2012

A MOLECULAR GENETIC ASSESSMENT OF SEX RATIOS FROM BREEDING, MIGRATORY AND OVERWINTERING COMMON LOONS

Abigail Leigh Debiak
Northern Michigan University

Follow this and additional works at: https://commons.nmu.edu/theses

Recommended Citation
https://commons.nmu.edu/theses/379
A MOLECULAR GENETIC ASSESSMENT OF SEX RATIOS FROM BREEDING, MIGRATORY AND OVERWINTERING COMMON LOONS

By

Abigail Leigh Debiak

THESIS

Submitted to
Northern Michigan University
In partial fulfillment of the requirements
For the degree of

MASTER OF SCIENCE

Office of Graduate Education and Research

2012
This thesis by Abigail Leigh Debiak is recommended for approval by the student’s thesis committee in the Department of Biology and by the Assistant Provost of Graduate Education and Research.

Committee Chair: Dr. Alec Lindsay

First Reader: Dr. Katherine Teeter

Second Reader: Dr. Kurt Galbreath

Third Reader: Dr. John Bruggink

Department Head: Dr. John Rebers

Asst Provost of Graduate Education and Research: Dr. Brian Cherry
In order to catalog your thesis properly and enter a record in the OCLC international bibliographic data base, Olson Library must have the following requested information to distinguish you from others with the same or similar names and to provide adequate subject access for other researchers.

<table>
<thead>
<tr>
<th>NAME:</th>
<th>Debiak</th>
<th>Abigail</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Last)</td>
<td></td>
<td>(First)</td>
<td></td>
</tr>
<tr>
<td>(Middle initial)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DATE OF BIRTH:</th>
<th>June</th>
<th>9</th>
<th>1987</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Month)</td>
<td></td>
<td>(Day)</td>
<td>(Year)</td>
</tr>
</tbody>
</table>
ABSTRACT

A MOLECULAR GENETIC ASSESSMENT OF SEX RATIOS FROM BREEDING, MIGRATORY AND OVERWINTERING COMMON LOONS

BY

ABIGAIL LEIGH DEBIAK

Biased sex ratios have been documented in several species of birds and mammals and have been attributed to numerous environmental, physiological, and behavioral factors. Isolating associated factors is often difficult, as sex ratios can vary dramatically among species as well as between different populations of the same species. Moreover, the degree to which juvenile sex ratios influence adult sex ratios is often complicated by factors including dispersal and sex-biased mortality incurred post-fledging. I used a PCR-based sex identification technique to evaluate juvenile sex ratios within breeding populations of common loons (Gavia immer) in Michigan (Seney National Wildlife Refuge – SNWR, and Isle Royale National Park - ISRO) and northern Wisconsin, as well as among loons killed in the Great Lakes during migration and among loons collected at overwintering sites off the Florida coast. Results of these analyses reveal a non-significant though shared trend of proportionally more male juveniles fledged from all three breeding populations. More female birds were represented in the overwintering sample, although also not significantly so, and sex ratios of birds killed on migration were at parity. I tested whether several parental and territory quality characters influenced chick sex at SNWR, but none were significantly correlated with offspring sex. Additionally, at SNWR, the male-bias recorded at fledging was not strong enough to account for the significant male-bias in adult return rates that has been observed at the refuge since 1998.
The completion of this project is thanks to the advice, expertise, and assistance of many. I primarily thank my thesis advisor and committee chair, Dr. Alec Lindsay for his consistent support, guidance, and endless patience. Moreover, his contributions to the molecular and statistical aspects of this project were essential to its success. I would also like to gratefully acknowledge the rest of my thesis committee: Dr. Katherine Teeter, Dr. Kurt Galbreath, and Dr. John Bruggink and thank them for their support and for reviewing this work.

Damon McCormick, Joe Kaplan, and Keren Tischler of Common Coast Research and Conservation were instrumental to this project, and I am extremely grateful to them for providing many of the blood and tissue samples I used, years worth of field data from Seney National Wildlife Refuge, as well as their expert advice along the way.

I would also like to thank all past and current members of the Lindsay Lab for their support, assistance, and accommodation throughout the duration of this project. Thanks also to Northern Michigan University and the Department of Biology for financial support through an Excellence in Education Grant as well as to the College of Arts and Sciences for funding travel costs to present this work at the 2012 North American Ornithological Conference in Vancouver, Canada.
I would finally like to recognize my family, friends, and fellow graduate students for their assistance, encouragement, and unwavering moral support. Thank you for bearing with me in times of frustration and providing inspiration everyday.

This thesis follows citation guidelines recommended by the Ecological Society of America’s journal Ecology. Instructions for authors submitting articles to this journal can be found at http://esapubs.org/esapubs/preparation.htm.
## TABLE OF CONTENTS

List of Tables .................................................................................................................... vii
List of Figures .................................................................................................................... viii

Introduction to Common Loon Biology .............................................................. 1
  Populations and Habitat Selection ................................................................. 2
  Reproduction and Chick-Rearing ................................................................. 2
  Migration ............................................................................................................. 3
  Conservation Concerns ...................................................................................... 4
    Botulism ........................................................................................................... 4
    Mercury ............................................................................................................ 4
    Human Development and Disturbance ........................................................ 5

Molecular Genetic Studies of the Common Loon ................................................. 6
  A molecular genetic assessment of sex ratios from breeding, migratory and overwintering common loons .......................................................... 8

A molecular genetic assessment of sex ratios from breeding, migratory and overwintering common loons .......................................................... 8

Introduction ............................................................................................................. 8

Methods .................................................................................................................... 12
  Sample Collection and DNA Extraction ....................................................... 12
  PCR Amplification and Sequencing .............................................................. 13
  Statistical Analyses ......................................................................................... 14

Results ...................................................................................................................... 18

Discussion .............................................................................................................. 24

Summary and Conclusions .................................................................................... 30
Literature Cited........................................................................................................31

Appendices............................................................................................................36
LIST OF TABLES

Table 1: Chi-square goodness-of-fit test of common loon sex ratios.........................19
Table 2: Chi-square test results comparing hatch and fledge ratios at SNWR..............20
Table 3: Juvenile sex ratios observed in Wisconsin from 1999-00 and from 2003-05.................................................................20
Table 4: Linear regression results assessing the proportion of males fledged at SNWR as a function of measures of productivity and parental quality.................................................................22
Table 5: Binary logistic regression results using parental mass to explain variation in offspring sex...............................................22
LIST OF FIGURES

Figure 1: Example gel image generated from PCR amplification of common loon sex chromosomes………………………………………………………………………………..18

Figure 2: Linear regression results assessing the proportion of males fledged at SNWR as a function of measures of productivity (a-d) and parental quality (e-f)……………………………………………………………………………21

Figure 3: Monte Carlo simulation test of sex bias in ABJ return rates…………………………23
INTRODUCTION TO COMMON LOON BIOLOGY

Scientists and birders alike have long been interested in the common loon (*Gavia immer*) for its research potential, for its role as a flagship conservation species, and for its popularity as a symbol of wilderness. As a highly territorial, long-lived, top-level piscivorous species that often returns to the same pristine boreal breeding areas year after year, the common loon is viewed as a useful indicator species of regional aquatic health and overall habitat quality (Evers et al. 2010). The reliable and recurrent observability of loons on their breeding territories has led to extensive behavioral and physiological research on loons during the breeding season (Meyer et al. 1995, Piper et al. 1997, Evers et al. 1998, Meyer et al. 1998, Norcera and Taylor 1998, Kenow et al. 2003, Merrill et al. 2005, Weinandt 2006, Kenow et al. 2007, Evers et al. 2008, Piper et al. 2012), but far less is known about loon biology associated with wintering grounds and migratory stopover sites. In birds, the conservation implications of biases in sex ratios are also largely understudied, in part due to the past inability to easily assess hatching and fledging sex ratios in juvenile birds. The purpose of this study was to examine sex ratios of common loons sampled from birds of breeding, migratory, and overwintering locations in eastern North America. I compared sex ratios from each study location and assessed factors that potentially influence the sex of offspring within a breeding population. This introduction reviews prior research on common loon life history characteristics, environmental factors that influence loon conservation, and past molecular genetic studies of loons.
Populations and Habitat Selection

Because common loons are highly territorial, population densities on breeding territories are directly related to suitable habitat availability. While freshwater lakes of 24 hectares (ha) or larger are preferable for breeding pairs, Ruggles (1994) found that common loons in Alaska were nesting on lakes as small as 12 ha. Larger lakes with irregular shorelines and multiple small islands may provide enough suitable habitat to support multiple breeding pairs. As loons are ground nesters, the coves and inlets created by undulating shorelines and offshore islands provide areas with necessary nest protection from wind, waves, and certain predators (Vermeer 1973, McIntyre 1983, Evers et al. 2010). Additionally, common loons require lakes with clear water and an adequate amount of prey (McIntyre 1983, Evers et al. 2010). Unlike breeding territories, characteristics of wintering grounds are not well described. As with breeding territories, however, marine coastal bays, coves, and inlets seem to be preferable to wintering common loons (Evers et al. 2010). Haney (1990) found that loons wintering off the southeastern coast of the United States preferred less-turbid waters near shore, less than 20 meters deep.

Reproduction and Chick-Rearing

Common loons are a serially monogamous species, and mated pairs have been observed to remain together for multiple breeding seasons (Evers et al. 2010). Genetic studies have shown that extra pair copulations among the species are rare, as breeding adults remain in close proximity to one another throughout the pre-laying period (Piper et al. 1997). Nesting typically begins in early spring (April-May). Clutch sizes range from one to two eggs per nest, and re-nesting may occur if an entire clutch is lost. Incubation is performed
by both members of a mated pair and lasts for approximately 28 days. Juvenile chicks remain with at least one parental adult for 12-15 weeks post-hatch and begin their fall migration shortly following the departure of the second parent (Evers et al. 2010). While on breeding grounds, juvenile survival may be influenced directly or indirectly by factors including lake acidity, mercury exposure, and predation (Meyer et al. 1998, Merrill et al. 2005, Evers et al. 2010).

**Migration**

Common loons are considered medium-distance diurnal migrants that breed on large freshwater lakes throughout the northern United States and Canada during summer months and winter in marine habitats near the oceanic coasts (Evers et al. 2010). Although adult birds migrate twice yearly in the spring and in the fall, sub-adult birds typically remain on wintering grounds until their third spring, when they usually reach sexual maturity. Spring and fall migration for breeding adult pair members, as well as their offspring, is performed independently from one another (Evers et al. 2010). Studies that used band recoveries, tracking radar (Kerlinger 1982), and more recently satellite transmitters (Kenow et al. 2002) have provided substantial insight into migratory patterns and behaviors of the common loon. Notably, for birds breeding in eastern North America, and possibly for birds breeding in the central Canadian provinces, the Great Lakes serve as important stopover sites for migrating loons en route to and from wintering habitats along the Atlantic and Gulf of Mexico coasts (Kenow et al. 2002).
Conservation Concerns

*Botulism:* Type E botulism (*Clostridium botulinum*) is an anaerobic bacterium that thrives in freshwater, marine, and terrestrial habitats (Yule et al. 2006). Outbreaks of this bacterium were first recorded in the Great Lakes in the 1960s and have since become almost annually recurrent (Kaufmann and Fay 1964, Yule et al. 2006). Multiple genetically distinct strains of *C. botulinum* are known to exist in the sediment layers of the Great Lakes, and it is speculated that their presence may be associated with that of the zebra and quagga mussels (*Dreissena spp.*) (Yule et al. 2006, Hannett et al. 2011). The Great Lakes are an important migratory stopover site for many loons of eastern North America, and as top-level piscivores, it is likely that many of these loons become burdened with type E botulism by ingesting diseased fish (Brand et al. 1988). Once afflicted with the bacterium, paralysis of the neck muscles often results in the drowning of the diseased bird (Hannett et al. 2011). This may have a significant conservation impact for the species, as large scale outbreaks of type E botulism have resulted in die-offs of thousands of loons (Brand et al. 1988, Yule et al. 2006).

*Mercury:* Atmospheric deposition of anthropogenic mercury and the subsequent bioaccumulation through diet has a demonstrably negative impact on common loon physiology. Mercury is most commonly bioaccumulated in the form of methylmercury (MeHg) and in elevated levels is neurotoxic to the species. Several studies have indicated that MeHg loads of aquatic environments and the associated negative impacts are indirectly correlated with lake pH, and that MeHg loads are greatest in eastern North American populations (Meyer et al. 1995, Evers et al. 1998, Kenow et al. 2003). Because
MeHg levels accumulate over time, MeHg loads are often greater in adults than juveniles (Evers et al. 1998). However, MeHg adversely affects loons of all age classes as elevated MeHg burdens negatively influence reproductive fitness and fledging rates, chick mass, juvenile immune function, and overall adult productivity (Meyer et al. 1998, Nocera and Taylor 1998, Kenow et al. 2003, 2007, Evers et al. 2008).

*Human Development and Disturbance:* In addition to disease and MeHg bioaccumulation, other anthropogenic factors such as shooting (Richardson et al. 2000), recreational pressures (Franson and Cliplef 1992), and human development around critical habitat (Lindsay et al. 2002) threaten common loon populations both regionally and continentally. Several studies have shown lead poisoning (ingestion of lead fishing weights) to be among the top causes of loon mortalities throughout the U.S. (Locke et al. 1982, Franson and Cliplef 1992, Pokras and Chafel 1992, Stone and Okoniewski 2001, Sidor et al. 2003). Moreover, increasing human presence and development of shoreline nesting habitat may deter breeding pairs from utilizing traditional nesting sites (Lindsay et al. 2002, Newbrey et al. 2005).

Combined, these factors have contributed to an overall northward shift of common loon populations throughout much of the North American breeding range. States such as Illinois, Indiana, Iowa, and Ohio that historically supported breeding loon populations are now devoid of the species, and, in Michigan the common loon is a state-listed “Threatened” species. Despite the observed population declines, the Midwest region
continues to support much of the entire breeding loon population within the United States (Evers et al. 2010).

**Molecular Genetic Studies of the Common Loon**

The genetic structure of common loon populations is not well understood (McMillan et al. 2004), yet using genetic markers to measure dynamics such as inbreeding, selection, and gene flow within and among populations is important for providing a more complete picture of common loon life history. Molecular analysis may also prove useful in understanding connections between breeding, migratory, and overwintering groups, as well as in predicting the possible increased susceptibility of certain populations to disease and physiological dysfunction due to environmental contaminants. Dhar et al. (1997) found low levels of genetic diversity in a molecular study of common loons breeding in New England and in Michigan, although they reported that their randomly amplified polymorphic DNA (RAPD) markers and mitochondrial restriction fragment length polymorphisms (RFLPs) showed geographical differentiation between the two populations. Levels of genetic variation within the populations differed, as the Michigan population showed less genetic diversity than the New England population (Dhar et al. 1997). McMillan et al. (2004) identified seven polymorphic microsatellite loci within the common loon genome that showed only slight variation between individuals from ten separate locations in North America, which was not useful for population analysis. More recently, similarly low levels of genetic variation were found when analyzing mitochondrial DNA (mtDNA) from overwintering common loons killed in an oil spill off southern Europe (Bartolome et al. 2011). Although their study showed low levels of
genetic diversity and subdivision throughout the entire species range, that may be an artifact of small sample size and the use of only three loci of a single linkage group.

Even fewer molecular studies have been conducted assessing aspects other than population genetic diversity of the common loon. In one such study, multilocus DNA fingerprinting was paired with visual observations to reaffirm monogamy within populations of loons from the upper Great Lakes region (Piper et al. 1997). In another, molecular techniques were used to assess blood parasite loads in loons from northern Wisconsin (Weinandt 2006). Interestingly, Weinandt (2006) found that the presence of a *Leucocytozoon* parasite was directly correlated with blood mercury levels within the birds. My study is the first to use molecular genetic techniques to determine the sex of individual loons, it provides the first molecular genetic assessment of juvenile sex ratios within breeding populations, and it is the first to evaluate whether there is sex-bias in migratory and overwintering groups of common loons.
A MOLECULAR GENETIC ASSESSMENT OF SEX RATIOS FROM BREEDING, MIGRATORY AND OVERWINTERING COMMON LOONS

INTRODUCTION

One of the first hypotheses regarding variability in sex ratios of birds suggested that under the influence of natural selection, sex ratios within a population should be largely balanced by the reproductive advantage of the rarer sex to contribute greater numbers of similarly sexed offspring to the population (Fisher 1930). However, recent studies have illustrated that various environmental, physiological, genetic, and behavioral factors can skew sex ratios within a species or population in favor of either sex; examples of biases in hatching and/or fledging sex ratios have been noted in several avian species (Donald 2007). Additionally, even if juvenile sex ratios are largely balanced within a population, factors including seasonal resource availability, mate selection, and the amount of parental investment allotted to separate clutches or to individuals within a clutch have been suggested to influence the sex of offspring hatched or fledged by individual adult pairs (Wiebe and Bortolotti 1992, Clotfelter 1996, Svensson and Nilsson 1996).

If existent, sex biases among juvenile birds within a population can be difficult to detect, and predictors of variation may differ between populations of the same species (West et al. 2002, Donald 2007). Although juvenile sex ratios can be skewed in favor of either sex, in species that experience even slight sexual size dimorphism, skewed sex ratios often become more pronounced post-hatch when increased energetic requirements of the larger sex, facultative parenting, or other ecological or physiological factors result in sex-biased mortality (Benito and González-Solís 2007).
Biases in adult sex ratios among birds are typically more prominent than offspring sex ratios. Moreover, in a quantitative assessment of 201 published avian adult sex ratios, Donald (2007) found 83% of statistically significant skewed sex ratios to be male-biased, with males outnumbering females by approximately 30-35%. Additionally, in most cases, biased adult sex ratios seemed to directly correlate with the International Union for Conservation of Nature (IUCN) threat-of-extinction status of a species (Donald 2007).

Skewed sex ratios among monogamous species should be of special conservation concern as sex-biased populations can face greater extinction probabilities than similarly biased populations with other mating systems (Bosé et al. 2007). For small populations of monogamous birds with limited territory availability, stochastic fluctuations in sex ratios may result in pronounced alle effect, increasing the probability of extinction (Bessa-Gomes et al. 2004). This is likely due largely to the limited mate availability typical of small populations (Legendre et al. 1999).

The common loon is a highly territorial, monogamous, long-lived migratory species with limited territory availability. Additionally, as a high trophic-level piscivore, the common loon is of conservation interest and a state-listed “Threatened” species due to its susceptibility to various environmental threats, including avian botulism (Brand et al. 1988), the bioaccumulation of toxins (Evers et al. 1998), and human development (Lindsay et al. 2002). Recently, characteristics of natal territory, including lake size and pH, have been shown to directly influence juvenile survival within populations (Piper et al. 2012). Loons are often considered to be attractive subjects for long-term field research
because of their reliable and recurrent observability while on their breeding territories, and extensive records of common loons breeding at Seney National Wildlife Refuge (SNWR) dating back to 1987 have allowed for a detailed longitudinal study of the genetic and behavioral characteristics of the species. This long-term study at SNWR, performed by Common Coast Research and Conservation (CCRC), has documented hatch and fledge rates that exceed modeled values expected for a stable population. As a possible metapopulation “source” for juvenile loons, the SNWR population is of particular value for examining common loon hatching and fledging sex ratios.

One objective of my research was to use genetic methods to determine hatching and fledging sex ratios of loon chicks at SNWR during 1996-2010. Using blood collected from juvenile loons by CCRC researchers, a series of DNA extractions and PCR amplifications were performed to assess sex ratios and to test for deviations from parity. Sex ratios were also analyzed for two other breeding regions in the Midwest: northern Wisconsin and Isle Royale National Park. To evaluate sex-biased mortality during migration and overwintering, birds killed during fall migration near Pinery Provincial Park, Ontario, as well as dead or moribund birds collected off the Florida coast (respectively) were included in these analyses. Examining sex ratios among multiple different groups of individuals was an important addition to this study, as factors including dispersal and sex-biased mortality incurred post-fledging can dramatically alter sex ratios and in turn affect population stability. A complete understanding of the unique characteristics of and the linkages between breeding, migratory, and overwintering
groups may help to better illuminate loon conservation concerns throughout the species range.

Additionally, at SNWR there is a male-biased sex ratio among the returning adult loons that were banded as juveniles on the refuge (D. McCormick, pers. comm.). Two possible explanations for this sex-bias in adults banded as juveniles (ABJs) are: 1) there may be female-biased mortality within this population or 2) there may be female-biased dispersal. Female-biased dispersal is not uncommon in birds (Bensch et al. 1998, Wheelwright and Mauck 1998). Greenwood (1980) presented a hypothesis that natal dispersal among female birds would be greater than natal dispersal of male birds, based on the assumption that males incur more benefits by acting as the philopatric sex since males generally defend territories. By establishing their territory in a familiar locale, males should experience higher overall fitness than they would if they dispersed to unknown habitats. Although the long-term nesting and productivity data collected at SNWR supports Greenwood’s (1980) hypothesis, I was also able to use my genetic data to determine if juvenile sex ratios at the refuge contribute to the observed male-bias in adult return rates and to determine whether there was sex-biased mortality on migration or overwintering habitats.
METHODS

Sample Collection and DNA extraction

As part of a long-term monitoring study at Seney National Wildlife Refuge (Schoolcraft CO, MI) between 1996 and 2010, adult loon pairs and their chicks were captured by CCRC colleagues (for methylmercury-based research purposes) using a standard night-lighting technique (Evers 1993). Adults and chicks were captured during July through October when chicks were at least four weeks old (fledging occurs at 11-14 weeks). During capture activities, birds were color-banded and measured for basic morphometric data, and blood and feather samples were taken from each bird. Although the sex of adult birds can be reliably determined in the field using morphological and behavioral characters, the sex of chicks is almost always unknown at the time of capture.

In addition to the chick bloods obtained at SNWR ($N=134$), blood samples were obtained from loon chicks hatched on lakes in northern Wisconsin (Vilas, Forest and Oneida counties: $N=119$) during the 2004 and 2005 breeding seasons, and at Isle Royale National Park (ISRO) in 1994, 1997, 1998, 2003, and 2004 ($N=14$). All of these samples were collected by researchers using the blood samples for methylmercury-based research questions. Tissue samples were collected from moribund or deceased overwintering juvenile birds on the Gulf coast of Florida in 1998 ($N=21$). Tissue samples were also collected from loons killed during a botulism outbreak on Lake Huron while on migration in November of 1999 ($N=96$). All bloods and tissues were stored in heparin or EDTA/DMSO buffers at -20°C until DNA was extracted from each using a silica-based filter purification DNA extraction kit (DNeasy kit; Qiagen, Valenica, CA, USA).
PCR Amplification and Sequencing

To assay the sex of chicks I amplified sex-chromosome specific markers using a PCR protocol modified from Itoh et al. (2001). Primers AWSO5 and NRD4 targeted a 289bp fragment of the conserved EE0.6 region of the W chromosome (female specific) and primers SINT-F and SINT-R targeted a 133bp fragment of the spindlin gene of the Z chromosome (shared by males and females). PCR reactions were run in 25 µl volumes using: 0.5 units of Bullseye HS Taq polymerase, 0.5mM MgCl₂, 1X HS buffer II, 0.16 mM dNTPs (Midscı Inc.), 0.5 µM of each primer and approximately 25 ng/µl of DNA template. Amplification was performed in an Eppendorf Masteırcycler Gradient thermocycler with the following thermal profile: an initial 15-minute denaturation at 95°C, followed by 35 cycles of 95°C denaturation for 80 seconds, 62.5°C annealing for 45 seconds, and 72°C extension for 60 seconds. The thermal profile concluded with a final five minute extension at 72°C and storage at 4°C until gel electrophoresis.

Amplification products were separated on 1.0% low-melt agarose Tris-Borate-EDTA gels (70V for ~60 min), stained with ethidium bromide (EtBr), and visualized under ultraviolet light. Each set of PCR reactions included negative (dH₂O) and positive controls. Positive controls were DNA samples from field-verified adult male and adult female loons.

To verify the identity of the amplification products from the positive controls and a subsample of the unknown juvenile birds, PCR products amplified from the Z and W chromosomes were sequenced from two adult loons of each sex (field confirmed) using the PCR protocol above, except in 50uL reaction volumes. PCR products were excised
from the agarose gel with a scalpel and cleaned using a Qiagen QIAQuick® Gel Extraction Kit. PCR products were sequenced in both directions using an ABI Prism® Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing products were cleaned using Sephadex columns, dried completely before re-suspension in deionized formamide and loaded onto an ABI Prism® 3100-Avant Genetic Analyzer. Forward and reverse sequences were reconciled against each other, ambiguous base-calls were reconciled by eye, and primers were trimmed from the consensus sequence using Geneious software (Biomatters Ltd v. 5.5.6) (Appendix A).

**Statistical Analyses**

Hatching and fledging data were recorded in the field for all loon pairs nesting on pools at SNWR between 1996 and 2010. To evaluate whether different factors influenced the sex of offspring, sex ratios were compared between different groups of chicks depending on: clutch size (one-egg vs. two-egg clutches), parental pair, parental male, parental female, and natal territory. To assess the impact of pre-fledging mortality on juvenile sex ratios, I calculated sex ratios of different subsets of chicks hatched and fledged between 1996 and 2010: chicks from single-egg clutches (“1-of-1” chicks), chicks of two-egg clutches where both chicks were sexed (“2-of-2” chicks), and surviving chicks of two-egg clutches where the other chick died before capture (“1-of-2” chicks). To eliminate the possible influence of pre-fledging sex-biased mortality on the overall hatch ratio, after separating sexed juveniles into clutch types, I examined the sex ratio of only clutches where “2-of-2” chicks were caught. When possible, each of these ratios were calculated for the group of chicks that hatched, as well as those that survived to fledge. Once
calculated, all sex ratios were expressed as the proportion of males within the population or subset group (Wilson and Hardy 2002). Overall sex ratios for loons captured/colle clected in northern Wisconsin, Ontario, ISRO, and Florida were also recorded and expressed in the same way as those from SNWR. Although not directly assayed in my study, I also included in my analyses juvenile sex data collected from chicks used in a dosing study of methylmercury effects on Wisconsin loon chicks in 1999, 2000, and 2003 (Kenow et al. 2003, Kenow et al. 2007).

Deviations in sex ratios from an expected 1:1 (male: female) were tested using a Chi-square goodness-of-fit test. To assess whether any of the observed hatch or fledge ratios at SNWR differed significantly from each other, a series of two-by-two contingency tables were constructed. For each table, male and female hatched and/or fledged chicks served as the categorical variables.

Six separate general linear regression models were constructed to evaluate the relationship between the fledging sex ratio and: (a) the productivity of adult loon pairs, (b) the productivity of adult male loons, (c) the productivity of adult female loons, (d) the productivity of refuge pools (an indirect indicator of natal territory quality), (e) the mass of the parental male, and (f) the mass of the parental female at SNWR. The independent variables in models (a) through (d) were the productivity values ( = the total number of chicks fledged from adult pair/adult male/adult female/pool divided by the total number of possible fledge years), while the independent variable in models (e) and (f) was the mass of the adult bird. The dependent variable in each test was the proportion of male
chicks fledged for each adult pair/adult male/adult female or pool ( = the number of male chicks fledged by each pair, adult male, adult female, or pool divided by the total number of chicks fledged by each pair, male, female, or pool), and dependent variables were arcsine square root transformed.

I evaluated the possible influence of parental quality on the sex of offspring using binary regression. The masses of the parental males and females were used as covariates of the sex of each chick hatched by that parent, which was considered the dependent variable. When the mass of either parent was obtained in multiple years, the value used in the model was that obtained in the year that the chick was hatched. If the mass of the adult was not obtained in the year that the chick was hatched, I used the average value of all observed measurements for that adult.

Across the 1998-2011 breeding seasons, 30 adults banded as juveniles (ABJs) returned to the SNWR breeding area. Observations began in 1998, as chicks that were fledged in 1996 could potentially begin returning to the refuge in 1998. Sexually mature loons usually return to breeding territories three, but occasionally two years post fledging. To determine whether the ABJ male sex-bias (23 males, 7 female ABJs) observed at SNWR between 1998 and 2011 is attributable to the fledging sex ratio, I ran 10,000 replications of a simulation model, where each replication randomly sampled 30 individuals fledged from SNWR between 1996-2008 to simulate the number of ABJs that have been observed at SNWR through 2011. The values from the simulation replicates created a null distribution of male returns out of 30 ABJs. This distribution was used to determine
the likelihood of the observed sex-bias (23 males out of 30) being attributable to the sex ratio documented in the birds fledged from 1996-2008.
RESULTS

From 1996 through 2010, CCRC collaborators at SNWR documented 184 and 154 common loon chicks hatched and fledged, respectively. Blood samples were collected from 137 and 135 of the hatched and fledged chicks, respectively. My molecular sexing protocol was successful in determining the sex of 134 blood samples, representing 72.8% of total hatched chicks, 87.0% of total fledged chicks, and across years there was considerable variation in sex ratios (Appendix B). Typical agarose gel visualization of PCR amplification products is shown in Figure 1.

Across all years at SNWR, the overall proportion of male chicks hatched at the refuge was 0.537 (72 males: 62 females) and the overall proportion of male chicks fledged was 0.538 (71 males: 61 females) (Table 1, Appendix B). The overall male-bias in hatching
and fledging from 1996-2010 did not differ significantly from 0.5 ($p=0.388$ and $p=0.384$, respectively) (Table 1). Sex ratios were determined for subsets of hatched and fledged chicks grouped by clutch-type (“1-of-1,” “1-of-2,” “2-of-2”), but chi-square tests indicated no significant difference between the degree of male-bias in each of these three groups of chicks (Table 2). None of the other observed sex ratios – from breeding birds sampled from northern Wisconsin and Isle Royale National Park, from migrating birds killed on Lake Huron, or from wintering birds sampled off the coast of Florida – showed any significant sex ratio biases (Table 1). Although not collected explicitly for use in this study, sex ratios were not significantly male-biased ($p=0.230$) (Table 1, Table 3) in the Kenow et al. (2007, K. Kenow, pers. comm.) study. Collectively considering all sampled loons from this breeding region, the number of male juveniles ($N=189$) was not significantly different ($p=0.096$) from the number of female juveniles ($N=158$).

### Table 1. Chi-square goodness-of-fit test of common loon sex ratios.

<table>
<thead>
<tr>
<th>Age Class</th>
<th>Sex Ratios from Different Locales</th>
<th>Population Type</th>
<th>N</th>
<th>Males</th>
<th>Females</th>
<th>Proportion of Males</th>
<th>X² value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile</td>
<td>Breeding</td>
<td>SNWR</td>
<td>132</td>
<td>71</td>
<td>61</td>
<td>0.538</td>
<td>0.758</td>
<td>0.384</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Breeding</td>
<td>N. Wisconsin ('99, '00, '03)</td>
<td>82</td>
<td>44</td>
<td>38</td>
<td>0.537</td>
<td>0.439</td>
<td>0.508</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Breeding</td>
<td>N. Wisconsin ('04-'05)</td>
<td>119</td>
<td>65</td>
<td>54</td>
<td>0.546</td>
<td>1.017</td>
<td>0.313</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Breeding</td>
<td>Isle Royale National Park</td>
<td>14</td>
<td>9</td>
<td>5</td>
<td>0.643</td>
<td>1.143</td>
<td>0.285</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Breeding</td>
<td><strong>combined breeding groups</strong></td>
<td>347</td>
<td>189</td>
<td>158</td>
<td>0.545</td>
<td>2.769</td>
<td>0.096</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Wintering</td>
<td>Florida</td>
<td>21</td>
<td>9</td>
<td>12</td>
<td>0.429</td>
<td>0.429</td>
<td>0.513</td>
</tr>
<tr>
<td>Adult</td>
<td>Migratory</td>
<td>Pinery Provincial Park, Ont.</td>
<td>96</td>
<td>49</td>
<td>47</td>
<td>0.510</td>
<td>0.042</td>
<td>0.838</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hatch Ratios at SNWR</th>
<th>1-of-1</th>
<th>2-of-2</th>
<th>1-of-2</th>
<th>Overall Hatch Ratio¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile</td>
<td>40</td>
<td>38</td>
<td>22</td>
<td>134</td>
</tr>
<tr>
<td>134</td>
<td>72</td>
<td>72</td>
<td>62</td>
<td>537</td>
</tr>
<tr>
<td>Overall Hatch Ratio¹</td>
<td>0.900</td>
<td>0.222</td>
<td>0.500</td>
<td>0.746</td>
</tr>
<tr>
<td>0.343</td>
<td>0.637</td>
<td>1.000</td>
<td>0.388</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fledge Ratios at SNWR</th>
<th>1-of-1</th>
<th>2-of-2</th>
<th>1-of-2</th>
<th>Overall Fledge Ratio²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile</td>
<td>40</td>
<td>71</td>
<td>21</td>
<td>132</td>
</tr>
<tr>
<td>132</td>
<td>23</td>
<td>37</td>
<td>11</td>
<td>61</td>
</tr>
<tr>
<td>Overall Fledge Ratio²</td>
<td>0.900</td>
<td>0.127</td>
<td>0.048</td>
<td>0.758</td>
</tr>
<tr>
<td>0.343</td>
<td>0.722</td>
<td>0.827</td>
<td>0.384</td>
<td></td>
</tr>
</tbody>
</table>

¹ Calculated using all hatched chicks of known sex
² Calculated using all fledged chicks of known sex
Linear regression analyses suggested that as male/female/pair productivity increased, the proportion of female offspring increased, while the reverse was true for refuge pool productivity (Figure 2). However, these associations were not significant, as indicated by ANOVA results for each regression test (p>0.05 for each test) (Table 4). As the mass of

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Males</th>
<th>Females</th>
<th>(X^2) value</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatch Ratio 1-of-1</td>
<td></td>
<td>23</td>
<td>17</td>
<td>0.231</td>
<td>1</td>
<td>0.630</td>
</tr>
<tr>
<td>Hatch Ratio 2-of-2</td>
<td></td>
<td>38</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hatch Ratio 1-of-2</td>
<td></td>
<td>11</td>
<td>11</td>
<td>0.052</td>
<td>1</td>
<td>0.819</td>
</tr>
<tr>
<td>Hatch Ratio 2-of-2</td>
<td></td>
<td>38</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hatch Ratio 2-of-2</td>
<td></td>
<td>38</td>
<td>34</td>
<td>0.017</td>
<td>1</td>
<td>0.896</td>
</tr>
<tr>
<td>Overall Hatch Ratio</td>
<td></td>
<td>72</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hatch Ratio 2-of-2</td>
<td></td>
<td>38</td>
<td>34</td>
<td>0.019</td>
<td>1</td>
<td>0.890</td>
</tr>
<tr>
<td>Overall Fledge Ratio</td>
<td></td>
<td>71</td>
<td>61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fledge Ratio 1-of-1</td>
<td></td>
<td>23</td>
<td>17</td>
<td>0.146</td>
<td>1</td>
<td>0.702</td>
</tr>
<tr>
<td>Fledge Ratio 1-of-2</td>
<td></td>
<td>11</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fledge Ratio 1-of-1</td>
<td></td>
<td>23</td>
<td>17</td>
<td>0.299</td>
<td>1</td>
<td>0.585</td>
</tr>
<tr>
<td>Fledge Ratio 2-of-2</td>
<td></td>
<td>37</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fledge Ratio 1-of-2</td>
<td></td>
<td>11</td>
<td>10</td>
<td>0.000</td>
<td>1</td>
<td>0.983</td>
</tr>
<tr>
<td>Fledge Ratio 2-of-2</td>
<td></td>
<td>37</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fledge Ratio 1-of-1</td>
<td></td>
<td>23</td>
<td>17</td>
<td>0.170</td>
<td>1</td>
<td>0.680</td>
</tr>
<tr>
<td>Overall Fledge Ratio</td>
<td></td>
<td>71</td>
<td>61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fledge Ratio 1-of-2</td>
<td></td>
<td>11</td>
<td>10</td>
<td>0.014</td>
<td>1</td>
<td>0.904</td>
</tr>
<tr>
<td>Overall Fledge Ratio</td>
<td></td>
<td>71</td>
<td>61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fledge Ratio 2-of-2</td>
<td></td>
<td>37</td>
<td>34</td>
<td>0.052</td>
<td>1</td>
<td>0.820</td>
</tr>
<tr>
<td>Overall Fledge Ratio</td>
<td></td>
<td>71</td>
<td>61</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Juvenile sex ratios observed in Wisconsin from 1999-00 and from 2003-05.

<table>
<thead>
<tr>
<th></th>
<th>Year</th>
<th>N</th>
<th>Male</th>
<th>Female</th>
<th>Proportion of Males</th>
<th>(X^2) value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatching Sex Ratios¹</td>
<td>1999</td>
<td>24</td>
<td>14</td>
<td>10</td>
<td>0.583</td>
<td>0.667</td>
<td>0.414</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>36</td>
<td>14</td>
<td>22</td>
<td>0.389</td>
<td>1.778</td>
<td>0.182</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>22</td>
<td>16</td>
<td>6</td>
<td>0.727</td>
<td>4.545</td>
<td>0.033*</td>
</tr>
<tr>
<td>Fledging Sex Ratios</td>
<td>2004</td>
<td>58</td>
<td>34</td>
<td>24</td>
<td>0.586</td>
<td>1.724</td>
<td>0.189</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>61</td>
<td>31</td>
<td>30</td>
<td>0.508</td>
<td>0.016</td>
<td>0.898</td>
</tr>
<tr>
<td>Total</td>
<td>201</td>
<td>109</td>
<td>92</td>
<td>0.542</td>
<td>1.438</td>
<td>0.230</td>
<td></td>
</tr>
</tbody>
</table>

¹ Data provided by K. Kenow, pers. comm.
* Indicates significance with alpha = 0.05

Linear regression analyses suggested that as male/female/pair productivity increased, the proportion of female offspring increased, while the reverse was true for refuge pool productivity (Figure 2). However, these associations were not significant, as indicated by ANOVA results for each regression test (p>0.05 for each test) (Table 4). As the mass of
parental males and parental females increased (individually), the proportion of male offspring fledged increased (Figure 2). However, as with the productivity data, these associations were non-significant (Table 4).

![Figure 2](image)

**Figure 2.** Linear regression results assessing the proportion of males fledged at SNWR as a function of measures of productivity (a-d) and parental quality (e-f). Bubble sizes are proportional to the frequency of observations observed at each data point.
The binary logistic regression models revealed no relationship (Nagelkerke $R^2 = 0.048$, $\chi^2 = 11.510$, df = 8, $p = 0.174$) between the sex of the offspring and the mass of the adult male or the adult female (Table 5).

From 1998 through 2011, 23 males and seven female ABJs were observed at SNWR. Of those birds, the sex of all seven female birds was genetically determined (with one definitively field-confirmed and three tentatively field-confirmed), while 22 of the 23
male birds were genetically established (with 22 tentatively field-confirmed and one of the 23 solely field-confirmed). To test whether the SNWR male sex-bias at hatching and fledging can explain the male-bias in juvenile return rates (D. McCormick, pers. comm.) I ran a simulation model that randomly sampled 30 individuals fledged from SNWR between 1996-2008 to simulate the number of ABJs that have been observed at SNWR through 2011. From 10,000 simulation runs, a male-bias as great or greater than that observed (23 males out of 30) was recorded only 161 times ($p=0.0161$) (Figure 3).
DISCUSSION

Although proportionally more chicks were hatched and fledged at SNWR between 1996 and 2010, the observed male-bias was not significant. As the fledging sex ratio incorporates 86% of loon chicks fledged from the refuge between 1996 and 2010, it is likely this estimation represents the actual ratio of chicks fledged from the entire SNWR population during that period. Calculations of a hatching sex ratio among loons at SNWR were complicated by the fact that there were about 47 hatched chicks that went unsampled and therefore un-sexed. A female sex-bias in pre-fledging mortality could lead to a greater proportion of males in the sample of birds that were actually caught and subsequently sexed. To test this and eliminate the possible influence of pre-fledging female-biased attrition, I separated sexed juveniles into clutch types (Table 1: hatch “1-of-1,” “1-of-2,” and “2-of-2”) and examined the sex ratio of only clutches where “2-of-2” chicks were caught. Examining sex ratios only within “2-of-2” clutches allowed me to assess juvenile sex ratios independent of the possible influence of juvenile sex-biased attrition. Like in the overall hatch ratio, in the “2-of-2” clutches proportionally more males were hatched, but again, the bias was not significantly different from 0.5 (Table 1: “2-of-2” hatch).

When considered individually, the hatch ratios for each clutch type (hatch “1-of-1,” “1-of-2,” “2-of-2”) at SNWR were all non-significant. Additionally, non-significant chi-square results confirmed that the observed hatch ratios were not significantly different from one another or from the combined overall hatch ratio (Table 2). As the observed individual hatch ratios did not differ significantly from one another, these data support
the conclusion that sex-biased attrition before capture does not have a significant influence on the observed fledging rate.

Like at SNWR, proportionally more males were observed among common loon chicks captured throughout northern Wisconsin in 2004 and 2005 (Table 1), as well as when fledging data from 1999, 2000, and 2003 were added to this dataset (Table 3); however, the biases observed were not significantly different from 0.5 ($p=0.313$ and 0.230, respectively). The apparent male-biased fledge ratio observed at ISRO was not significant either, although small sample size may have been an influencing factor ($N=14$). When considering the sex-bias in juveniles sampled from across the study area (SNWR, ISRO, WI), the male-bias approached statistical significance ($p=0.096$), but this may also be an artifact of an inflated sample size. Although none of the individual breeding population sex-biases were statistically significant, all showed a similar pattern of proportionally more males than females (Table 1). These data may suggest that common loon breeding populations, at least within this region, are fledging proportionally more male offspring than female offspring.

The sex ratio among adult birds collected at Pinery Provincial Park, Ontario during the fall migratory period was nearly balanced at 0.510. The deceased loons collected in Ontario died as a result of avian botulism, a disease agent that presumably has no sex-bias in its action, thus this group of birds is likely a representative sample of all loons migrating over the lake at that time. Conversely, I found proportionally more females (although non-significant) among the overwintering birds of the Florida sample.
Although the sex ratio of juveniles in the Florida sample may be influenced by small sample size ($N=21$), my data indicate that female-biased mortality may occur among juveniles on wintering grounds. Sex ratios among the migrating and overwintering groups warrant further research on whether sex-specific physiological or environmental stresses exist for adult and sub-adult common loons. Further studies also should be conducted to assess whether sex ratios differ between separate populations or geographical regions or if similar trends are inherent throughout the species range.

Although several studies have indicated that the sex of offspring within a population may be influenced by factors such as variation in seasonal resources (Cooch et al. 1997, Oddie 2000) and parental condition (Velando et al. 2002, Weimerskirch et al. 2005, Blanchard et al. 2007), the non-significant outcomes from my regression analyses suggest that external factors including natal pool productivity, clutch size, and parental quality are not significant predictors of offspring sex at SNWR. It is possible that offspring sex may have varied by year, however year of hatch/fledge was not included as an independent variable since in some years a high proportion of chicks were never sampled (see Appendix B).

Little research has been done to examine variation in adult, or tertiary, sex ratios within common loon populations. I found that juvenile sex ratios were not significantly biased in either direction and thus may be supportive of a near equal tertiary sex ratio within breeding populations, as proposed by Fisher (1930). The nearly equal ratio of male:female adult birds killed while migrating over Lake Huron also seem to support Fisher’s
hypothesis. Still, proportionally more males were fledged from all three breeding locations. This observation, though not statistically significant, may be important for maintaining even sex ratios later in life if males incur greater rates of mortality post-fledging. Although the nearly equal adult mortality within the migratory sample and the non-significant female-bias among overwintering birds in my study do not support male-biased attrition post-fledging, it is possible that male loons may be adversely affected by the higher energetic costs associated with being the larger sex. As the larger sex, poor resource availability may adversely affect male loons more than female loons, resulting in increased rates of male mortality (as in the lesser snow goose, *Chen caerulescens*; Cooch et al. 1997). Additionally, mortality among males may increase due to the elevated energetic costs associated with the physiological effects triggered by sex-specific androgens among males. If this is the case among loons, these energetic costs combined with the ability of androgens to compromise immune function could account for elevated mortality rates among males during periods of limited resource availability or adverse conditions (Grossman 1985, Schwabl 1993).

From this study alone, it is not possible to tell whether male-biased sex ratios are characteristic of breeding populations, or if adult sex ratios are alternatively balanced by male-biased mortality. If adult sex ratios among loons do not follow the one-to-one ratio suggested by Fisher (1930), we could expect to continue to observe elevated numbers of adult male loons in breeding populations. This would not be abnormal among avian populations and may be a result of evolutionary forces (Donald 2007). In a monogamous mating system, such as that of loons, male-biased adult sex ratios may act to promote
monogamy within a population by promoting male-male competition when female partners are limited (Ligon 1999). This has been studied within a kiwi (Apteryx spp.) population, where mate fidelity decreased as adult sex ratios shifted to become female-biased (Taborsky and Taborsky 1999).

Even if juvenile male-biased sex ratios exist among breeding loons, it is unlikely that this factor alone accounts for the significant male-bias in adult return rates to SNWR. In accordance with Greenwood’s (1980) hypothesis, it is likely that among loons, a greater proportion of adult males return to natal breeding grounds while the majority of adult females disperse elsewhere. This hypothesis was supported by the results of the ABJ simulation model, which demonstrated that the observed sex-bias in the ABJ return rate is unlikely to have resulted from the sex-bias in fledging rate alone ($p=0.0161$). Although juvenile females seem to be dispersing from SNWR, there is no lack of breeding females at the refuge, indicating that adult females hatched elsewhere must immigrate into the refuge population.

Similar sex-biased dispersal to that observed at SNWR has been documented across several avian orders and may be an evolutionarily adaptive trait that reduces the probability of mating with a related individual, and as a consequence limits inbreeding within populations and promotes gene flow between populations. Although this explanation is appealing for explaining these observations of loons at SNWR and northern Wisconsin, further research is needed to evaluate whether and to what extent
other factors including sub-adult female-biased attrition on migration or on wintering grounds may contribute to the adult returns to breeding territories.
SUMMARY AND CONCLUSIONS

As a highly territorial, monogamous species of special concern within the state, it is important to understand the role that sex ratios play within isolated populations of common loons and the factors that may influence the sex of offspring from year to year. Although non-significant, proportionally more males are being fledged from among breeding populations sampled in this study. Productivity measures of breeding adults and natal territories do not seem to influence the sex of chicks at SNWR. Additionally, the male-bias at fledging observed at SNWR was not dramatic enough alone to account for the male-biased adult return rate to the refuge, indicating that there may be female-biased dispersal in common loons. Further research should be conducted to understand dispersal behavior in loons as well as the physiological and environmental stressors encountered by loons on migratory and wintering habitats that may influence mortality in a sex-biased fashion.


APPENDIX A

Appendix A. “Z” and “W” sex chromosome sequences amplified from common loon blood samples via PCR assays. Images were produced using Geneious software (Biomatters Ltd v. 5.5.6).

a) “Z” chromosome sequencing data
b) “W” chromosome sequencing data
Appendix B. Sex breakdown of hatched and fledged chicks by year at SNWR (1996-2010).

<table>
<thead>
<tr>
<th>Year</th>
<th>Hatched</th>
<th>Fledged</th>
<th>Number Sampled</th>
<th>% of Hatched Chicks Sexed</th>
<th>% of Fledged Chicks Sexed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>1996</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>50%</td>
<td>75%</td>
</tr>
<tr>
<td>1997</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>33%</td>
<td>75%</td>
</tr>
<tr>
<td>1998</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>82%</td>
<td>82%</td>
</tr>
<tr>
<td>1999</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>89%</td>
<td>100%</td>
</tr>
<tr>
<td>2000</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>2001</td>
<td>16</td>
<td>13</td>
<td>5</td>
<td>31%</td>
<td>38%</td>
</tr>
<tr>
<td>2002</td>
<td>17</td>
<td>13</td>
<td>12</td>
<td>71%</td>
<td>92%</td>
</tr>
<tr>
<td>2003</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>86%</td>
<td>100%</td>
</tr>
<tr>
<td>2004</td>
<td>11</td>
<td>9</td>
<td>7</td>
<td>64%</td>
<td>78%</td>
</tr>
<tr>
<td>2005</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>80%</td>
<td>94%</td>
</tr>
<tr>
<td>2006</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>86%</td>
<td>100%</td>
</tr>
<tr>
<td>2007</td>
<td>24</td>
<td>20</td>
<td>19</td>
<td>79%</td>
<td>95%</td>
</tr>
<tr>
<td>2008</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>57%</td>
<td>67%</td>
</tr>
<tr>
<td>2009</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>2010</td>
<td>12</td>
<td>8</td>
<td>10</td>
<td>67%</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>184</td>
<td>154</td>
<td>134</td>
<td>73%</td>
<td>86%</td>
</tr>
</tbody>
</table>