritus*) nesting on the Great Lakes were nearly listed on the Endangered Species Act in the 1970s. With protection from persecution, banning of DDT, and increased prey availability on breeding and wintering grounds, e.g. invasive species introductions and catfish aquaculture, the cormorant population in the Great Lakes basin has risen to 1.4 million birds of which there are more than 100,000 breeding pairs. Consequently, cormorants are involved in human-wildlife conflicts involving impacts to fisheries, vegetation destruction, and perhaps most importantly disease spread to poultry. Despite their large population, knowledge of intercolonial movements and connectivity of cormorant colonies is lacking. Likewise, whether cormorants are reservoirs for disease has yet to be confirmed.

In my first chapter, I surveyed the interior population of double-crested cormorants for exposure to and current infection of APMV-1 and AIV. This study supports the idea that cormorants are a reservoir for APMV-1, but are not important in the spread of AIV.

In my second and final chapter, I explored the connectivity of cormorant colonies and general attendance patterns of cormorants nesting in the North Channel of Lake Huron near Blind River, Ontario. The level of colony connectivity has implications for effectively managing cormorant populations and understanding how disease is spread and maintained.

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CHAPTER 1

PREVALENCE OF AVIAN PARAMYXOVIRUS 1 AND AVIAN INFLUENZA VIRUS IN DOUBLE-CRESTED CORMORANTS IN EASTERN NORTH AMERICA

INTRODUCTION

Newcastle disease (ND) and avian influenza (AI) have the highest economic impact on the global poultry industry (Nayak et al. 2009, Suarez 2010). Low-virulence forms of both avian paramyxovirus serotype 1 (APMV-1), the etiological agent of ND, and avian influenza virus (AIV), the etiological agent of AI can cause reduced egg production, respiratory illness, and lethargy in chickens. More virulent strains of both pathogens can cause near 100% mortality in chicken flocks (Alexander 1997, Easterday et al. 1997).

AIV is a negative-sense, single-stranded RNA virus of the Family *Orthomyxoviridae*, Genus *Influenza A virus*. AIV is classified into subtypes that are determined by various combinations of hemagglutinin (HA) and neuraminidase (NA) proteins on the virus surface; there are 16 H and 9 N subtypes. Two pathotypes of AIV are recognized, highly pathogenic AIV (HPAIV) and low pathogenic AIV (LPAIV), based on the pathogenicity in poultry (Stallknecht 2003).. The primary reservoir for AIV are wild birds in the orders Anseriformes (ducks, geese, swans) and Charadriiformes (shorebirds, gulls, terns) (Stallknecht 2003, Olsen 2006) and has been isolated from 105 species (Artois et al. 2009). Wild hosts are typically asymptomatic for AIV (but see Latorre-Margalef et al. 2009). AIV is shed via feces, body fluids, and eggs, and is transmitted by the fecal-oral route as well as aerosolized bodily secretions (Stallknecht and Brown 2007). AIV is shed in the feces of waterfowl, and can persist in surface water, for at least a month (Stallknecht and Brown 2007).

APMV-1 is a negative-sense, single-stranded RNA virus of the Family *Paramyxoviridae*, Genus *Rubulavirus*. APMV-1has been detected in over 200 bird species; in most cases the birds

were asymptomatic, resulting in no clinical signs (Kaleta and Baldauf 1988). Three pathotypes of APMV-1 are recognized, lentogenic (low-virulence), mesogenic (moderate-virulence) and velogenic (high-virulence), based on disease produced by the virus isolate in poultry (Alexander 1997). Cormorant species (*Phalacrocorax* spp.) and rock pigeons (*Columba livia*) are the only species associated with epizootics worldwide and each species maintains velogenic APMV-1 strains within their populations (Leighton and Heckert 2007). APMV-1 is shed via feces, body fluids, and eggs, and is transmitted by the fecal-oral route as well as aerosolized bodily secretions (Leighton and Heckert 2007). The virus is able to persist in the environment over wide temperature ranges. APMV-1 is shed in the feces for up to a month post infection (Kuiken 1998).

Double-crested cormorants (*Phalacrocorax auritus*, hereafter cormorant) are piscivorous seabirds that nest in dense colonies ranging from tens to thousands of nesting pairs. They are widely distributed throughout North America (Hatch and Weseloh 1999). The interior population of cormorants breeds throughout the Canadian prairie provinces and the Great Lakes Basin, and winters in Louisiana, Mississippi, Alabama, and Arkansas, U.S. (King et al. 2010).

The primary reservoirs for AIV are wild birds in the orders Anseriformes and Charadriiformes (shorebirds, gulls, and terns; Stallknecht 2003, Olsen 2006). Two pathotypes of AIV are recognized, highly pathogenic AIV (HPAIV) and low pathogenic AIV (LPAIV), based on their pathogenicity in poultry (Stallknecht 2003). AIV has been detected in cormorants worldwide; but it is less prevalent among Pelecaniformes compared to other aquatic avian orders, such as Anseriformes (Stallknecht and Brown 2007). Suss *et al*. (1994) surveyed wild bird populations for AIV in Eastern Germany from 1977 to 1989 and isolated AIV from 18 of 4,500 (H6N1, 0.4% prevalence rate) great cormorants (*P. carbo*) sampled. To date, there are no published studies documenting the prevalence of AIV in cormorants in North America.

The most significant APMV-1 outbreaks with the only documented widespread mortality in wild birds (Wobeser 1997) occurred in cormorant chicks (Heckert et al. 1996, Glaser et al. 1999, Kuiken 1999); hence, cormorants are considered to be an important reservoir for APMV-1. Young-of-the-year (< 16 weeks of age) birds experience high mortality while older cormorants remain primarily asymptomatic (Kuiken et al. 1999). The first documented APMV-1 epizootic in cormorants in North America occurred in 1975 in Quebec, Canada (Cleary 1977). More recently, outbreaks have occurred in Canada and the United States during the early 1990's (Wobeser et al. 1993, Glaser et al. 1999) with biennial outbreaks occurring in the last decade (Sleeman 2010). It is clear that cormorants play an important role as reservoir hosts, but it is unclear how APMV-1 is maintained and propagated among the different cormorant populations.

Previous studies have found that cormorants have high seroprevalence of NDV antibodies but few studies have done large scale testing of both antibody and virus presence. There are no published studies about prevalence of AIV prevalence in cormorants but the lack of detection of AIV in USDA Wildlife Services AIV surveys of cormorants culled in Michigan suggests they are not likely to be important in AIV transmission. Individual birds were tested for the presence of pathogen-specific antibodies and active viral infections for both diseases. I test the following hypotheses in the study: (1) the interior population of cormorants are reservoirs for NDV both on the breeding grounds and (2) cormorants are not maintaining AIV on the breeding grounds.

MATERIALS AND METHODS

Study Sites

Cormorants were sampled in Michigan, U.S. and Ontario, Canada in May – July 2009 and 2010. In Michigan, I sampled cormorants culled during ongoing cormorant population

management efforts by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services (USDA/APHIS/WS) and/or the Grand Traverse Bay Band of Ottawa and Chippewa Indians. Culled birds were processed in the field within 4 hours of being harvested. Sites varied by year thus in cases when a site was not sampled in both years of the study, the sample year is given in parenthesis. These sites were a colony near the Ludington Pumped Storage Plant (43.893, -86.455) on Lake Michigan, in Ludington, MI; Bellow Island (45.099, -85.567) (2010) in Suttons Bay, Lake Michigan, near Suttons Bay, MI; and Goose Island (45.926, -84.433) (2009) and Green Island (45.837, -84.745) (2009) Islands located in the Straits of Mackinac, near Hessel, MI and St. Ignace, MI, respectively.

In Ontario, birds were sampled at three geographically distinct locations described by Chastant (2008), including Lake of the Woods (LOW), near Kenora, in southwest Ontario (49.663, -94.507); North Channel of Lake Huron (NC), near Blind River, in south-central Ontario (46.108, -83.026); and Eastern Lake Ontario (ELO), near Kingston, in southeast Ontario (44.191, -76.543).

Capture Techniques

In Ontario, adult breeding cormorants (breeders) were captured on active nests just prior to egg laying in May 2009-2010, using modified Victor #3 Softcatch leghold traps set on nests near the center of the colonies. The traps were modified in the following ways: Victor #3 coil springs were replaced with weaker Victor #1.5 coil springs, chain was replaced with aircraft cable, shock-cord and a box and stake swivel (King *et al.* 1998). I placed captured birds in burlap bags and removed them out of sight of the colony for processing and sampling for pathogens. Breeders were not uniquely marked during this study and therefore it is possible the same individual was sampled in both years unknowingly. Chicks were captured by herding crèches

into a corral constructed with wooden stakes and plastic snow fencing and banded with a USFWS aluminum band and plastic field-readable alpha-numeric band and placed in burlap bags while awaiting disease sampling. Chicks close to fledging age, (minimum 3.5-4.5 weeks) as determined by primary feather length (>2inches), were sampled for APMV-1 and AIV. In locations where islands consisted of solid granite, corrals could not be used, thus I placed chicks in burlap bags where they awaited banding processing. Upon being sampled for pathogens they were released back into the colony.

I also sampled culled cormorants after removal from the colonies. In the USA, cormorants were culled using methods described by Dorr *et al*. (2010) and accepted by the USDA, Animal and Plant Health Inspection Service (APHIS), Wildlife Services, and the Grand Traverse Bay Band of Ottawa and Chippewa Indians. In Ontario, birds were culled by Meeker's Aquaculture at Lake Wolsey, Manitoulin Island, Canada.

The ageing of cormorants can be difficult especially with culled individuals. I did not consistently record plumage coloration; but, given that the timing of culls in Michigan was early in the season, prior to hatching and fledging, all culled birds in 2009 and 2010 were classified as after-hatch-year (AHY). In the live-captured birds in Ontario, adults were captured on their nests thus all these mature adults were classified as “breeders”. Fledgling cormorants were captured between 3-5 weeks from hatching; hence, I classify all these individuals as “chicks”.

Sample Collection

Blood (2-3mL) was collected in 3-mL vacutainer tubes from the brachial vein or the medial metatarsal vein of live cormorants using a 22-gauge (breeders) or 26-gauge (chicks) syringe. In culled AHY cormorants, blood was collected from the opened body cavity in the order of highest preference via 1) heart puncture, 2) pooled blood in the thoracic cavity, and 3)

pooled blood or cutting the hepatic artery in the abdominal cavity, to minimize contamination with gastrointestinal flora and digestive enzymes. Serum was separated from whole blood using a centrifuge in the field and placed on ice in the field. Serum was stored at -20°C in the lab until antibody assays were performed. In addition, oropharyngeal and cloacal swabs were collected and placed together in 3-mL of brain heart infusion broth in cryovials and placed on dry ice in the field. Once in the lab the swabs were transferred to a -80°C freezer until real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays were performed for pathogen-specific viral RNA.

Serology

I analyzed serum for the presence of AIV specific antibodies using multi-species blocking ELISA (bELISA; Idexx Laboratories, Inc., Maine, U. S.) per manufacturer's protocols with the exception that the plate reader was equipped with a 630nm filter rather than the specified 650nm filter (verified by Idexx Laboratories Technical Support). The use of the 630nm filter, resulted in lower optical density readings; however, these readings were proportional across the entire plate and did not affect the results. Sample to negative (S/N) ratios less than 0.50 were considered positive while those ratios greater than 0.60 were considered negative and ratios between 0.50-0.60 were considered undetermined and were retested.

Presence of APMV-1 antibodies was detected using bELISA kits (Svanova Biotech AB, Uppsala, Sweden) per manufacturer's protocols. Percent inhibition (PI) values greater than 40% were considered positive while PI values less than 30% were considered negative, and PI values between 30-40% were considered undetermined and were retested. Samples considered positive and undetermined by the APMV-1 antibody bELISA were verified using a hemagglutination

inhibition (HI) assay developed and performed by the National Veterinary Services Laboratory (NVSL) in Ames, Iowa. Samples with titers of 1:8 and greater were considered positive.

Virology

APMV-1 viral RNA was purified using QIAamp Viral RNA kits (QIAGEN, Inc., Valencia, CA) per manufacturer's protocol. A swab sample with 1-µL of undiluted APMV-1-transcribed RNA (NVSL) added served as a positive control. In 2009-2010, oral and cloacal swabs were tested for APMV-1 and AIV by rRT-PCR following the protocols described by Wise *et al*. (2004) and Spackman *et al*. (2003), respectively. In both years, only the matrix protein (M) gene primers and probes described in Wise *et al.* (2004) were used for the APMV-1 rRT-PCR assay. However, in 2010, the final reaction volume was 10-µL instead of 25-µL prescribed by Wise *et al.* (2004). The cycle threshold (CT) values used to determine positive results were CT≤40 for AIV and CT≤38 for APMV-1 rRT-PCR assays. In 2009, the Diagnostic Center for Population Animal Health (DCPAH), East Lansing, MI performed both APMV-1 and AIV analyses. Samples positive for AIV were further analyzed by the DCPAH using a second rRT-PCR assay to determine if H5 or H7 subtypes were present per the protocol in Spackman *et al*. (2003).

In 2010, the DCPAH performed the AIV analysis and the Research Technology Support Facility (RTSF) Genomics Core at Michigan State University. East Lansing, MI conducted the APMV-1 analysis. APMV-1-transcribed RNA (NVSL) was used as a positive control and to determine the limit of detection. Samples positive for APMV-1 were verified by virus isolation and sequencing using protocols developed and conducted by the NVSL.

RESULTS

I sampled 461 breeders and 326 chicks in 2009 -2010 in Ontario and Michigan. In addition, I sampled 105 culled AHY birds culled in Michigan in late July and early August (Table 1).

Serology

I report the seroprevalence of APMV-1 antibodies here in percent positive by HI of the total number of samples analyzed. Chicks were seronegative for APMV-1 antibodies in 2009, while 4.4% were seropositive in 2010 (Table 1). Breeder cormorants were seropositive for APMV-1 antibodies, 48.7% in 2009 and 58.3% in 2010. Additionally, culled AHY birds were seropositive for APMV-1 antibodies, 38.9% in 2009 and 33.3% in 2010. APMV-1 antibody titers in breeder cormorants ranged from 1:8 to 1:32 in 2009, whereas in 2010, titers ranged from 1:8 to 1:128. Chick APMV-1 antibody titers ranged from 1:8 to 1:32, in 2010 (Table 1). A single culled AHY cormorant was seropositive for AIV antibodies; this bird was sampled in 2010 and was also seropositive for APMV-1 antibodies.

Results by APMV-1 bELISA assay as compared to HI did differ in that the percent of cormorants considered APMV-1 seropositive was greater by bELISA than by HI assay (Table 1). Of the 557 APMV-1 positive or undetermined serum samples verified by the NVSL, 240 were negative (<1:8 titer) for APMV-1 antibodies. There was no relationship between PI values from the bELISA results and the end-point titers from the HI assays.

Virology

All birds sampled in 2009 were negative for active infections of AIV and APMV-1. Likewise, in 2010 all birds were AIV negative. However, nine chicks tested positive for APMV-1 by rRT-PCR, eight in ELO and one in NC. Cycle threshold (CT) values ranged from 30.7 to

34.5 (Table 2). Three of the APMV-1 positive ELO chicks exhibited ataxia, paresis and torticollis, all classic clinical signs of APMV-1 infection.

DISCUSSION

While AIV is rare, particularly H5 and H7, I find that exposure to NDV is common in cormorants within the Great Lakes and LOW. Furthermore, I observed an APMV-1 outbreak in chicks as well as breeder cormorants with very high APMV-1 titers. Hence, our study suggests that cormorants may play a role in the maintenance of APMV-1 in the environment given their high seroprevalence during each year of the study.

Over the course of this study I found a single AIV seropositive adult cormorant, suggesting cormorants have an insignificant role in the maintenance of AIV in wild bird populations in the Great Lakes Basin and LOW. Additionally, previous studies have isolated AIV from cormorant species only occasionally (Süss et al. 1994). Therefore, the remainder of this discussion will focus on APMV-1 in cormorants that breed in the Great Lakes Basin and LOW.

Despite two decades of APMV-1 epizootics on cormorant colonies in Canada and the U.S., significant knowledge gaps exist. The focal question recurring in studies of ND in cormorants is how the virus is maintained in the system. Adult cormorants remain asymptomatic when infected with APMV-1 while chick cormorants are highly susceptible to the virus, hence highly visible, periodic, widespread epizootics on the breeding grounds. APMV-1 maintenance may be a function of 1) age and immune status of individuals in the population, 2) constant transmission events, and 3) environmental persistence of the virus.

Age-related susceptibility of cormorants likely influences the maintenance and transmission of APMV-1 (McFerran and McCracken 1988). For instance, in the sporadic

epizootics of APMV-1 in the Canadian provinces, the Upper Midwest, Nevada and California, U. S., and throughout the Great Lakes basin, young birds (< 16 weeks of age, typically ~4 weeks of age) experience high mortality (up to 92%) from virulent APMV-1 infection (McFerran and McCracken 1988, Wobeser et al. 1993, Meteyer et al. 1997, Kuiken et al. 1998, Glaser et al. 1999, Kuiken 1999, Sleeman 2010). In agreement with these previous studies, I observed dead and dying chick cormorants, but not breeder cormorants in 2010 that tested positive for APMV-1 and/or APMV-1 antibodies. Given the high reproductive success of cormorants in the Great Lakes, a high proportion of susceptible individuals enter the population each year, preventing any long lasting herd immunity and likely drive the recent biennial outbreaks of APMV-1 (Van Boven et al. 2008).

APMV-1 may also circulate undetected among cormorant populations due to continuous transmission events on wintering grounds. However, I observed a high APMV-1 seroprevalence among adult cormorants in addition to a lack of actively infected adults on the wintering or breeding grounds, which suggests that continuous transmission is an unlikely explanation for the maintenance of APMV-1. Interestingly, studies with poultry seropositive for APMV-1 demonstrated that the virus could still infect, replicate within, and be excreted from mucosal membranes of otherwise clinically healthy birds (Holmes 1979). Likewise, Stone *et al*. (1980) found that the respiratory tracts of chickens were unprotected from APMV-1 infections and capable of shedding virus even with the presence of high HI titers (1:80 to 1:160). Similarly, studies of free-ranging, closed flocks of village chickens in southeast Asia, where half of the flock possessed protective HI antibodies to APMV-1, showed that the virus persisted in the closed flock for at least two years (Samuel and Spradbrow 1989). Antibody persistence is variable, and APMV-1 HI antibodies decline in 3 – 4 months post infection (p.i.), becoming

undetectable in 8 – 12 months (Beard and Hanson 1984). In contrast, one study observed that HI antibody titers declined gradually within 10 weeks p.i. and were assumed to be undetectable by 18 weeks (Kuiken 1998). Thus, if cormorants are similar to poultry in their susceptibility for reinfection, and are infectious despite the presence of APMV-1 antibodies, continuous transmission throughout the wintering period may easily go undetected without any susceptible chicks to exhibit morbidity and/or mortality.

APMV-1 is able to persist in the environment in a wide range of temperatures, mediums, and pH. Under experimental conditions Olesiuk (1951) found at temperatures of 20°C to 30°C, the virus was viable in soil samples for 66 days, in feces for 83 days, and in feather down for 163 days. While at temperatures of 3°C to 6°C, APMV-1 was viable in soil samples for 235 days, and in feces and feather down for at least 538 days. APMV-1 remains infectious for at least 538 days in soil, feces and down at -26°C. Furthermore, APMV-1 remained infectious from pH 2 to 11 (Moses et al. 1947) and is resistant to direct sunlight (Skinner and Bradish 1954). APMV-1 remained viable and infectious in lake water for 11 to 19 days under experimental conditions (Boyd and Hanson 1958). Although, survival time and infectious status of APMV-1 in field conditions is likely reduced, the virus likely persists in the environment from year to year (Olesiuk 1951). The ability of APMV-1 to survive and remain infectious in a wide variety of substrates and environmental conditions is evidence that the virus may overwinter from year to year in dried feces, nests and soil on breeding colonies. In this case, susceptible chick cormorants could be infected via virus persisting in the environment on the breeding colony. However, this is likely not the case since recent APMV-1 outbreaks failed to occur in consecutive years, as one would expect if virus overwintered on breeding colonies. For instance, recent APMV-1 outbreaks occurred in 2008 and 2010, but not in 2009 (Sleeman 2010).

Cormorants may also be responsible for the dispersal and long-range movement of the virus during the migratory period. In previous studies, researchers have suggested that cormorants are exposed and infected with APMV-1 along the northern migration route prior to arrival at breeding grounds (Meteyer et al. 1997, Glaser et al. 1999). Since APMV-1 infected cormorants have been shown to shed virus for up to 28 days (Kuiken 1999) and cormorants migrating from summer to winter grounds can complete the trip in as few as six and as many as 42 days (Scherr et al. 2010), there is potential for cormorants to become infected on wintering grounds and still be infectious upon arrival at breeding colonies. This possibility should be tested by collecting samples from actively migrating cormorants.

Despite the high seroprevalence of APMV-1 in cormorants, it is unknown whether APMV-1 has any significant effect on population size. Cormorants have recently experienced a population resurgence resulting in about 100,000 pairs of birds nesting in the Great Lakes Basin and 1.4 million birds total (Weseloh et al. 1995, Werner and Hanisch 2003). Given the current size and reproductive output of the interior breeding population of cormorants and the sporadic spatial pattern of APMV-1 outbreaks, it is unlikely that APMV-1 is regulating the population. The high mortality experienced by juvenile cormorants infected with APMV-1 is akin to egg-oiling management practices, which have been deemed ineffective as the sole management regime to decrease cormorant populations (Blackwell et al. 2002, Chastant 2008). While interior population-wide demographic parameters are lacking, using demographic parameters from cormorants nesting in Lake Ontario from 1979 to 2000, Blackwell *et al* (2002) constructed a stage-classified projection matrix of cormorant population growth and found that survival of cormorants age 3 and older contributed the most to the growth of the population. Cormorants older than 16 weeks are resistant to infection by APMV-1, therefore sporadic epizootic outbreaks

of APMV-1 are not likely to have an effect on the overall interior population of cormorants.

APMV-1 epizootic events on cormorant colonies have occurred in conjunction with mortality and morbidity of co-nesting species, e.g. American white pelicans, ring-billed gulls, Franklin's gulls, herring gulls, Caspian terns, and black-crowned night-herons (Wobeser et al. 1993, Kuiken et al. 1998, Glaser et al. 1999). Many of these species often exhibited clinical signs congruent with APMV-1 infection in cormorants. However, APMV-1 was only isolated from a single pelican and ring-billed gull in the 1990 epizootic in Canada, and from a Caspian tern in 1995 in Canada (Wobeser et al. 1993, Canadian Cooperative Wildlife Health Centre 1995). In addition, a single seropositive ring-billed gull with low APMV-1 titer was found during the 1992 Canadian APMV-1 outbreak (Glaser et al. 1999). Over the course of this study, I found no evidence of anomalous morbidity or mortality in colonial waterbirds co-nesting with affected cormorants. It is likely that APMV-1 does not pose a significant risk to other species of birds; the reasons for which warrant further investigation.

An interesting finding in the current study was the difference in the bELISA and HI assay results from the same samples. Blocking ELISAs (bELISA) have rarely been used in the detection of APMV-1 antibodies in wild birds. While HI assays have been and are still considered to be the gold standard in APMV-1 antibody detection, bELISAs for APMV-1 antibody detection in serum serve as an economical high-throughput method for quickly detecting seroprevalence. Previous studies evaluated the relationship between the ability of ELISA and HI assays to detect APMV-1-specific antibodies in both commercial poultry flocks and laboratory animals (Brown et al. 1990, Bell and Lelenta 2002). Brown *et al*. (1990) and Bell and Lelenta (2002) found a high level of correlation (kappa = 0.84 and coefficient of correlation = 0.914, respectively) between the bELISA and HI tests for determining APMV-1-specific

antibody titers. Blocking ELISAs have rarely been used in the detection of APMV-1 antibodies in wild birds, but they are highly sensitive and can detect antibodies at titers lower than the standard < 1:8 series dilution titer of the traditional HI assay. In our study, 29% (153 out of 524) of the serum samples positive by bELISA were negative by HI assay. Furthermore, there was no relationship between PI values from the bELISA and HI titers. Marquardt *et al*. (1985) showed that the correlation between ELISA and HI assay results decreased as post-infection time increased and when inoculation routes were varied. In fact, the correlation was lower in birds infected via the intranasal-intraocular versus the intratracheal route. In the wild, cormorants can be exposed to APMV-1 in a number of different ways, such as via the fecal-oral route, in the water, or during territorial or mate defense disputes (Alexander 1997). Furthermore, the timing of infection of breeder cormorants in the wild is unknown. These reasons may account for the lower congruency between bELISA and HI assay results. Hence, caution should be used when using results from bELISA assays to measure level of protective antibodies in wild birds.

Whether cormorants maintain an APMV-1 reservoir is a question of critical importance, since they may introduce the pathogen into poultry operations, where the impact of an outbreak would be devastating. The cormorant's ability to transmit APMV-1 to poultry was demonstrated during the 1992 epizootic, where the same virulent APMV-1 strain infecting cormorants was transmitted to and isolated from free-range turkey flocks in North Dakota, U.S. (Heckert et al. 1996). Furthermore, there is evidence that APMV-1 exists on wintering grounds (Allison et al. 2005). Besides, locations of cormorants reported by Scherr *et al*. (2010) were in close proximity to broiler operations in Mississippi, the state with the largest poultry industry in the USA. A spillover event to poultry stocks would be economically devastating to the country with far-reaching effects. Given the demonstrated ability of cormorants to transmit APMV-1 to

commercial poultry flocks (Heckert et al. 1996) and the high mortality experienced by poultry infected with APMV-1 (Alexander 1997), it is important to understand how the virus is maintained among wild birds and may spread to livestock.

I documented high APMV-1 seroprevalence in breeder cormorants in all years sampled, but our results indicate breeder cormorants do not play a significant role in the maintenance and transmission of AIV. The high APMV-1 seroprevalence rates observed among breeders further implicates cormorants as important reservoir hosts for APMV-1; however, it remains unclear how APMV-1 is maintained year after year without causing infectious outbreaks every year. The reoccurring APMV-1 outbreaks, observed among breeding cormorants in the Great Lakes region, may suggest that seasonal maintenance occurs via continuous transmission events and not through the environmental persistence of the virus. A dedicated ongoing surveillance program for APMV-1 in cormorants on breeding and wintering grounds as well as migratory routes is necessary to begin to predict future outbreaks. In a satellite-tracking study of cormorants nesting in Georgian Bay, Ontario, Scherr *et al*. (2010) reported Lake Erie as a significant staging area for cormorants preparing for fall migration. Therefore, I highly recommend expanding surveillance to known staging and stopover sites elucidated in previous studies, such as Scherr *et al*. (2010). Additionally, further satellite tracking studies on a larger portion of the interior breeding population of cormorants may be useful in finding other significant staging and stopover sites.

APPENDIX

Table 1. Seroprevalence of avian paramyxovirus-1 antibodies in breeder and chick double-crested cormorants sampled in 2009 and 2010. Only those samples positive or undetermined by ELISA were titered by HI assay. Titers less than 1:8 are considered to be nonprotective and thus negative. Birds sampled in 2010 may be recaptured birds from 2009, hence caution should be used when comparing years.

					Titer by HI†							%+ by ELISA	%+ by HI
Year	Age	n	Ab+[1]	Ab*i*[2]	NT[3]	<1:8[4]	1:8	1:16	1:32	1:64	1:128		
2009	Breeder	149	135	1	12	52	37	28	9			90.6	49.7
	Chick	76	0									0.0	0.0
	AHY	54	39	0	0	18	8	9	3	1		72.2	38.9
2010	Breeder	290	265	11	7	97	98	43	22	5	1	91.4	58.3
	Chick	250	37	34	0	60	6	3	2			14.8	4.4
	AHY	51	34	1	16	13	8	4	4	1		66.7	33.3

[1]Samples seropositive by b-ELISA

[2]Samples with undetermined antibodies by b-ELISA

[3]No test – seropositive samples by b-ELISA but not tested by HI due to autoagglutination

[4]Samples considered negative by HI

†HI tests were performed on all Ab+ and Ab*i* samples

Table 2. Virulent avian paramyxovirus-1 infection in nine double-crested cormorant chicks sampled during the 2010 breeding season. The range of cycle threshold (CT) values are reported from rRT-PCR. Titer results are based on hemagglutination inhibition (HI) assays. Clinical signs of ataxia, paresis, and torticollis were observed in 3 birds on Snake Island in ELO.

Location	Julian Day	Age	Range of CT Values	Titer by HI
Ontario				
Eastern Lake Ontario				
Pigeon Island	172	Chick	31.4 - 34.0	<1:8 – 1:32
Snake Island	172	Chick	30.7 – 34.5	0[1] - 1:16
North Channel, Lake Huron				
Magazine Rock	195	Chick	30.8	0[1]

[1]Negative by bELISA

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CHAPTER 2

COLONY VISITS, ATTENDANCE PATTERNS AND FACTORS INFLUENCING COLONY ATTENDANCE OF BREEDING DOUBLE-CRESTED CORMORANTS IN THE NORTH CHANNEL OF LAKE HURON

INTRODUCTION

Cormorants are colonial nesting, foot-propelled, diving seabirds. Of the six cormorant species that breed in North America, the double-crested cormorant (*Phalacrocorax auritus,* hereafter referred to as cormorant) has the widest range with five recognized subspecies occurring in distinct geographic regions: 1) Alaska, 2) Pacific coast, 3) U.S. and Canadian interior and northeast Atlantic coast, 4) southeastern U.S. and western Caribbean and 5) San Salvador (Hatch and Weseloh 1999, Wires and Cuthbert 2006). The U.S. and Canadian interior and northeast Atlantic coast subspecies *Phalacrocorax auritus auritus* is the most numerous comprising 60% of the cormorant population (Hatch 1995). It is separated into two breeding populations: 1) U.S. and Canadian interior metapopulation (hereafter referred to as interior population) and 2) northeast Atlantic coast.

Studying colony attendance of nesting seabirds allows us to gain information about daily activity budgets, foraging ecology, reproductive success, parental roles, habitat selection and use, effects of human disturbance, and to help guide management decisions pertaining to both seabird and fisheries conservation, and human-wildlife conflicts. The majority of recent colony attendance studies of cormorants in North America focused on foraging activity budgets of breeding adults (Custer and Bunck 1992, Stapanian 2002, Seefelt and Gillingham 2006, Coleman and Richmond 2007). Cormorants nesting on the Great Lakes forage three to 11 km from their breeding colonies (Custer and Bunck 1992, Stapanian 2002) and probably have little negative impact on sport fisheries near breeding colonies (Seefelt and Gillingham 2006, Coleman and

Richmond 2007). Other studies have investigated basic colony attendance patterns, parental roles, and responses to population management regimes, e.g. egg-oiling, culling of adults, and hazing, of cormorants (Léger and McNeil 1985, Coleman and Richmond 2007, Duerr et al. 2007)Léger and McNeil 1985, Coleman and Richmond 2007, Duerr et al. 2007).

Cormorants exhibit a bimodal colony attendance pattern with peaks in absence at 09:00 h and 15:00 h (Coleman and Richmond 2007), and males and females are equally invested in nest attendance and chick-rearing duties (Léger and McNeil 1985). Cormorants subjected to egg-oiling and subsequent egg predation by gulls experienced an increase in dispersal, up to 20%, from their nesting colony to nearby unmanaged colonies during the breeding season that management occurred (Duerr et al. 2007). Understanding parental roles of cormorants, where and when breeding cormorants forage, and how they respond to management actions, researchers can narrow down sites for future studies and assessments of human-cormorant conflicts. Furthermore, biologists can more efficiently control cormorants by managing a population instead of one or two colonies.

Diurnal foraging seabirds can maximize foraging opportunities by foraging nocturnally but are often limited by light conditions. Nocturnal foraging in otherwise diurnal foragers has been documented in a few seabird species (Regular et al. 2011, Zavalaga et al. 2011), two of which are cormorant species, great cormorants (*P. carbo*; (Gremillet et al. 2005) and double-crested cormorants (King, Harrel, et al. 1998). Interestingly, evidence of nocturnal foraging during moonlit nights by cormorant species in the United States, Argentina, Scotland, and South Georgia has been discovered during colony attendance studies (Wanless et al. 1999, Sapoznikow and Quintana 2002, Coleman and Richmond 2007). However, no studies have documented this behavior in cormorants in the Great Lakes region (Hatch and Weseloh 1999). Foraging at night

could have impacts on specific age classes of sport fish populations that undergo diel vertical migration near cormorant nesting colonies (Lantry et al. 2002, Suski and Ridgway 2009). Diel vertical migration due to predator avoidance and/or prey tracking is described for several species of fish, including sculpin, round goby (*Neogobius melanostomus*), emerald shiner (*Notropis atherinoides*), and alewife (*Alosa pseudoharengus*), all of which occur often in cormorant diet analyses in the Great Lakes (Janssen and Brandt 1980, Bur et al. 1997, Dopazo et al. 2008).

The interior population of cormorants in the United States is currently managed under United States Fish and Wildlife Service (USFWS) Depredation Orders (50 CFR 21.47, 50 CFR 21.48) for conflicts with commercial and sport fisheries, the aquaculture industry, and habitat and vegetation destruction on nesting colonies. In 2006, the USFWS and the Ontario Ministry of Natural Resources identified colony attendance and movement as a priority information need for further fine-tuning management strategies for cormorants in the Great Lakes Basin (Ontario Ministry of Natural Resources 2006, U.S. Fish and Wildlife Service 2009).

In 2009, the Great Lakes Basin had 108,592 breeding pairs of cormorants (Cuthbert and Wires n.d.) and despite being managed to alleviate conflict stemming from over-population, little is known about basic cormorant colony site attendance behaviors. Gaining knowledge of breeding cormorant attendance at their own nesting colonies and then visits to those adjacent breeding colony sites will help maximize management efforts and can provide insight into how diseases, e.g. APMV-1, are transmitted throughout a population. APMV-1 infections have plagued cormorant populations for over a decade (Kuiken 1999). The objectives of this study were to 1) determine how much time radio-tagged cormorants spend at nesting colonies and visits to nearby colonies; 2) determine whether breeding stage, sex, sky conditions, and moon phase influence attendance patterns; and 3) investigate breeding site fidelity.

I hypothesized that time spent on the colony is influenced by reproductive stage (e.g. incubation, chick-rearing and fledging) of the colony with cormorants predicted to stay near their colonies during active nesting (Werner et al. 2001, Seefelt and Gillingham 2006, Coleman and Richmond 2007). Specifically, I predicted that if time spent on the breeding colony is related to reproductive stages of the colony, then I expect 1) cormorants to spend more time at the colony during incubation and less time at the colony during chick-rearing due to higher energetic demands of the chicks; 2) the highest frequency of visits to a trout aquaculture facility to occur during the fledging stage; 3) that to supplement their own energetic demands, adult cormorants would leave the colony more often and/or for longer durations of time to forage on clear nights when the moon is at least half full.

MATERIALS AND METHODS

Study Sites

The North Channel of Lake Huron consists of hundreds of granite slab and outcropping islands, many of which are breeding sites for ground-nesting cormorants. The study sites were four such islands, Doucet Rock (DR), Fortin Rock (FR), Magazine Island (MI), and Robb Rock (RR), ranging in size from 0.4 to 1.5 ha and located within 18km of Blind River, Ontario, Canada (46.108, -83.026). An additional study site was a rainbow trout (*Oncorhynchus mykiss*) aquaculture facility, MTM Aquaculture (MTM), located 42 km south of FR on Lake Wolsey, Manitoulin Island, Ontario, Canada (45.802, -82.548).

Capture and Transmitter Attachment

In May 2009 and 2010, I captured adult breeding cormorants (n=19 in 2009, n=44 in 2010) on active nests at FR and MI just prior to egg laying using modified Victor #3 Softcatch leghold traps set on nests near the center of the colonies. The traps were modified in the

following ways: Victor #3 coil springs were replaced with weaker Victor #1.5 coil springs, chain was replaced with aircraft cable, shock-cord and a box and stake swivel (King, Paulson, et al. 1998). I placed captured birds in burlap bags and removed them out of sight of the colony for processing. VHF transmitters (SirTrack, North Liberty, Iowa, USA; Advanced Telemetry Systems (ATS), Inc., Isanti, Minnesota, USA; 27g, < 3% body weight) were deployed on birds using backpack harnesses crafted out of 6 mm Teflon ribbon (Bally Ribbon Mills, Bally, Pennsylvania, USA, Part# 8476) (King et al. 2000). The transmitters featured 18-month battery lives, pulse rates of 50 pulses per minute (ppm), and no mortality switch or duty cycle. Prior to release, I banded the birds with uniquely numbered aluminum leg bands, measured tarsus, culmen and flattened wing cord lengths, and, in 2010, collected blood from the brachial or medial metatarsal vein using a 22-gauge needle and syringe for DNA sexing. In both years, I used a morphometric model developed by Glahn and McCoy (1995) to sex birds. In 2009, I verified the model using DNA samples collected from culled cormorants nesting on Green and Goose Islands, Straits of Mackinac, MI.

Data-logging Stations

Data-logging stations were deployed on DR, FR, MI, RR and MTM. The detection radius for each station was 2 km and the total study area was 260 km^2. Data-logging stations consisted of a receiver (model R4000) and a data collection computer (model DCC D5041A) (ATS, Inc., Isanti, Minnesota, USA), a 12-volt deep-cycle marine battery charged by a solar panel, and an antenna mounted on a 6 m mast. Five-element Yagi antennas were placed on MI and FR, while omni-directional antennas were placed on DR, RR and MTM. I placed the data-logging stations on small islands adjacent to the breeding colony or secluded locations on the nesting colony where researcher activity would not disturb nesting birds. The data-loggers scanned 24 hours per

day with a 20-minute scan cycle, and recorded Julian day, hour, minute, frequency, ppm, and signal strength. The data-logging stations collected data from 1 May to 30 September 2010 and data was downloaded from the logging stations monthly.

Data Management

Various aspects of the study required some data to be either extrapolated from collected data or acquired from outside sources in the case of weather and astronomical variables, i.e. sky conditions, and moonrise and moon phase data. Absence data was built from the presence data in a binary fashion with absent assigned a "0" and present assigned a "1". Because the data-logging stations cycled on 20-minute recording intervals, I recorded a bird as absent in the database if the data-logging station did not record it during a 20-minute interval. I also assigned the sex of the birds in a binary fashion.

I defined the breeding stages as time intervals where the majority of cormorants in the colony were in the same stage, incubating (21 May- 20 June), chick-rearing (21 June – 1 August), and fledging (2 August – 15 September), based on 2009 observations on MI and FR in addition to published breeding and reproductive biology data for colonies elsewhere on the Great Lakes (Seefelt and Gillingham 2008). Most of the cormorants leave the breeding colonies to stage for migration by 15 September. I defined breeding site fidelity as the return of a cormorant to the original breeding colony in subsequent years where it was first captured and tagged.

Civil sunrise and sunset, and moonrise and moonset and moon illumination (in percent) data were obtained from the United States Naval Observatory (USNO) Astronomical Applications Data Services (www.usno.mil/USNO/astronomical-applications) by specifying the latitude and longitude coordinates (46.108, -83.026) for Blind River, Ontario. I figured the moon variable in a binary fashion based on the aforementioned datasets. If the sun had set and the

moon had risen, and the moon was greater than or equal to half full I assigned a "1"; if the sun had set and the moon had risen, and the moon was less than half full I assigned a "0". I decided to include three replicates of each moon phase to account for correlation of colony attendance with the breeding stages. For purposes of analyzing the influence of moon phases on colony attendance, I chose three replicates of each moon phase and chose to include two days prior and after the actual date that the moon was in First Quarter, Full, Third Quarter, and New phases. For example, the first Full moon of the study occurred on Julian Day 147, so I chose to include Julian Days 145 to 149 in the analysis. Finally, I obtained sky conditions collected by the Gore Bay, Ontario, Canada weather station, located roughly 30 km from the study site, from Weather Underground, Incorporated (www.wunderground/com/history, Ann Arbor, MI, USA).

Statistical Model

I used two generalized linear mixed models (GLMM) (PROC GLIMMIX, SAS 9.2, SAS Institute Inc., Cary, NC, USA) and in both models specified the between-within method of computing denominator degrees of freedom, Newton-Raphson with ridging optimization technique to help with model convergence, a binary distribution, and the logit link function. Statistical significance was determined at $\alpha = 0.05$ level for both models. I included only cormorants captured and tagged in 2010 in our model because I could not be sure of the breeding status of 2009 captured birds. In the first model I modeled presence (event = "1") as a binary response variable. Breeding stage (1=incubation, 2=chick-rearing, 3=fledging), sex (0=male, 1=female), nesting colony (FR, MI), and moon phase (0=less than half full, 1=greater than or equal to half full) were analyzed as random effects to address autocorrelation. Wind speed was included as a continuous variable; however, the model wouldn't converge with weather variables included, so I omitted the weather component from this model. Individual birds were analyzed

by subject in the random statement in PROC GLIMMIX to account for individual variation; this specification also nested breeding stage, sex, and moon phase within individual birds.

In the second model, I modeled presence (event="0") as a binary response variable. Conditions (0=clear skies, 1=less than half covered skies, 2=greater than half covered skies) and moon phase (first, full, last, new) were analyzed as random effects to address autocorrelation. Individual birds were analyzed by subject in the random statement in PROC GLIMMIX to account for individual variation.

RESULTS

Colony Visits

In 2010, forty-four adult breeding cormorants were tagged, 22 each on MI and FR, and by chance the sex ratio was 1:1 on each colony. I detected cormorants more often at their nesting colonies compared to detections at other colonies in the study area, and they spent the majority of their time within the study area. Ninety-three percent (40 of 44) of cormorants visited at least two other colonies other than their nesting colony throughout the study (Figure 1). A single cormorant attended only its nesting colony, whereas two birds visited all three nearby colonies throughout the breeding season in addition to MTM (Figure 1). Only FR birds were detected at MTM; these detections occurred during the fledging stage. Fortin Rock cormorants attended their nesting colony 15% more frequently than birds nesting on Magazine Island (Figure 2). The proportion of time FR birds spent at other colonies decreased with distance from the nesting colony (17% at DR, 4% at MI, 1% at RR, less than 1% at MTM), whereas MI cormorants' visits to nearby colonies had no relationship with distance from their nesting colony (3% at FR, 3% at RR, 21% at DR; Figure 2).

GLMM 1

Nesting Colony

The GLMM output including numerator and denominator degrees of freedom, *F* statistics and corresponding *p*-values of the GLMM 1 are given in Table 3. Cormorant detections at nesting colonies occurred in a bimodal pattern with peaks at dawn and dusk (Figure 3). I found no significant difference in overall attendance patterns between birds nesting on FR and MI ($F_{1,40} = 0.94$, $p = 0.3393$; Figure 3).

Breeding Stage

Attendance patterns differed significantly between breeding stages ($F_{2,68} = 22.09$, $p < 0.0001$; Figure 4). Cormorants attended their nesting colonies most often during the incubation stage followed by near equal colony attendance during the chick-rearing and fledging stages (Figure 4). Visits to MTM Aquaculture occurred with highest proportion during the fledging stage (Figure 5). I logged a single FR male and FR female cormorant at MTM Aquaculture facility late in the fledging stage for a number of days before they left the area for the season. Furthermore, in the fledging stage of a pilot study in 2009, one adult radio-tagged cormorant was killed during culling operations at MTM, while one other 2009-tagged cormorant carcass was recovered elsewhere near Lake Wolsey on Manitoulin Island, Canada.

The highest proportion of unknown locations for FR and MI cormorants occurred during the chick-rearing stage (Figure 6). Cormorants spent overnight periods at nearby colonies, i.e., DR and RR colonies, throughout the breeding season (Figure 7). Doucet Rock was the most important overnight location for birds nesting on FR and MI (Figure 8). However, FR cormorants used DR the most during overnight hours in the fledging stage, whereas MI birds used DR the most during overnight hours in the incubation stage (Figure 8).

Sex

Attendance patterns between male and female cormorants did not differ significantly ($F_{1,40} = 2.87$, $p = 0.0980$; Figure 9). Females attended the breeding colony more frequently than males on average, especially during the early- to mid-morning through late-evening hours, and throughout the fledging stage (Figure 10). Male cormorants were absent more than females most frequently during the chick-rearing and fledging stages (Figure 10). Attendance patterns of male and female cormorants differed significantly by nesting colony ($F_{1,40} = 4.83$, $p = 0.0338$; Figure 11). Males and females nesting on MI spent equal amounts of time on MI. However males spent more time on FR and RR whereas females spent more time at DR when away from the nesting colony (Figure 11). Females nesting on FR attended their nesting colony 17% more frequently than males (Figure 11). FR males attended MI and RR more frequently than females when away from the nesting colony, and both males and females attended DR nearly the same amount of time, about 25% (Figure 11). A single male and female visited MTM during the fledging stage, although the female remained at MTM for a longer duration of time than the male (Figure 11).

Moon Phase

Attendance at colonies was highest during daylight hours. Differences in attendance patterns between nights where the moon was less than half full and nights where the moon was greater than or equal to half full were statistically significant ($F_{1,40} = 58.32$, $p < 0.0001$; Figure 12). Attendance by cormorants at nesting colonies was lower on nights where the moon was greater than or equal to half full (Figure 12). I found no statistically significant interaction effect between male and female cormorant attendance at their nesting colonies and moon phase ($F_{1,40} = 0.65$, $p = 0.4253$).

GLMM 2

Moon Phase

The GLMM output including numerator and denominator degrees of freedom, *F* statistics and corresponding *p*-values of the GLMM 2 are given in Table 4. Absence from the nesting colony was significantly different by moon phase ($F_{3,728} = 232.55$, $p < 0.0001$; Figure 13). Absence during First Quarter and New phases of the moon did not differ significantly from one another, however all other comparisons were significantly different (Figure 13).

Sky Conditions

Absences across all moon phases on nights with clear skies differed significantly ($F_{6,728} = 6.51$, $p < 0.0001$; Figure 14). When the sky was less than half covered by clouds, all comparisons of moon phases were significantly different with the exception of First Quarter and New phases, and Full and Last Quarter phases of the moon (Figure 14). The First Quarter and New phases when the sky greater than half covered by clouds did not differ significantly, however all other comparisons did (Figure 14).

Site Fidelity

During a pilot study in 2009, I captured and tagged 19 adult breeding cormorants; eight on MI and 11 on FR. Two birds were recovered from Manitoulin Island located 42km southeast of FR in 2009. One bird had been culled at a trout farm, MTM Aquaculture, on Lake Wolsey, while the other bird was found in a decomposed state on the shore of Manitoulin Island near MTM Aquaculture.

I detected 13 of the 17 birds (76%) tagged in 2009 during the 2010 breeding season (six were tagged on MI, seven were tagged on FR). The earliest return date I recorded was 5 May 2010. Cormorants tagged at FR in 2009 exhibited higher breeding site fidelity than those birds

tagged at MI in 2009 (Figure 15). Ninety-five percent of the detections in 2010 for cormorants tagged on FR in 2009 occurred at FR and just two percent of the detections occurred at MI, whereas only 37% of the detections in 2010 for birds tagged on MI in 2009 occurred at MI in 2010 and 60% of the detections occurred at FR in 2010 (Figure 15).

DISCUSSION

Overall, the cormorant colonies in the North Channel of Lake Huron seem to be very well connected to one another in terms of how often breeding adult cormorants visit nesting colonies other than their own nesting colony. The study provides evidence of a local-population modified after Anderson and King (2005). Cormorants attended their nesting colonies most often during the incubation stage. While there was no significant difference between males and females, females attended their nesting colonies more frequently than did males. Attendance patterns by moon phase differed significantly, with attendance higher on nights illuminated by a moon greater than or equal to half full. Finally, the site fidelity of breeding adult cormorants in the North Channel of Lake Huron was 76%. In the following paragraphs, I discuss my findings in terms of known behaviors of double-crested cormorants and other cormorant species nesting elsewhere and management implications for current and ongoing control of double-crested cormorant populations in the United States.

These findings provide evidence that the islands serving as cormorant colony sites in the North Channel of Lake Huron should be considered a "local-population" modified after Anderson and King (2005). These colonies occur at a large spatial extent thus meeting the "local-population" criteria set forth by Anderson and King (2005). Ninety-three percent of cormorants visited at least two colonies other than their nesting colony throughout the breeding season, and they spent the majority of time within the study area. The OMNR has identified 48 separate

island colonies in the North Channel of Lake Huron (Ridgway et al. 2006). Currently, the cormorant population in the North Channel of Lake Huron is not managed by the Canadian government. The numbers of nesting cormorants on each of these colonies vary from year to year and are influenced by disturbances to the colony by humans and predators, e.g. bald eagles (*Haliaeetus leucocephalus*). Duerr *et al*. (2007) found that when disturbances were combined, e.g. egg-oiling and gull egg depredation, movement to nearby colonies increased from 4 - 7% to 23 - 40%. Managers charged with managing cormorant colonies similar to those found in the North Channel of Lake Huron should consider the possibility of a local-population to maximize the effect of management efforts. A joint management plan between U.S. and Canadian governments may be most efficient in controlling cormorant populations on the Great Lakes where local-populations have the potential to span federal borders.

According to the GLMM 1, further support for a local-population is evidenced in the similar attendance patterns of cormorants nesting on FR and MI. Fortin Rock and MI are only about 6 km apart and are located near several other smaller nesting colonies. With such high degree of dispersal between colonies, it isn't surprising that behaviors of individuals on each colony were so similar.

Similar to other studies of double crested-cormorants (Léger and McNeil 1985; Coleman and Richmond 2007) and great cormorants (Johansen *et al*. 2001), cormorant attendance exhibited a bimodal pattern with peaks just after dawn and in the hours before dusk. Diel vertical migration of prey items consumed by great cormorants was a likely explanation for foraging bouts during twilight in the mornings and evenings (Johansen et al. 2001). The majority of cormorants in this study left the nesting colonies during twilight in the mornings and returned to the colony just before dark in the evenings. Timing of departures and returns correspond to

possible foraging bouts that would take advantage of prey items that use diel vertical migration as a predator avoidance strategy.

The study findings support my hypothesis that attendance patterns are influenced by the varying energetic requirements of the different breeding stages and my first prediction that attendance would be highest during the incubation stage due to the intrinsic requirements of egg incubation and nest protection. My results support findings for cormorants nesting in Vermont, USA (Duerr et al. 2012) and other species of cormorants as well (Kato et al. 2001, Gremillet et al. 2005). Cormorants nesting on Lake Champlain exhibited highest colony attendance during the incubation stage and the lowest attendance during the fledging stage (Duerr et al. 2012). Japanese cormorants (*P. filamentosus*) have fewer and shorter foraging trips during the incubation stage and increased foraging trips of longer duration during the chick-rearing stage (Kato *et al*. 2001).

The chick-rearing and fledging stage decreasing attendance patterns of this study support findings by Duerr *et al*. (2012) that showed similar decreasing attendance patterns with breeding season progression, and swimming and foraging activities of cormorants were highest during the chick-rearing and fledging stages. Similarly, Gremillet (1997) found that chick-rearing adult great cormorants in France increased the number of foraging trips per day in response to the greater demand for food by their chicks.

That cormorants visited MTM Aquaculture more frequently during the fledging stage of the breeding season supports my second prediction that the highest frequency of visits to a trout aquaculture facility would occur during the fledging stage if time spent on the colony is influenced by the varying energetic requirements of the breeding stages. During the fledging stage, adults are feeding fledged chicks, teaching chicks how to acquire prey items, and

preparing for the impending southern migration. Duerr *et al*. (2012) found that foraging distances from nesting colonies were highest during the "post-breeding stage" which overlaps with the fledging stage of this study. Adult cormorants have about three weeks after they're independent from their chicks to increase body mass for migration (Nelson 2005). The MTM Aquaculture trout facility provides an area of high fish concentrations both inside the net pens and outside the net pens where wild fish congregate to forage on pelleted food not consumed by the trout. Trout aquaculture facilities throughout the North Channel of Lake Huron are likely attractive foraging locations for cormorants to quickly replenish fat stores during pre- and post-migration staging. That two of 44 tagged cormorants in my study visited MTM during the fledging stage, two 2009-tagged cormorants were recovered during the fledging stage in 2009 either at or near MTM, and the culling logs of MTM indicate cormorant numbers at the aquaculture facility peak during pre- and post-migration, there is evidence that aquaculture facilities likely influence cormorant movements in the North Channel of Lake Huron.

Cormorants' visits to nearby colonies during overnight hours might be explained by the requirements of each breeding stage. Care of chicks during the fledging stage is generally given be a single parent (Léger and McNeil 1985). Incubation requires only one parent present at a nest; however, both parents participate in chick-rearing duties. Chicks are brooded for 2 to 3 weeks (Nelson 2005) thereafter the parents generally only return to the colony to feed chicks and brood chicks overnight (personal observation). Chick-rearing is a particularly tense time for parents due to the constant mobbing of parents by hungry chicks. Therefore as these data show, it is advantageous for one parent to roost elsewhere overnight (personal observation).

The findings of this study are congruent with Léger and McNeil (1985); they found no significant difference in the time male and female cormorants spent attending nests or feeding

chicks in Quebec, Canada. I found male cormorants were absent on average more than females during the chick-rearing and fledging stages, which also supports Léger's and McNeil's (1985) study that at about 5 weeks post hatching male cormorants assume the role of feeding and rearing chicks. Male cormorants in this study may have the responsibility of procuring meals for chicks and teaching chicks how to fish. My findings are in contrast of studies of blue-eyed shags (*P. atriceps*), a close relative of double-crested cormorants, where Bernstein and Maxson (1984) and Wanless *et al*. (1995) reported sexually distinct colony attendance. Bernstein and Maxson (1984) found that males attended nests from 0:00-12:00 hours while females were away foraging and vice versa when females attended nests. Sexually distinct patterns were observed colony-wide for all breeding stages of blue-eyed shags (Bernstein and Maxson 1984). This study found evidence of possible synchronized nest care shift changes during the incubation stage at roughly 06:00 when females' attendance decreased and males attended the colony with higher frequency (Figure 10).

The differences in attendance I found between male and female cormorants at their respective nesting colonies may be an effect of the APMV-1 outbreak on MI. I observed in a concurrent study an outbreak of APMV-1on MI and isolated APMV-1 from chicks hatched on the island (See Chapter 1). Exposure of adults to an active APMV-1 outbreak could have altered colony attendance behaviors whereby males and females perceived decreased nesting success and prospected for new nesting sites.

The results of this study do not support my third prediction that cormorants would leave the colony more often on moon illuminated nights when the moon was at least half full in order to supplement their own energetic demands during the breeding season, in fact, just the opposite was true. It is possible that if cormorants are foraging nocturnally, that they avoid foraging on

moonlit nights due to decreased prey availability in response to higher light conditions as Klomp and Furness (1992) found in Cory's shearwaters (*Calonectris diomedea*) nesting in the Azores.

A few birds did leave their nesting colonies during overnight hours and were not detected on other colonies, therefore the possibility of nocturnal foraging cannot be completely discounted. Coleman and Richmond (2007), too, found evidence of possible nocturnal foraging in a few birds involved in their study. Again, many of the prey items commonly found in cormorants' diets in the North Channel of Lake Huron use DVM as a predator avoidance strategy (Janssen and Brandt 1980, Bur et al. 1997, Dopazo et al. 2008). It is possible that cormorants have the ability to rely on tactile prey detection given the findings of close-quarter prey detection in great cormorants (Martin et al. 2008), and the density in which the round goby, a stationary predator, occur in the Great Lakes (Jude 1997, White et al. 2008). Additionally, King *et al*. (1998) observed at night a flock of cormorants foraging at the surface on shad (*Dorosoma* spp.) in an oxbow lake on the Mississippi River. Rock shags and blue-eyed shags left their nests at night for intervals of time consistent with those recorded for diurnal foraging trips of the respective species (Sapoznikow and Quintana 2002). Additionally, most of these nights were moon illuminated and thus were bright enough to allow for foraging as shown by Wanless *et al*. (1999) in a study of European shags and blue-eyed shags. Whether cormorants in the current study were foraging nocturnally is beyond the scope of this study as I did not conduct diet analysis nor did I follow cormorants to nocturnal forage areas. The skill and efficiency with which cormorants procure prey items has been demonstrated in several studies; I believe that foraging in low light conditions is not beyond the skill of cormorants nesting in the North Channel of Lake Huron and warrants further investigation.

Cormorants in this study exhibited similar breeding site fidelity as other cormorants and shags (Aebischer et al. 2008, Scherr et al. 2010). A study of cormorants nesting in Georgian Bay, Lake Huron, Canada revealed breeding site fidelity was 75 percent, nearly the same as the findings of the current study of 76 percent. European shags nesting in the UK had similar nesting site fidelity (76%) over a three year study (Aebischer et al. 2008). In both studies, cormorants and shags that failed to return to a nesting colony in subsequent years chose sites nearby that had been occupied by cormorants or shags in the prior year, yet further support for a local-population. Active population management in areas where many cormorant colonies exist in clusters, such as the ones in the North Channel of Lake Huron, should consider cormorants' propensities to return to nesting sites or active sites close to previous nesting sites even in the wake of active disturbance.

In summary, my findings suggest that cormorant nesting in the North Channel of Lake Huron have high nesting site fidelity and colonies are well-connected to one another with frequent movement of individuals between colonies, thus providing support for a local-population. Efforts to control cormorant populations should carefully consider the possibility of a lake-wide, and perhaps Great Lakes Basin-wide, local-population where management actions that occur at a single colony will likely push cormorants to other colonies or islands that have been used as nesting colonies by cormorants in recent history. Furthermore population control efforts in U.S. waters may disperse breeding cormorants to active colonies in Canada. A joint effort between U.S. and Canadian governments may prove most effective in curbing human-cormorant conflicts. Whether cormorants forage nocturnally in the Great Lakes is an area that warrants further study. Aquaculture facilities in the North Channel of Lake Huron appear to be important foraging areas during pre- and post-migration, and influence cormorant behavior and

movements. I documented that cormorants nesting in the North Channel of Lake Huron have variable attendance patterns that are most influenced by the intrinsic energetic requirements of each breeding stage.

APPENDIX

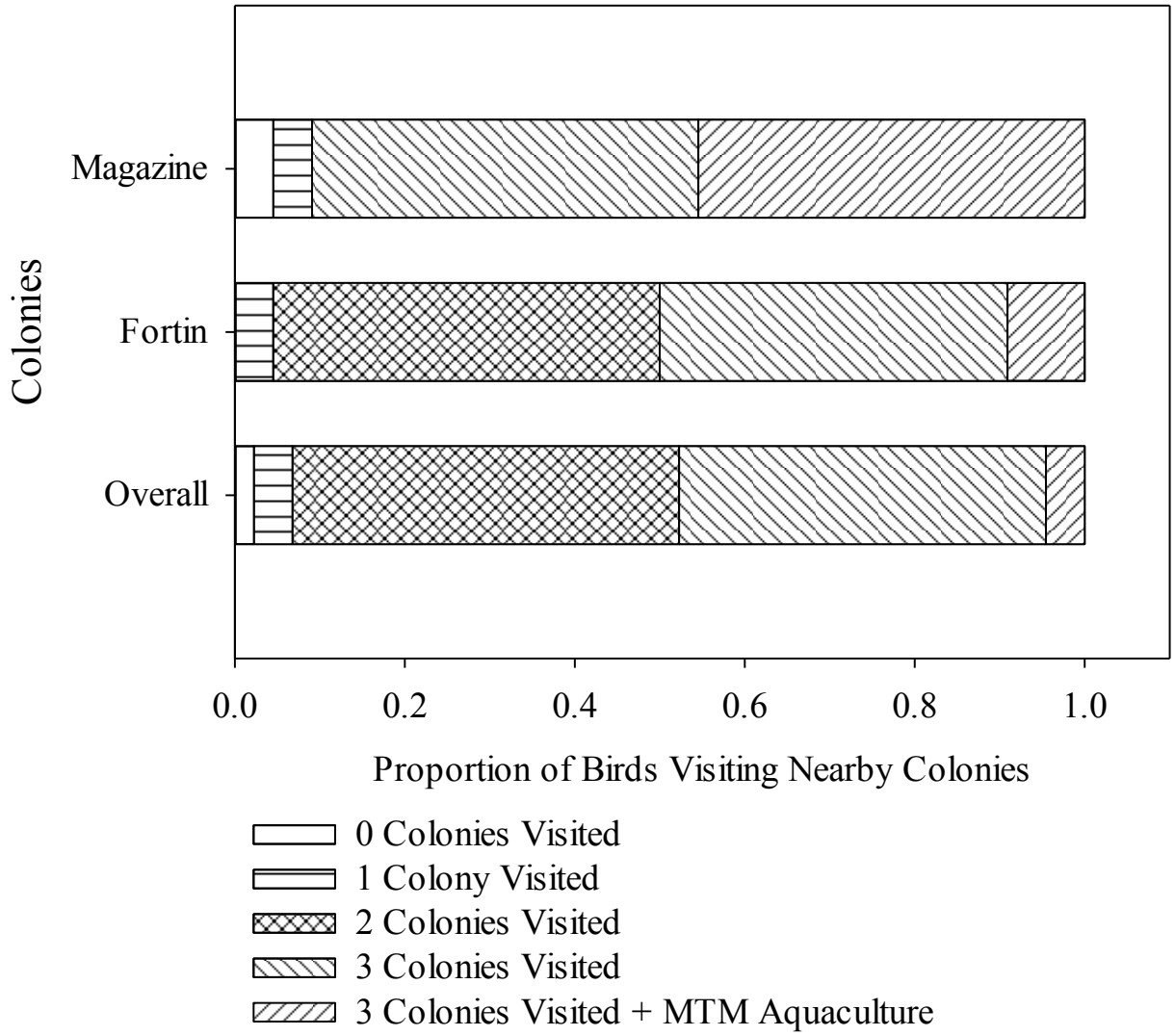

Figure 1. Proportion of double-crested cormorants visiting nearby colonies during 2010 breeding season in the North Channel of Lake Huron, Canada.

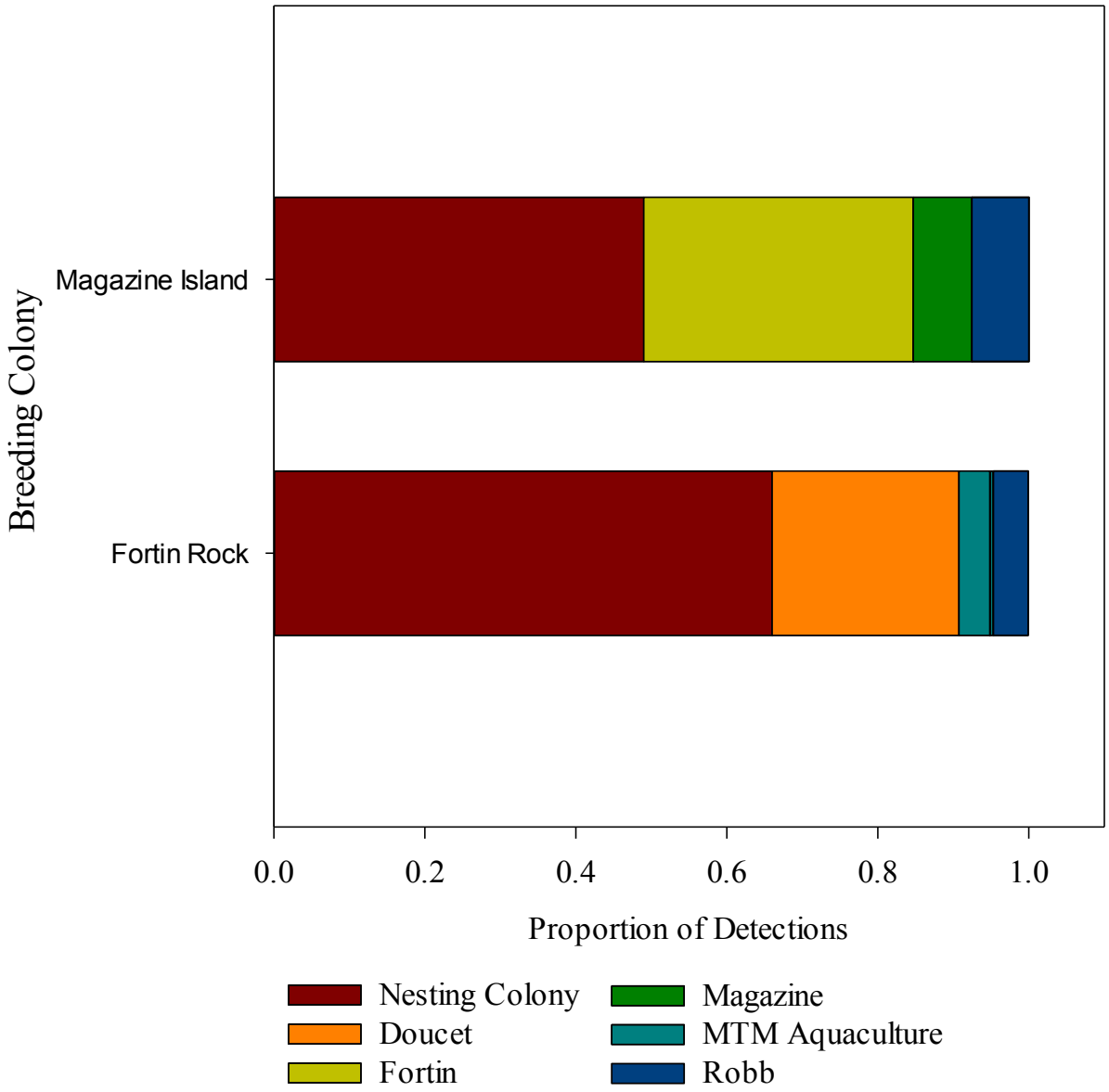

Figure 2. Proportion of detections of nesting double-crested cormorants at Fortin Rock and Magazine Island, North Channel of Lake Huron, Canada, from May to September 2010. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Table 3. Output of the final GLMM for predicting breeding adult double-crested cormorant colony attendance in the North Channel of Lake Huron, Canada, from May to September 2010, by nesting colony, sex, breeding stage and moon phase. Values provided in bold indicate the final model.

Breeding adult cormorants	Numerator Degrees of Freedom	Denominator Degrees of Freedom	*F*	*P*
Nesting colony	**1**	**40**	**0.94**	**0.3393**
Sex	**1**	**40**	**2.87**	**0.0980**
Nesting colony × sex	**1**	**40**	**4.83**	**0.0338**
Breeding stage	**2**	**68**	**22.09**	**<0.0001**
Nesting colony × breeding stage	2	68	0.14	0.8720
Sex × breeding stage	2	68	0.39	0.6796
Nesting colony × sex × breeding stage	2	68	0.80	0.4545
Moon phase	**1**	**40**	**58.32**	**<0.0001**
Nesting colony × moon phase	1	40	2.73	0.1065
Sex × moon phase	1	40	0.65	0.4253
Nesting colony × sex × moon phase	1	40	2.54	0.1186
Breeding stage × moon phase	2	65	84.53	<0.0001
Nesting colony × breeding stage × moon phase	2	65	31.94	<0.0001
Sex × breeding stage × moon phase	2	65	19.11	<0.0001
Nesting colony × sex × breeding stage × moon phase	2	65	50.60	<0.0001

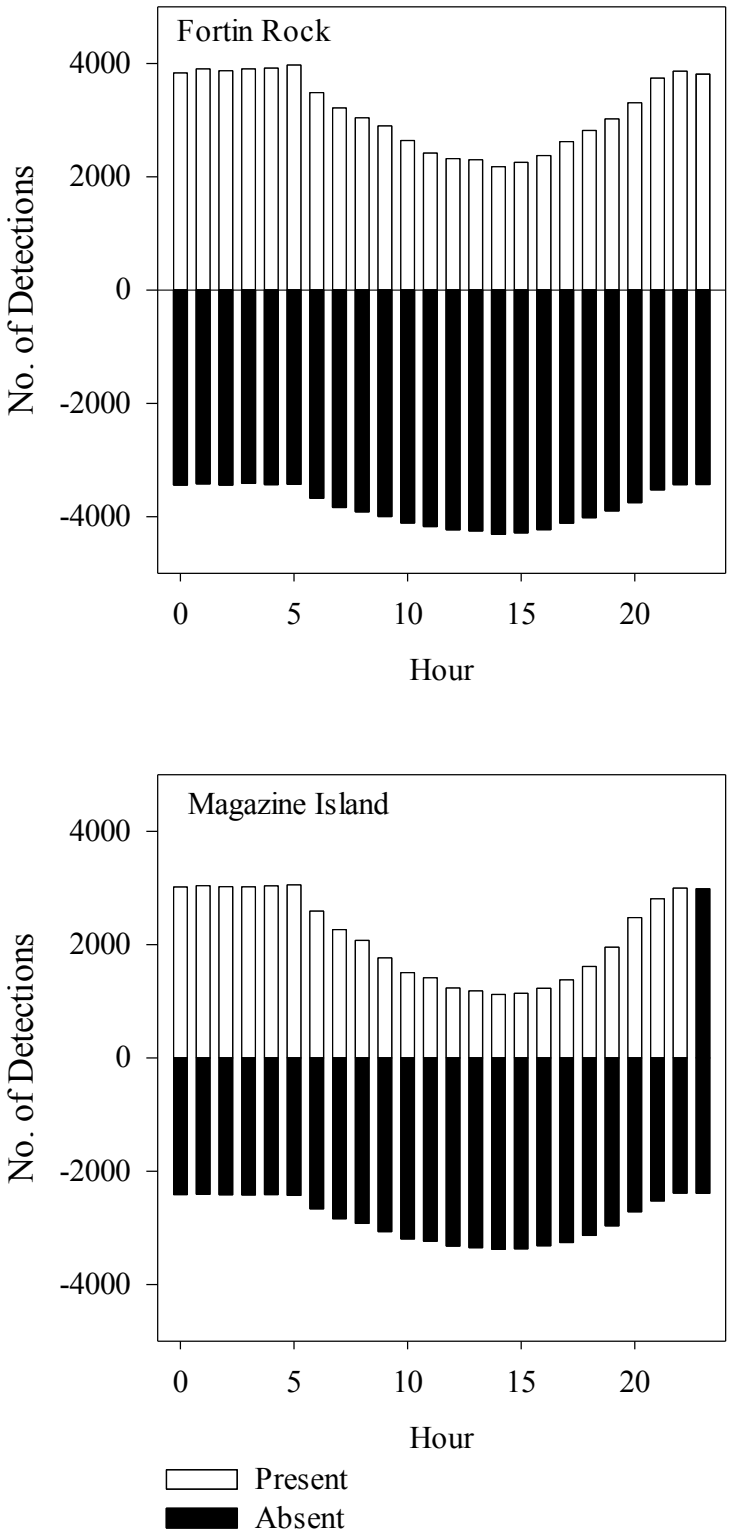

Figure 3. Double-crested cormorant nesting colony attendance at Fortin Rock and Magazine Island, North Channel of Lake Huron, Canada, from May to September 2010.

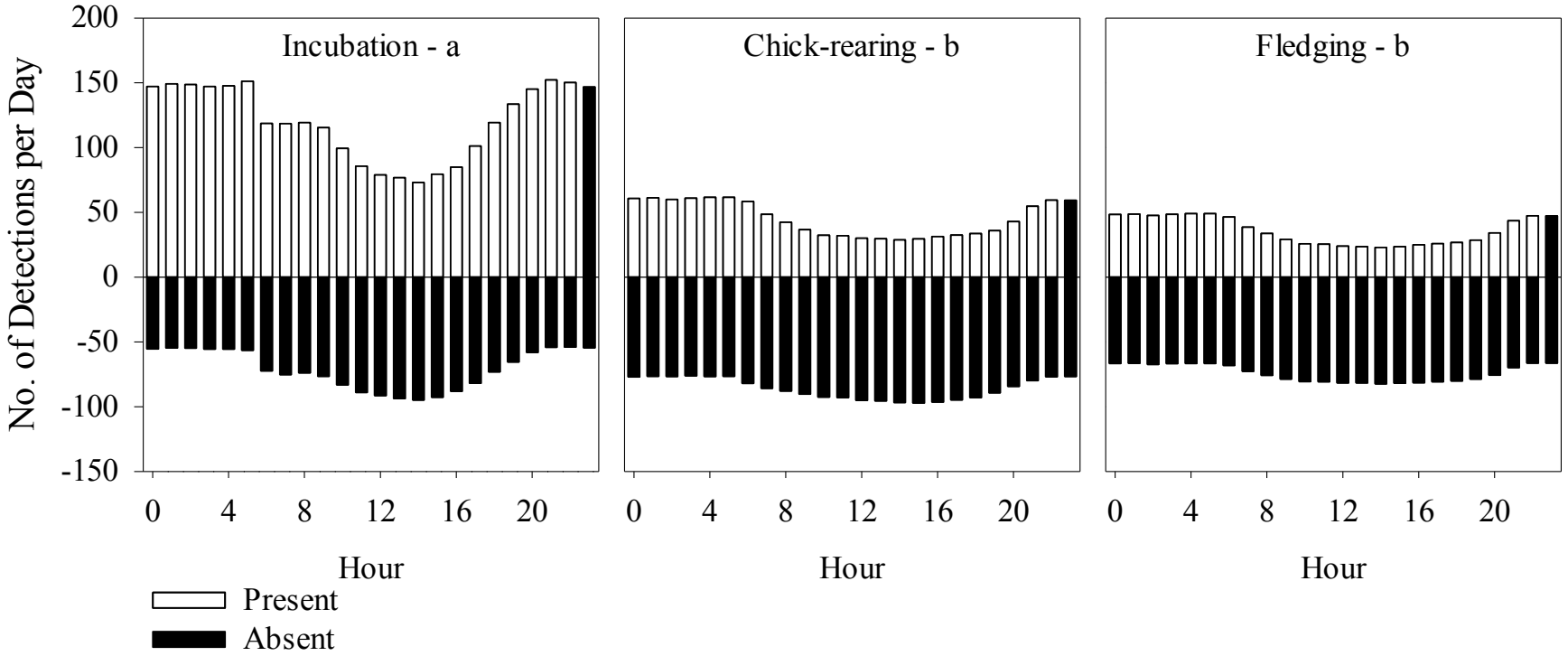

Figure 4. Colony attendance by double-crested cormorants nesting at Fortin Rock and Magazine Island, North Channel of Lake Huron, Canada, from May to September 2010.

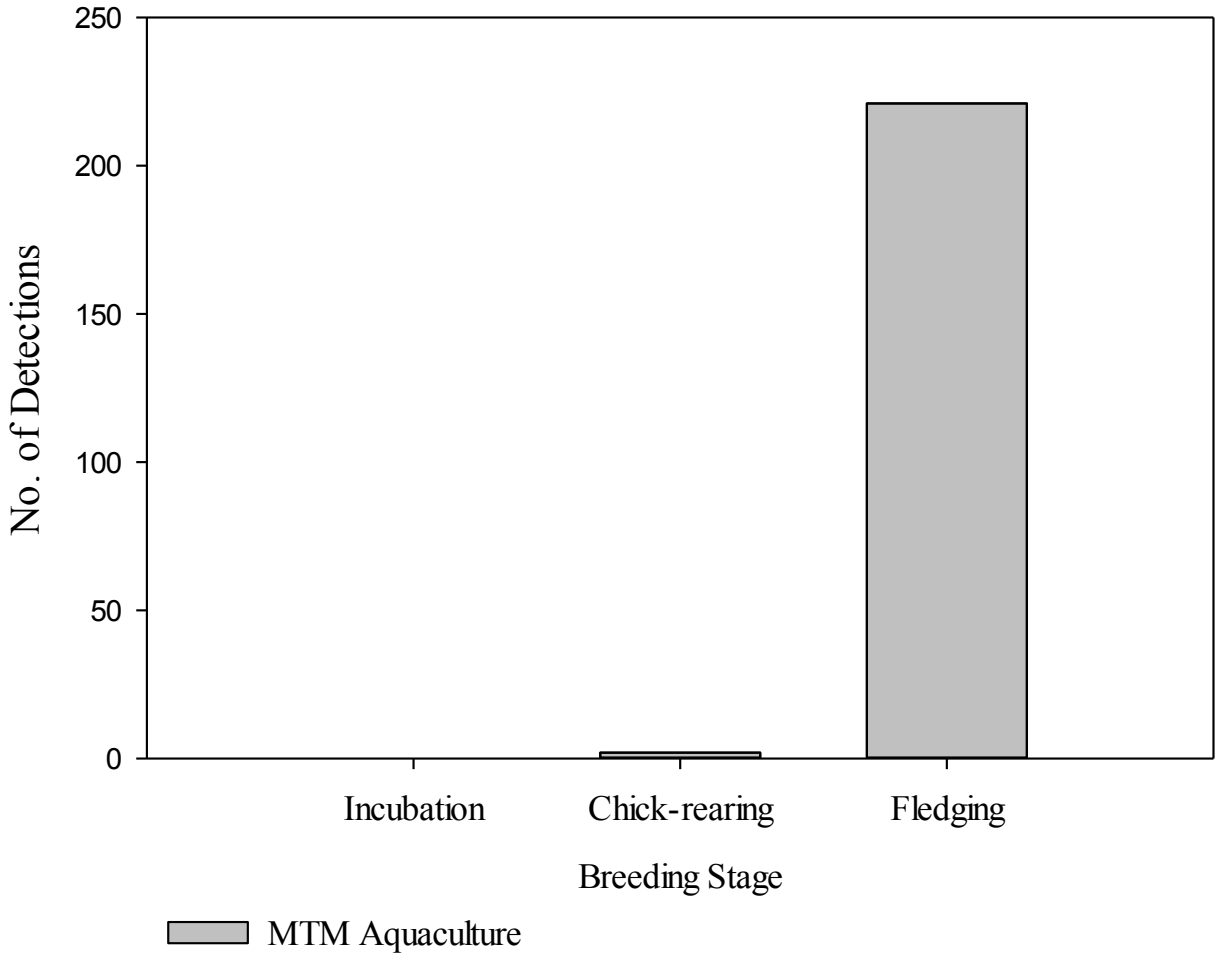

Figure 5. Visits to MTM Aquaculture facility by double-crested cormorants nesting on Fortin Rock, North Channel of Lake Huron, Canada, during each breeding stage from May to September 2010.

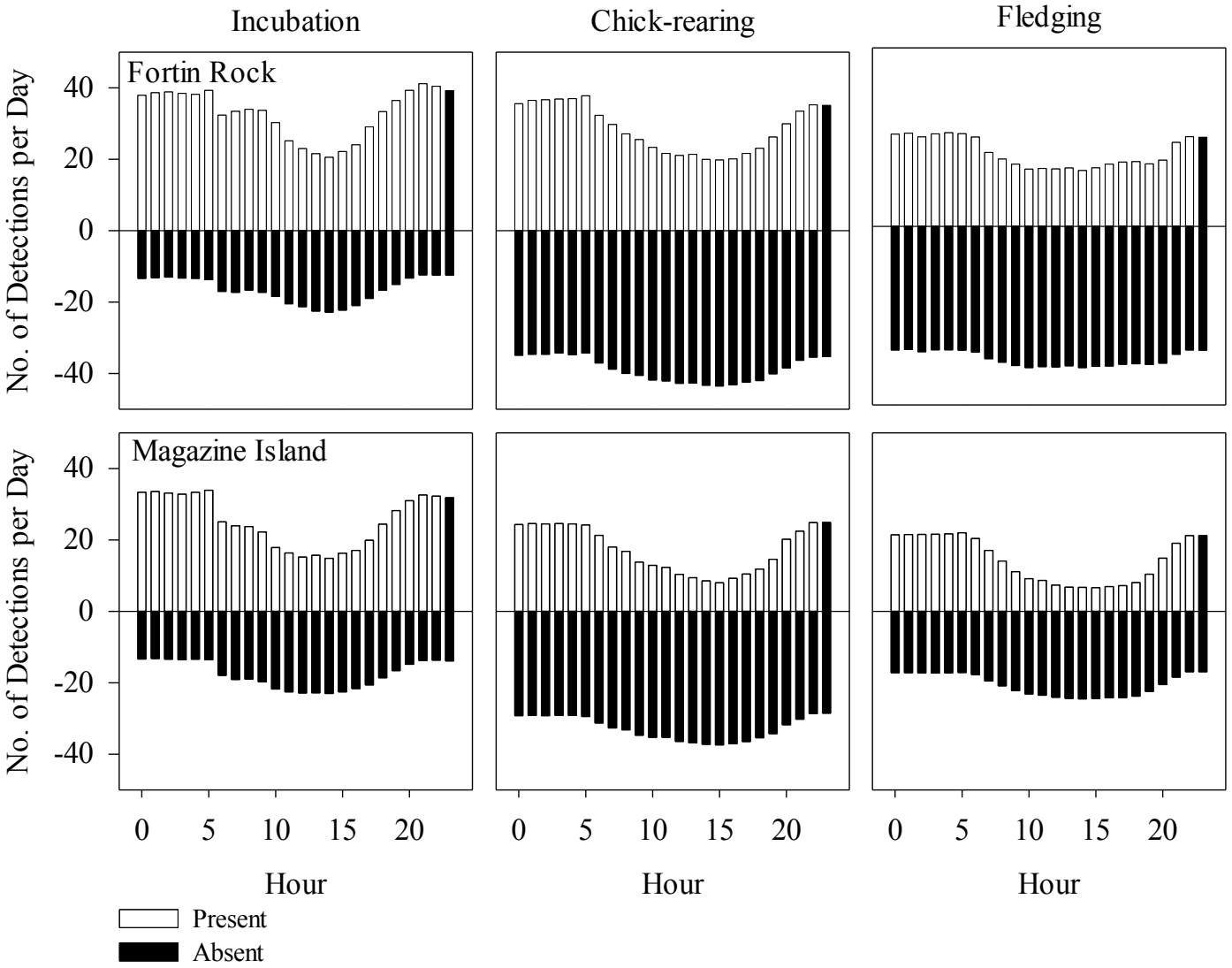

Figure 6. Daily detections per hour of double-crested cormorants at Fortin Rock and Magazine Island breeding colonies in the North Channel of Lake Huron, Canada from May to September 2010.

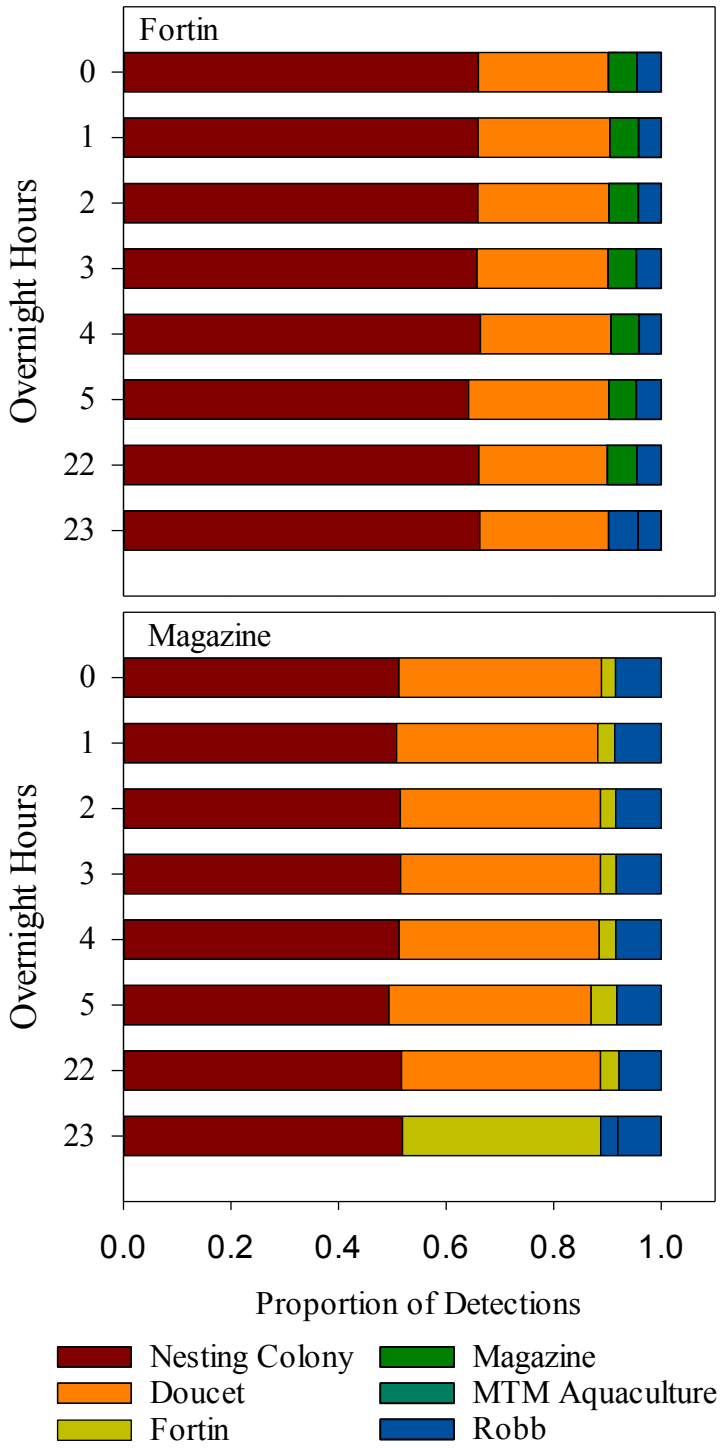

Figure 7. Proportion of overnight hourly detections of double-crested cormorants nesting on Fortin Rock and Magazine Island, North Channel of Lake Huron, Canada from May to September 2010.

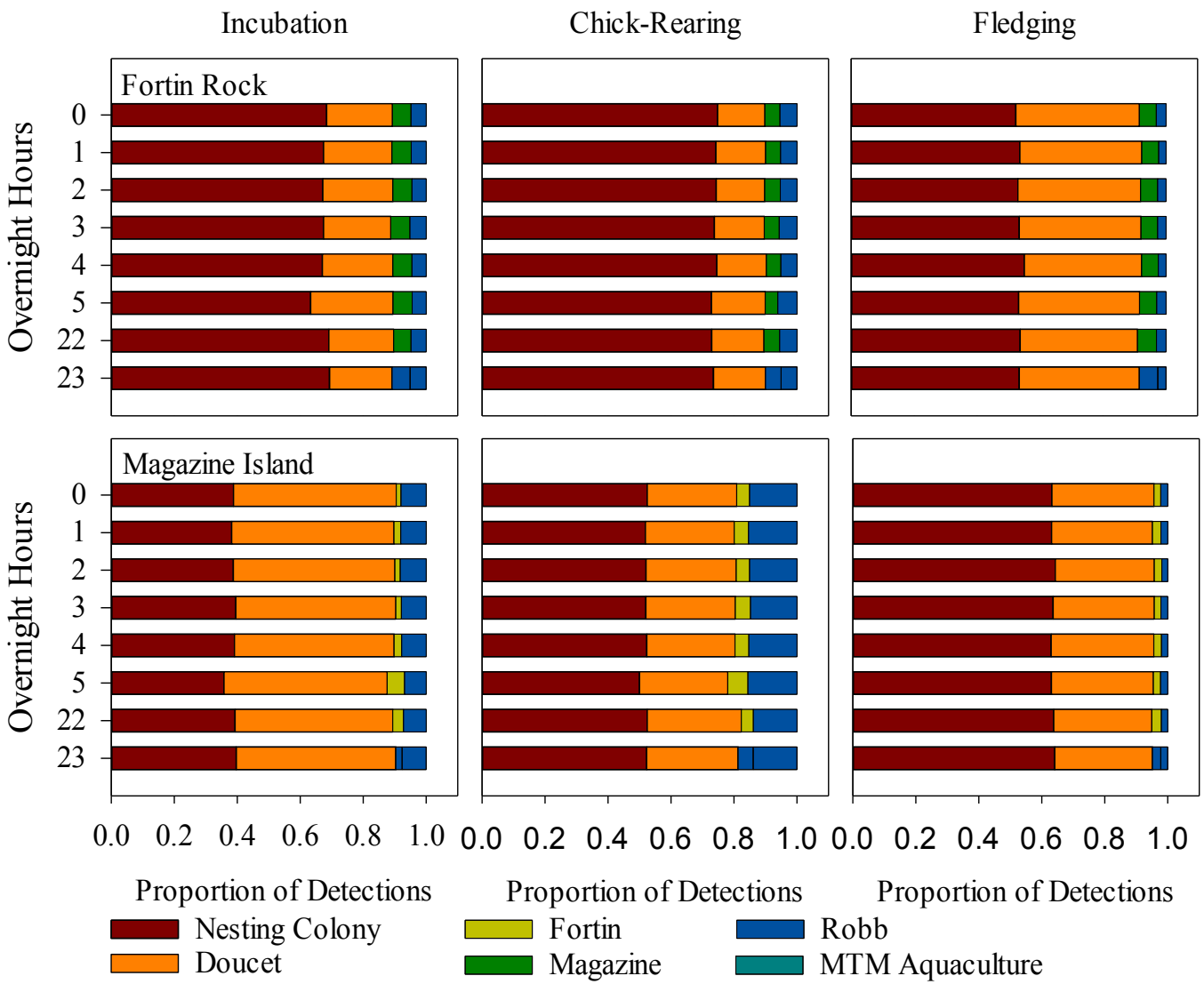

Figure 8. Proportion of overnight hourly detections of double-crested cormorants at Fortin Rock and Magazine Island breeding colonies and nearby colonies in the North Channel of Lake Huron, Canada from May to September 2010.

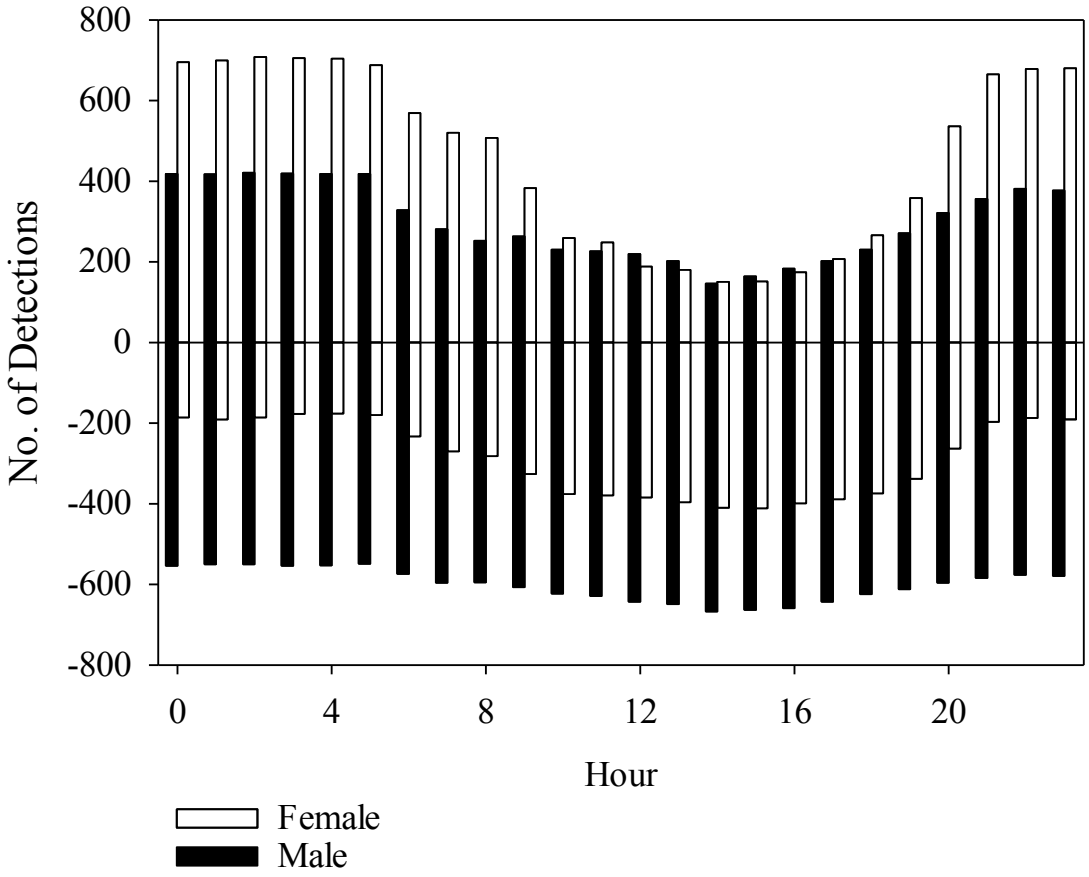

Figure 9. Overall, female double-crested cormorants nesting in the North Channel of Lake Huron, Canada from May to September 2010, attended their nesting colonies more frequently than males, though this difference was not statistically significant.

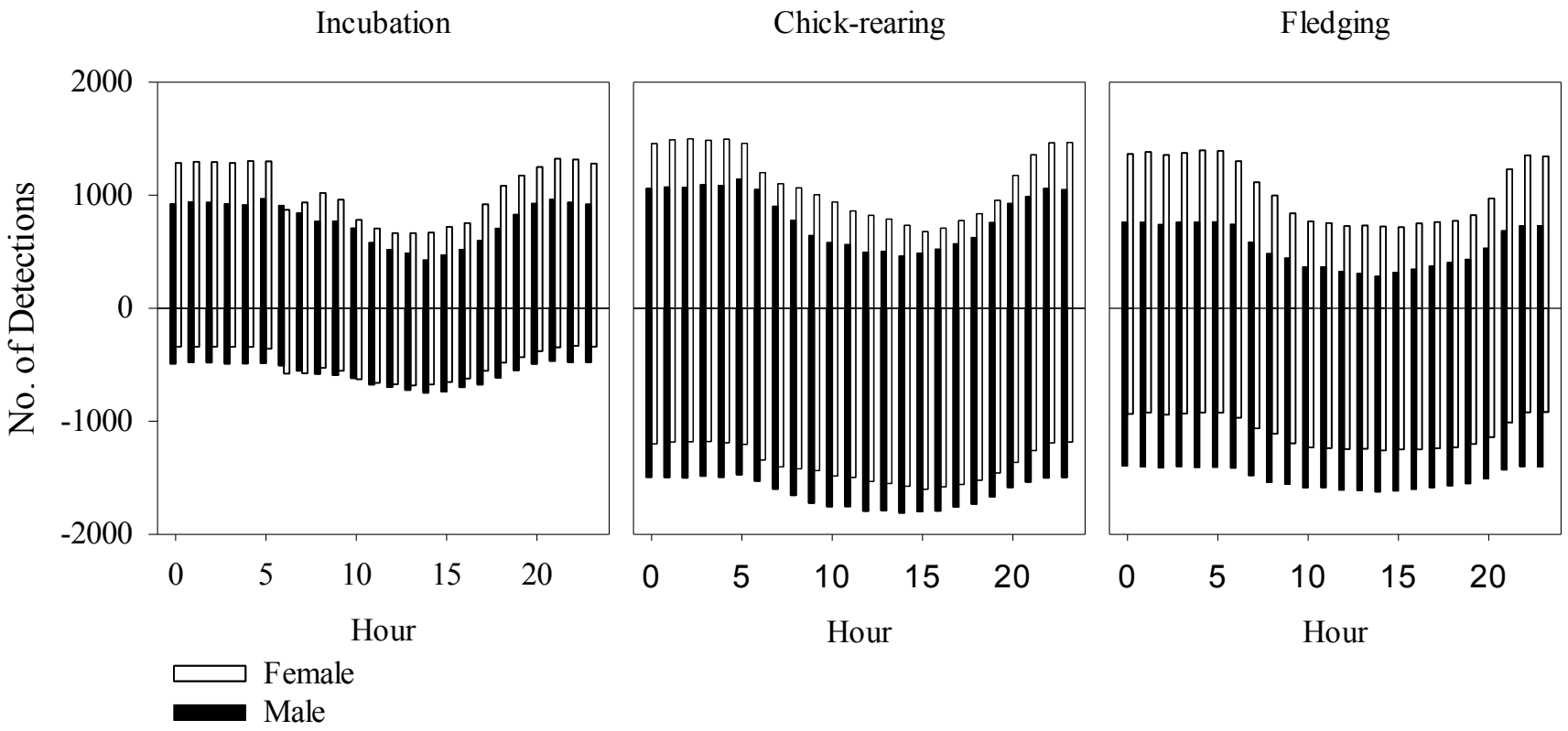

Figure 10. Attendance patterns of male and female double-crested cormorants nesting in the North Channel of Lake Huron, Canada, from May to September 2010, did not differ significantly by breeding stage.

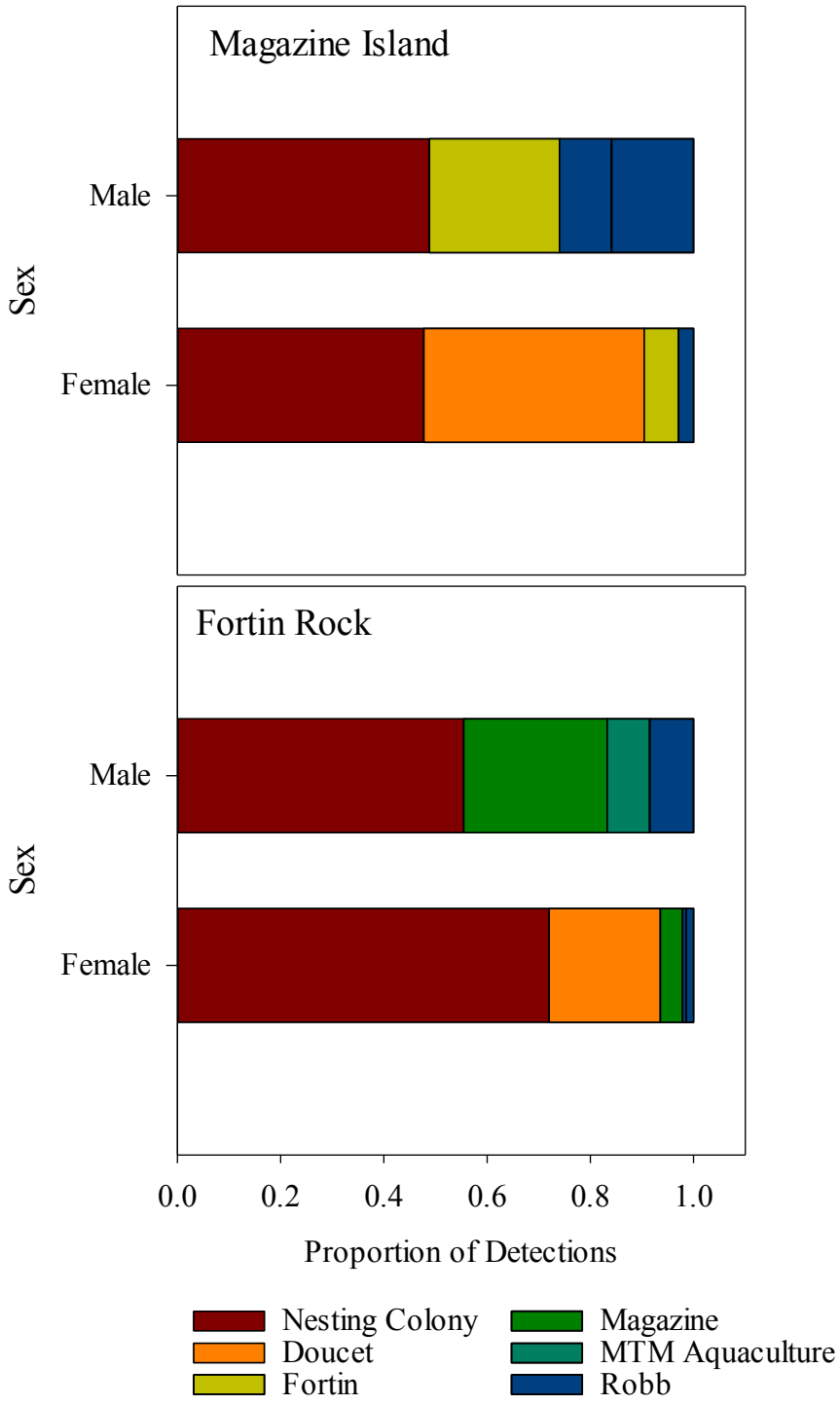

Figure 11. Attendance patterns of male and female double-crested cormorants nesting in the North Channel of Lake Huron, Canada, from May to September 2010, differed significantly by nesting colony.

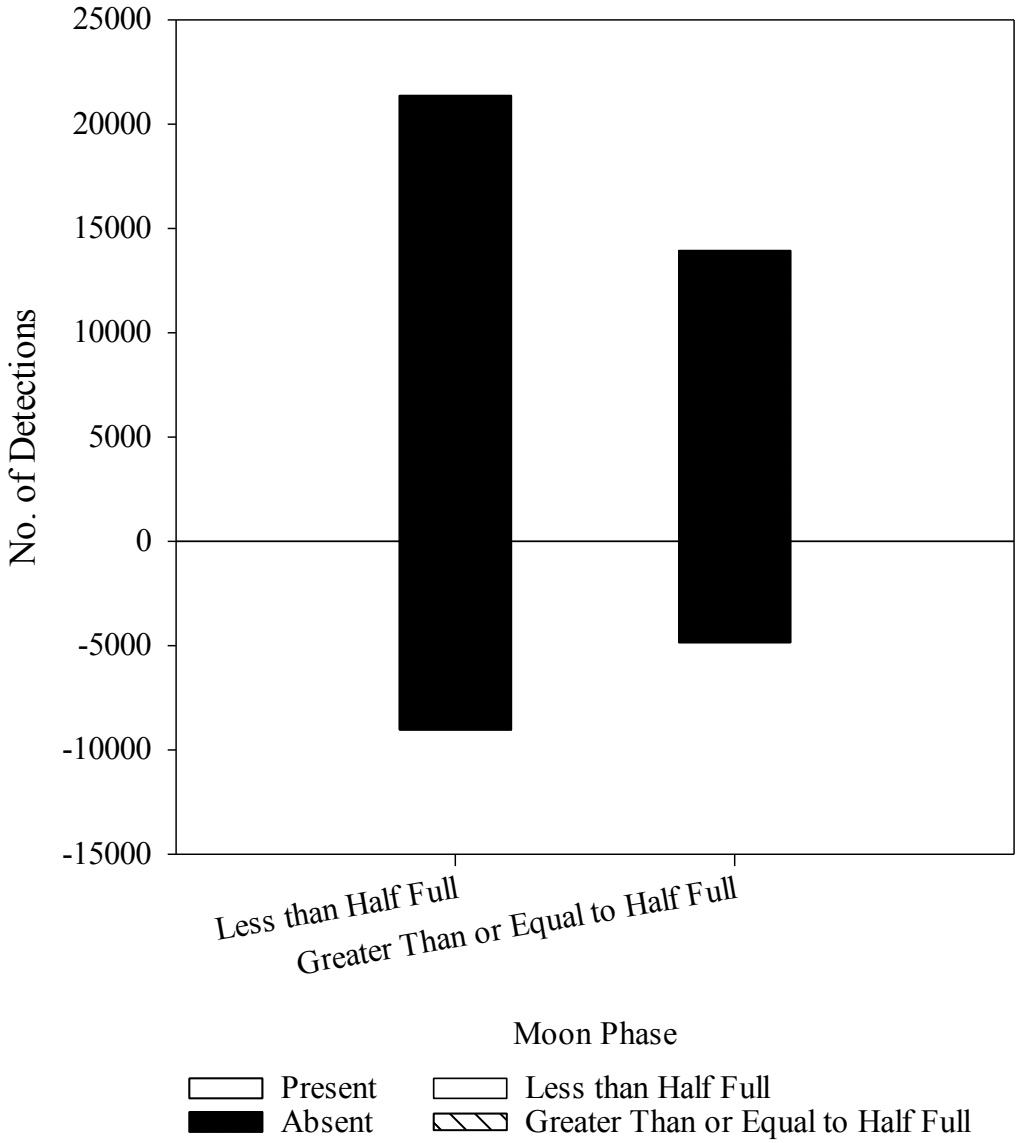

Figure 12. Differences in detections of nesting double-crested cormorants in the North Channel of Lake Huron, Canada, from May to September 2010, between nights when the moon is less than half full and greater than or equal to half full differed significantly.

Table 4. Output of the final GLMM for predicting breeding adult double-crested cormorant absence in the North Channel of Lake Huron, Canada, from May to September 2010, by moon phase and sky conditions. Values provided in bold indicate the final model.

Breeding adult cormorants	Numerator Degrees of Freedom	Denominator Degrees of Freedom	*F*	*p*
Moon phase	**3**	**728**	**232.55**	**<0.0001**
Conditions	2	364	0.96	0.3830
Moon phase × conditions	**6**	**728**	**6.51**	**<0.0001**

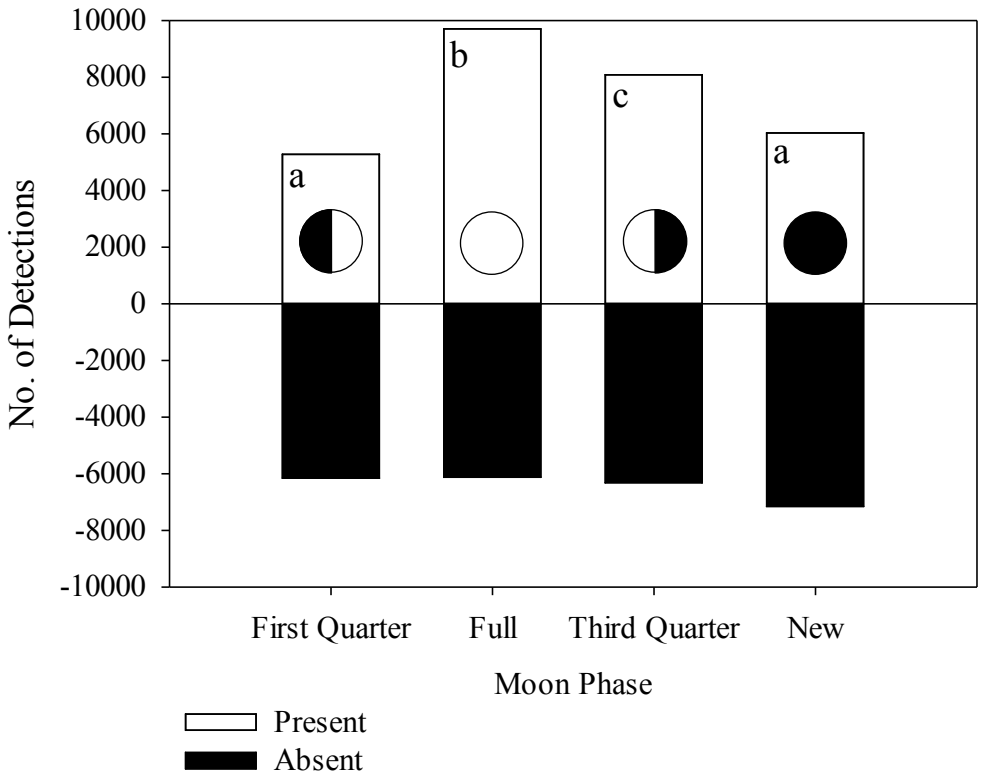

Figure 13. Differences in nightly detections of nesting double-crested cormorants in the North Channel of Lake Huron, Canada, from May to September 2010, by moon phase differed significantly. Lower case letters indicate statistically significant differences.

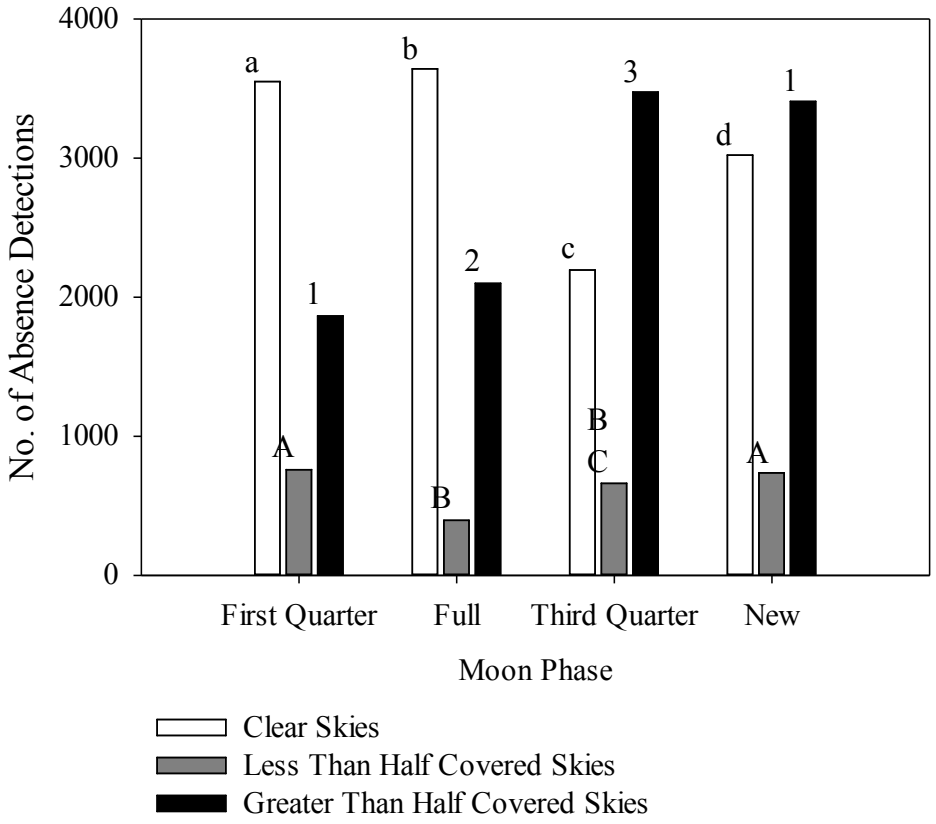

Figure 14. Differences in nightly detections of nesting double-crested cormorants in the North Channel of Lake Huron, Canada, from May to September 2010, by moon phase and sky conditions differed significantly. Lower case letters indicate statistically significant differences between moon phases during Clear Skies. Upper case letters indicate statistically significant differences between moon phases during Less Than Half Covered Skies. Numbers indicate statistically significant differences between moon phases during Greater Than Half Covered Skies.

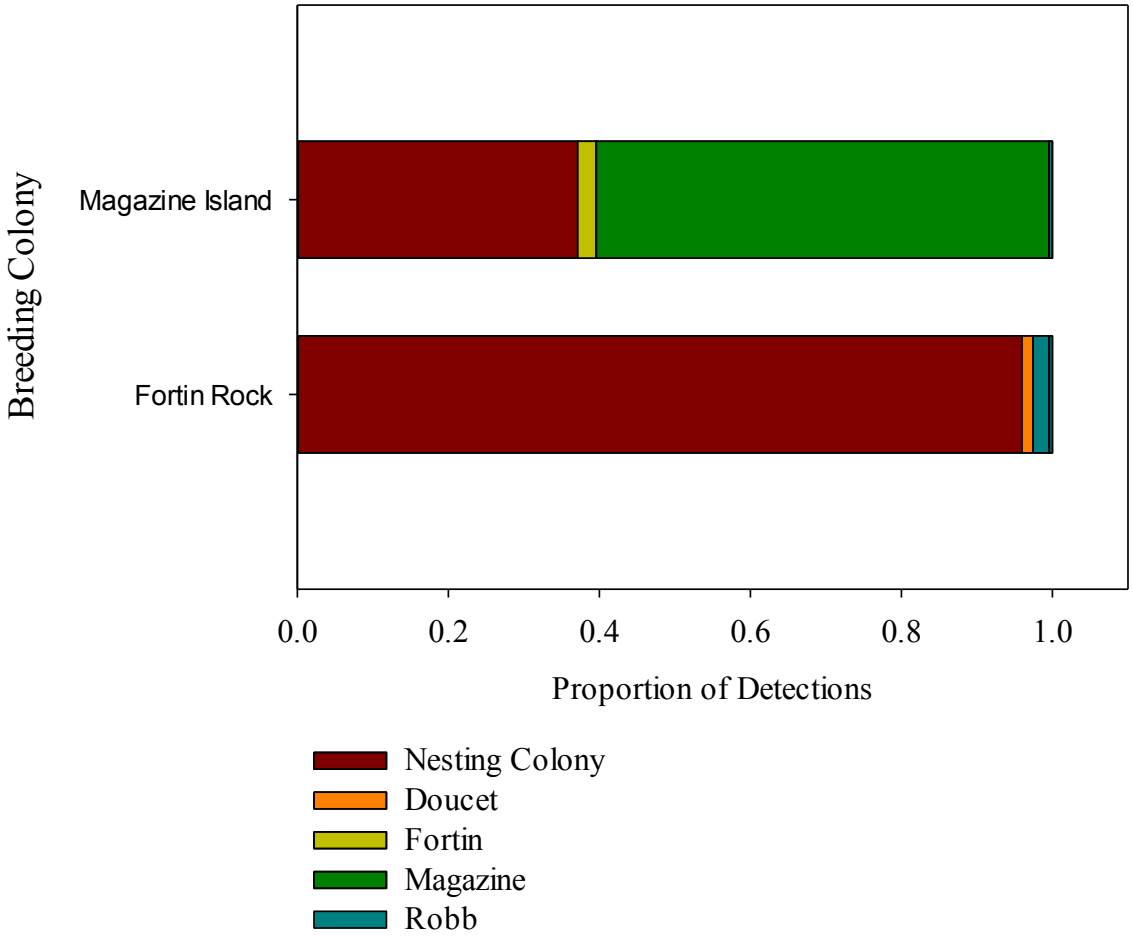

Figure 15. Proportion of detections of 2009 tagged double-crested cormorants (n=6 each at each colony) at Fortin Rock and Magazine Island nesting colonies and other colonies in the North Channel of Lake Huron, Canada, from May to September 2010.

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