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## **A Molecular Genetic Assessment of Sex Ratios from Pre-fledged Juvenile and Migrating Adult Common Loons (*Gavia immer*)**

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# A Molecular Genetic Assessment of Sex Ratios from Pre-fledged Juvenile and Migrating Adult Common Loons (*Gavia immer*)

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**Abstract.**—We used a polymerase chain reaction (PCR)-based sex identification technique to evaluate the sex ratios of pre-fledged juvenile Common Loons (*Gavia immer*) from three breeding populations in northern Michigan and northern Wisconsin, and of dead adult Common Loons migrating through Lake Huron. Results of these analyses show that more juvenile males than females fledged from all three breeding populations, although none of these biases differed significantly from parity. The sex ratio of migratory adults was at parity. No parental or territory quality indicators were significantly associated with the sex of chicks fledged from one intensely studied northern Michigan population at Seney National Wildlife Refuge. A significant male bias in adult re-observation of Common Loons banded as juveniles at the wildlife refuge was not solely attributable to the modest male bias recorded at fledging, and thus suggests a pattern of female-biased post-fledging mortality and/or female-biased adult dispersal in Common Loons. Received 20 January 2013, accepted 26 April 2013.

**Key words.**—botulism, Common Loon, dispersal, *Gavia immer*, Gaviidae, migration, natal philopatry, sex-bias, sex chromosomes, sex ratio.

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Fisher (1930) famously argued that if male and female offspring cost the same to produce, natural selection should drive a population sex ratio to parity. Even if sex ratios are not shaped by adaptive processes, simple Mendelian (meiotic) inheritance should lead to equal numbers of male and female offspring. However, empirical studies of many avian species (reviewed in Donald 2007) show that various environmental, physiological, genetic, and behavioral factors can skew hatching and fledging sex ratios in wild populations of birds. Juvenile sex-bias within avian populations can be difficult to detect, and predictors of sex ratio variation may differ between these populations (West *et al.* 2002; Donald 2007). In species that experience even slight sexual size dimorphism, skewed sex ratios can arise or become more pronounced post-hatch when facultative parenting, increased energetic requirements of the larger sex, or other ecological or physiological factors result in sex-biased mortality (Benito and González-Solís 2007). Additionally, even if population-level juvenile sex ratios are largely balanced, the sex of offspring hatched or fledged by individual adult pairs can be influenced by factors such as seasonal resource availability, mate selection, and

parental investment (Wiebe and Bortolotti 1992; Svensson and Nilsson 1996; Clotfelter and Yasukawa 1999).

Skewed sex ratios among monogamous species are of special conservation concern, as sex-biased populations can face greater extinction probabilities than similarly biased populations with alternative 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 Allee effects, 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). In birds, the conservation implications of biases in sex ratios are largely understudied, in part due to the inability to easily assess hatching and fledging sex ratios in juvenile birds.

The Common Loon (*Gavia immer*) is a highly territorial, serially monogamous, long-lived migratory species with limited breeding territory availability. Occupying freshwater lakes in boreal and near-arctic habitats of North America, the same male and female often successfully breed together for multiple seasons (McCormick *et al.* 2007;

Evers *et al.* 2010). Nesting typically begins in late April through mid-May, with nearly always one or two eggs per clutch. Incubation lasts for approximately 28 days and is performed by both members of a mated pair, as are most chick-rearing duties (feeding, defense, etc.). Juveniles remain with at least one parent for 12-15 weeks post-hatch, and begin their fall migration shortly following the departure of the second parent (Evers *et al.* 2010). For Common Loons breeding in eastern North America and possibly in the central Canadian provinces (U.S. Geological Survey Bird Banding Laboratory, unpubl. data), the Great Lakes serve as important stopover sites during migration to and from oceanic wintering habitats (Kenow *et al.* 2002). Wintering behavior is largely understudied, but most Common Loons from the Midwest likely spend the non-breeding season in the near-continental waters of the Gulf of Mexico and the Atlantic Ocean (Lee 1987; Haney 1990; Jodice 1993). Although adult birds migrate twice yearly in the spring and fall, sub-adult birds typically remain on wintering grounds until their third spring, when they usually reach sexual maturity.

As a high trophic-level piscivore, the Common Loon (loon) is of conservation interest due to its susceptibility to various environmental threats, including avian botulism (Brand *et al.* 1988), toxin bioaccumulation (Evers *et al.* 1998), and human development (Lindsay *et al.* 2002). While on the breeding grounds, juvenile survival may be influenced directly or indirectly by various factors including lake acidity, lake size, mercury exposure, and predation (Meyer *et al.* 1998; Merrill *et al.* 2005; Evers *et al.* 2010; Piper *et al.* 2012).

Monitoring data collected since 1987 have shown that Common Loons breeding within northern Michigan's Seney National Wildlife Refuge (NWR) hatch and fledge more chicks than expected for a stable population (Evers 2007; Mitro *et al.* 2008; Tischler 2011). Research at Seney NWR has also demonstrated a male-biased sex ratio among banded juveniles that subsequently return to the refuge as breeding adults: among 30 adults banded as juveniles (ABJs) documented at Seney NWR from 1998-2011,

23 were males and seven were females (D. McCormick, unpubl. data). At least three hypothetical mechanisms could lead to this pronounced male sex-bias: 1) male-biased hatching ratios; 2) female-biased mortality (either pre- or post-fledging); or 3) female-biased adult dispersal. Using a molecular genetic assay to determine the sex of juvenile and adult loons, we examined these possible mechanisms by testing the following null predictions: 1) juvenile hatching and fledging sex ratios should be at or near parity; 2) adult sex ratios should be at or near parity; and 3) male-biased hatching and fledging sex ratios of Seney NWR juveniles led to the skewed sex ratio observed among refuge ABJs. To test these hypotheses, we used our genetic assay to determine the sex of loon chicks hatched and fledged from Seney NWR during 1996-2010. Furthermore, to evaluate if sex ratios at Seney NWR were population-specific rather than an inherent property of Common Loon life history, we similarly analyzed samples from two additional breeding regions in the Midwest: northern Wisconsin and Isle Royale National Park. To examine adult sex ratios, we also analyzed samples from migrating adults that died during a botulism outbreak on southern Lake Huron.

## METHODS

### Sample Collection and DNA Extraction

From 1996-2010, loon chicks hatched on managed impoundments ("pools") at Seney NWR (Schoolcraft County, Michigan) were captured using a standard night-lighting technique (Evers 1993). These juveniles were uniquely color-banded, and blood samples were drawn from most. In addition to samples obtained from chicks at Seney NWR ( $n = 134$ ), blood was collected from loon chicks hatched on lakes in northern Wisconsin during the 2004 and 2005 breeding seasons (Vilas, Forest and Oneida Counties;  $n = 119$ ) and at Isle Royale National Park in northern Michigan (Keweenaw County) in five of the years from 1994-2004 ( $n = 14$ ). Tissue samples were collected from migrating adult loons that died during a botulism outbreak on southern Lake Huron in the autumn of 1999 (Pinery Provincial Park, Ontario;  $n = 96$ ). All blood and tissue samples were stored in heparin or EDTA/DMSO buffers at  $-20\text{ }^{\circ}\text{C}$  until DNA was extracted from each using a silica-based filter purification DNA extraction kit (DNeasy kit; Qiagen).

### Genetic Amplification and Sequencing

The sex of chicks and adults was assayed using a PCR (polymerase chain reaction) protocol modified from Itoh *et al.* (2001). Primers AWSO5 and NRD4 targeted a 289 bp fragment of the conserved EE0.6 region of the W chromosome (female specific), and primers SINT-F and SINT-R targeted a 133 bp fragment of the *spindlin* gene of the Z chromosome (shared by males and females). PCR reactions were run in 25  $\mu$ l volumes using 0.5 units of Bullseye HS Taq polymerase, 0.5 mM MgCl<sub>2</sub>, 1X HS buffer II, 0.16 mM dNTPs (Midsci Inc.), 0.5  $\mu$ M of each primer, and approximately 25 ng of DNA template. Amplification was performed in an Eppendorf Mastercycler Gradient thermocycler with the following thermal profile: an initial 15-min denaturation at 95 °C, followed by 35 cycles of 95 °C denaturation for 80 sec, 62.5 °C annealing for 45 sec, and 72 °C extension for 60 sec. The thermal profile concluded with a 5-min 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 (70 V for ~60 min), stained with ethidium bromide (EtBr), and visualized under ultraviolet light. Each set of PCR reactions included negative (dH<sub>2</sub>O) and positive (DNA samples from field-verified adult male and adult female loons) controls.

To verify the identity of the amplification products from the positive controls and a subsample of the unknown 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 50  $\mu$ L 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 the software package Geneious v5.5.6 (Biomatters Ltd. 2012). DNA sequences were submitted to the National Center for Biotechnology Information GenBank and are available through accession numbers KC491204 (Z chromosome-linked fragment) and KC491205 (W chromosome-linked fragment).

Although not directly assayed in this study, juvenile sex data collected from Wisconsin chicks ( $n = 82$ ) used in a dosing study of methylmercury effects in 1999, 2000, and 2003 (Kenow *et al.* 2003, 2007) were included for comparison purposes. At the end of that study, chicks were sacrificed and sex was diagnosed by internal anatomy.

### Statistical Analyses

Sex ratios were evaluated and presented as the proportion of males (Wilson and Hardy 2002). To assess the impact of pre-fledging mortality on juvenile sex ratios at

Seney NWR, they were evaluated for different subsets of chicks hatched and fledged from 1996-2010: chicks from single-egg hatches ("1-of-1" chicks), chicks of two-egg hatches where both chicks were sexed ("2-of-2" chicks), and surviving chicks of two-egg hatches where one chick died before capture and sampling ("1-of-2" chicks). When possible, each of these hatch-type ratios were calculated for the group of chicks that hatched, as well as for those that survived to fledge. The "2-of-2" sex ratios eliminate the possible influence of pre-fledging sex-biased mortality on the overall hatch ratio. All deviations of sex ratios from an expectation of parity (proportion of males = 0.5) were tested using Chi-squared goodness-of-fit tests. To assess whether any of the observed hatching or fledging sex ratios differed significantly from each other, a series of two-by-two contingency tables were constructed in which male and female hatched and fledged chicks served as categorical variables.

To determine whether parental or territory quality factors influenced the sex of fledged offspring (defined here as loon chicks at least 9 weeks of age), six general linear regression models were constructed to evaluate the relationship between fledging sex ratio at Seney NWR and: a) the mass of the parental male; b) the mass of the parental female; c) the productivity of Seney NWR pools; d) the productivity of parental loon pairs; e) the productivity of parental male loons; and f) the productivity of parental female loons. We used mass of the parents as an indirect indicator of parental quality for regressions (a) and (b) (Mager *et al.* 2007), and used the productivity of the Seney NWR pool (c) as an indirect indicator of natal territory quality. Productivity values used in models (c)-(f) were expressed as the total number of chicks fledged by a parental pair/parental male/parental female or from a Seney NWR pool divided by the total number of possible fledge years. The dependent variable for each regression was the proportion of male chicks fledged for each pair/male/female/pool. All dependent variables were arc-sine square-root transformed.

The influence of parental quality on the sex of offspring was evaluated using binomial logistic regression where the sex of hatched offspring was considered as a function of the masses of the parental males and parental females. 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, the average value of all observed mass measurements was used for that adult.

Across the 1998-2011 breeding seasons, 30 breeding ABJs returned to Seney NWR pools. Although sexually mature loons typically leave their wintering grounds and migrate north to breeding areas in their third spring post-fledging, they occasionally return in their second; as such, we included observations from 1998 onward, as chicks that were fledged in 1996 would be the first cohort mature enough to potentially return to Seney NWR in 1998. To determine whether the ABJ sex-bias (23 male and seven female ABJs) observed at Seney NWR from 1998-2011 was attributable to a male-biased

fledging sex ratio, we ran a simulation model (100,000 replications) to establish a null distribution of sex ratios for ABJ returns, where each replication randomly sampled 30 sexed juveniles fledged from Seney NWR from 1996-2009. 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 being attributable to the sex ratio documented in the loon chicks fledged from 1996-2009.

## RESULTS

From 1996 through 2010, 184 and 154 Seney NWR Common Loon chicks hatched and fledged, respectively, and blood samples were collected from 137 and 135 of those chicks, respectively. The sex of 134 chicks was successfully determined using the PCR-based technique, representing 72.8% of total hatched chicks, 87.0% of total fledged chicks, and 97.8% of available samples. There was notable annual variation across the sampling period in the total number of chicks sampled—from two chicks in 1997 to 19 chicks in 2007—and in the observed sex ratios (see Table 1).

The proportion of male chicks hatched and fledged at Seney NWR from 1996-2010

was 0.537 (72 males:62 females) and 0.538 (71 males:61 females), respectively. The male bias in hatching and fledging did not differ significantly from 0.5 ( $P = 0.388$  and  $P = 0.384$ , respectively; Table 2). Chi-square tests indicated no significant difference (all  $P > 0.5$ ) between the degree of male bias among each of three subsets of hatched and fledged chicks grouped by hatch-type (“1-of-1” vs. “1-of-2” vs. “2-of-2”), nor between these groups and the overall male bias in hatching and fledging sex ratios (all  $P > 0.6$ ). Similarly, more male chicks than female chicks were documented in northern Wisconsin and Isle Royale National Park (Table 2), but only one subset sample ( $n = 22$ ) from northern Wisconsin in 2003 showed a significant male bias (Table 3). In pooling all sampled juveniles from the region (Seney NWR, Wisconsin, and Isle Royale National Park), the number of male chicks ( $n = 189$ ) was not significantly different ( $P = 0.096$ ) from the number of female chicks ( $n = 158$ ). The sex ratio of migrant adults killed on Lake Huron was not different from parity (Table 2).

Although the proportion of male offspring appeared to correlate with increased mass of both male and female parents (Figs.

**Table 1. Annual productivity and sexing data for Common Loon chicks hatched and fledged at Seney National Wildlife Refuge, 1996-2010.**

Year	# Ha <sup>1</sup>	# Fl <sup>2</sup>	# Sam <sup>3</sup>	Sex of Hatched			Sex of Fledged		
				♂	♀	% Sexed	♂	♀	% Sexed
1996	6	4	3	3	0	50%	3	0	75%
1997	6	6	2	0	2	33%	0	2	33%
1998	11	11	9	5	4	82%	5	4	82%
1999	9	8	8	3	5	89%	3	5	100%
2000	14	14	14	8	6	100%	8	6	100%
2001	16	13	5	3	2	31%	3	2	38%
2002	17	13	12	6	6	71%	6	6	92%
2003	7	6	6	4	2	86%	4	2	100%
2004	11	9	7	4	3	64%	4	3	78%
2005	20	16	16	12	4	80%	12	3	94%
2006	14	12	12	6	6	86%	6	6	100%
2007	24	20	19	10	9	79%	10	9	95%
2008	7	6	4	1	3	57%	1	3	67%
2009	10	8	9	4	5	90%	3	5	100%
2010	12	8	10	3	5	67%	3	5	100%
Total	184	154	134	72	62	73%	71	61	86%

<sup>1</sup>Hatched chicks.

<sup>2</sup>Fledged chicks.

<sup>3</sup>Sampled chicks.

Table 2. Sex ratios of juvenile and adult Common Loons and chi-square values for tests of deviation of sex ratios from parity.

Groups	Age Class	Group	Location	<i>n</i>	♂	♀	Proportion of ♂	$\chi^2$ value	<i>P</i> -value
Groups	Juvenile	Breeding	Seney National Wildlife Refuge	132	71	61	0.538	0.758	0.384
	Juvenile	Breeding	Northern Wisconsin (1999, 2000, 2003)	82	44	38	0.537	0.439	0.508
	Juvenile	Breeding	Northern Wisconsin (2004, 2005)	119	65	54	0.546	1.017	0.313
	Juvenile	Breeding	Isle Royale National Park	14	9	5	0.643	1.143	0.285
Hatching <sup>1</sup>	Adult	Migratory	<i>Combined breeding groups</i> Lake Huron Botulism (1999)	347	189	158	0.545	2.769	0.096
	Juvenile	Breeding	1-of-1	96	49	47	0.510	0.042	0.838
	Juvenile	Breeding	2-of-2	40	23	17	0.575	0.900	0.343
	Juvenile	Breeding	1-of-2	72	38	34	0.528	0.222	0.637
	Juvenile	Breeding	1-of-2	22	11	11	0.500	0.000	1.000
Fledging <sup>1</sup>	Juvenile	Breeding	Overall Hatching Sex Ratio <sup>2</sup>	134	72	62	0.537	0.746	0.388
	Juvenile	Breeding	1-of-1	40	23	17	0.575	0.900	0.343
	Juvenile	Breeding	2-of-2	71	37	34	0.521	0.127	0.722
	Juvenile	Breeding	1-of-2	21	11	10	0.524	0.048	0.827
	Juvenile	Breeding	Overall Fledging Sex Ratio <sup>3</sup>	132	71	61	0.538	0.758	0.384

<sup>1</sup>Long-term annual productivity dataset from Seney National Wildlife Refuge.<sup>2</sup>Calculated using all hatched chicks of known sex.<sup>3</sup>Calculated using all fledged chicks of known sex.

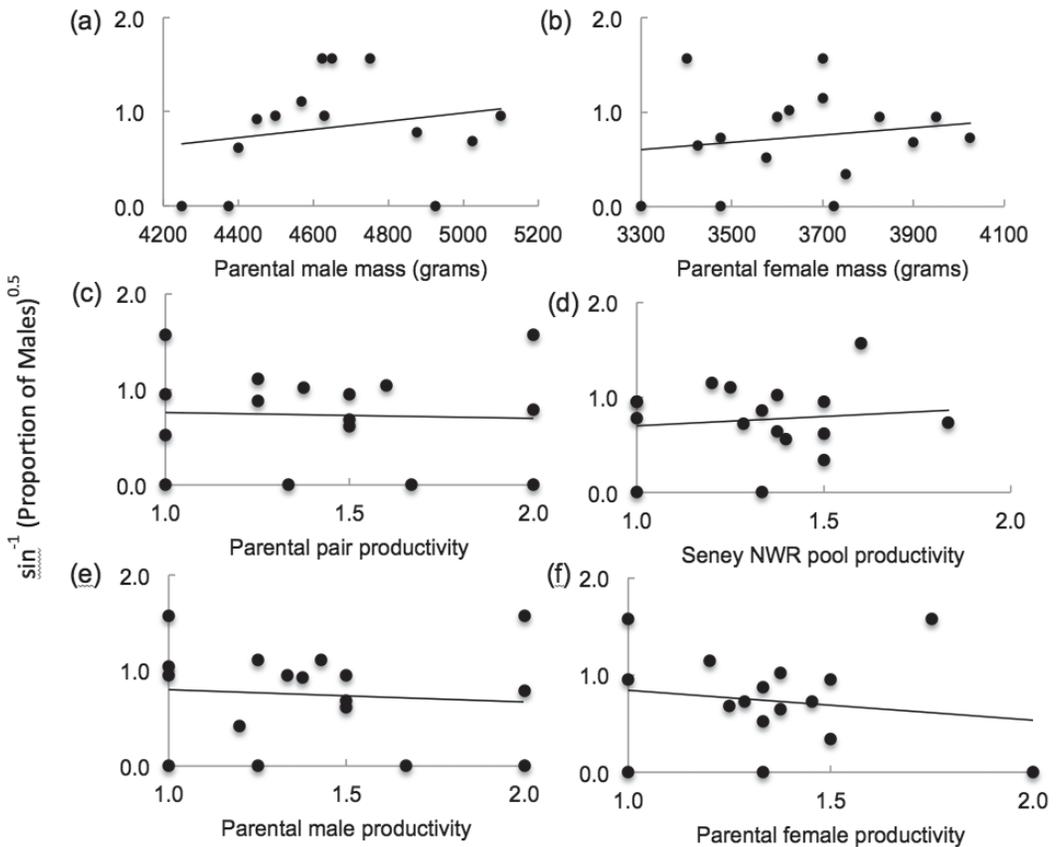
**Table 3. Juvenile sex ratios observed in hatched (1999-2003) and fledged (2004-2005) northern Wisconsin Common Loons and chi-square values for tests of deviation of sex ratios from parity. \* indicates significance with  $\alpha = 0.05$ .**

Sex Ratios <sup>1</sup>	Year	<i>n</i>	♂	♀	Proportion of Males	$\chi^2$ value	<i>P</i> value
Hatching	1999	24	14	10	0.583	0.667	0.414
Sex	2000	36	14	22	0.389	1.778	0.182
Ratios <sup>1</sup>	2003	22	16	6	0.727	4.545	0.033*
Fledging	2004	58	34	24	0.586	1.724	0.189
Sex Ratios	2005	61	31	30	0.508	0.016	0.898
Total		201	109	92	0.542	1.438	0.230

<sup>1</sup>Data provided by K. Kenow, pers. commun.

1a and 1b) and with increased pool productivity (Fig. 1c), these associations were not significant (Table 4). None of the linear regression analyses showed a significant correlation (Table 4) between pool/male/female/productivity (Figs. 1d, 1e, and 1f,

respectively) and the sex of offspring. The binomial logistic regression model revealed no relationship (Nagelkerke  $R^2 = 0.048$ ,  $X^2 = 11.510$ ,  $df = 8$ ,  $P = 0.174$ ) between the probability of having a male offspring and the mass of the adult male or the adult female.



**Figure 1. Scatterplots (with trend lines shown for each) of the proportion of juvenile male Common Loons fledged from Seney National Wildlife Refuge (NWR; vertical axes) plotted against (a) the mass of the parental male, (b) the mass of the parental female, (c) the average productivity of parental pairs, (d) the average productivity of Seney NWR pools, (e) the average productivity of parental males, and (f) the average productivity of parental females.**

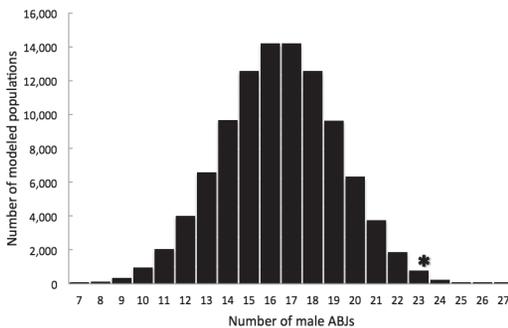
**Table 4. Linear regression results assessing the proportion of juvenile male Common Loons fledged at Seney National Wildlife Refuge as a function of measures of productivity and parental quality.**

Proportion of Males Fledged as Function of:	ANOVA Results					Regression Coefficient	
	<i>n</i>	<i>R</i> <sup>2</sup>	<i>F</i>	df	<i>P</i> -value	Individual Variation	Constant
Parental male mass	14	0.041	0.519	1	0.485	0.000	-1.200
Parental female mass	16	0.027	0.383	1	0.546	0.000	-0.669
Pool productivity	20	0.063	1.207	1	0.286	0.192	0.489
Parental pair productivity	26	0.059	1.383	1	0.252	-0.376	1.360
Parental male productivity	24	0.001	0.027	1	0.870	-0.056	0.744
Parental female productivity	18	0.064	1.093	1	0.311	-0.480	1.438

From 1998 through 2011, 23 male and seven female ABJs were observed at Seney NWR. In 100,000 iterations of the Monte Carlo simulation, a male bias equal to or greater than 23 was recorded in only 1.13% of the runs ( $P = 0.0113$ ) (Fig. 2).

#### DISCUSSION

Although proportionally more male chicks were hatched and fledged at Seney NWR from 1996-2010, the observed male bias was not statistically significant. As the fledging sex ratio dataset incorporates 86% of loon chicks fledged from Seney NWR from 1996-2010, it is likely this estimation is quite close to the actual sex ratio of chicks fledged from the entire Seney NWR popu-



**Figure 2. Monte Carlo simulation test of sex-bias in Common Loon adults banded as juveniles (ABJs) at Seney National Wildlife Refuge (NWR). Histogram shows the number of simulations (vertical axis) wherein a particular number of males returned among 30 total candidate ABJs (horizontal axis). Out of 100,000 simulations, only 1,128 (1.13%) iterations produced 23 or more males returned to the modeled population; \* notes the actual number of male ABJs (23) that were observed at Seney NWR from 1998-2011.**

lation during that period. Calculation of a hatching sex ratio among Seney NWR loons was complicated by the fact that 47 of the 184 chicks hatched from 1996-2010 went unsampled (primarily due to death before 5 weeks of age) and, therefore, unsexed. A female sex-bias in pre-fledging mortality could lead to a greater proportion of males in the sample of birds that were captured and subsequently sexed. To eliminate this possible influence of pre-fledging female-biased attrition, we separated sexed juveniles into hatch-types (hatch “1-of-1,” “1-of-2,” and “2-of-2”) and compared these to one another and to the overall sex ratio at hatch (Table 2). None of the groups demonstrated a sex ratio differing from parity, and none of the sex ratios significantly differed from one another (data not shown), thus providing no evidence of sex-biased attrition between hatching and fledging.

As at Seney NWR, we found proportionally more males among Common Loon chicks captured in northern Wisconsin in 2004 and 2005, although again the bias was non-significant ( $P = 0.313$ ; Table 2). Wisconsin chicks (1999-2003) used in the Kenow *et al.* (2003, 2007) studies were also analyzed, but the male bias observed in that group was similarly non-significant ( $P = 0.537$ ; Table 2). Although the sex ratio documented among Isle Royale National Park chicks ( $n = 14$ ) was the most highly skewed toward males (0.643), the small sample size precluded significance in this bias.

Although none of the sex ratios among individual breeding populations statistically differed from parity, all populations demonstrated a similar pattern of proportion-

ally more males than females (Table 2). Both Seney NWR and northern Wisconsin expressed an annual male bias in fledged chicks: Seney NWR had eight male-biased, five female-biased, and two even seasons, while Wisconsin had four male-biased and one female-biased seasons. Power analyses indicate that for the level of male bias we detected in most populations (0.54) to be significantly different from parity, a sample size of nearly 1,000 individuals would be necessary. Even when aggregating data from all juveniles ( $n = 347$ ) in the three study areas (Seney NWR, Isle Royale National Park and northern Wisconsin,), the male bias (0.545) only approached statistical significance ( $P = 0.096$ ). While the pattern of male bias in juvenile sex ratios is suggestive, our limited sample sizes preclude identifying this bias as statistically different from parity.

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 the regression analyses suggest that external factors including natal pool productivity, clutch size, and parental quality (expressed as mass) are not significant predictors of offspring sex at Seney NWR.

Little research has examined variation in adult, or tertiary, sex ratios within Common Loon populations. In part, this is because adult populations have largely been studied by focusing solely on territorial breeding pairs (Paruk 2006; Mitro *et al.* 2008; Mager *et al.* 2010), since non-territorial birds are hard to reliably locate and identify, and harder still to capture and sample. We 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. The sex ratio among migratory adult birds collected from southern Lake Huron was nearly balanced at 0.510. These loons died during a widespread outbreak of avian botulism, a disease agent that presumably has no sex-bias in its action;

thus, these adults are likely a representative sample of all breeding loons migrating through the lake at that time, and provide a less biased estimator of the tertiary sex ratio than studies of territorial breeders. Sex ratios among migrating and overwintering loons warrant further research to determine whether sex-specific physiological or environmental stresses exist for adults and sub-adults. Suggestively, Forrester *et al.* (1997) reported a slight female bias (0.520) among 327 age-undifferentiated Common Loons that died in Florida coastal waters from a variety of causes (primarily emaciation syndrome, oiling, and aspergillosis) during the winters of 1970 through 1994.

From this study alone, it is not possible to determine whether male-biased sex ratios are characteristic of Common Loon breeding populations. If adult sex ratios among loons do not follow the one-to-one ratio suggested by Fisher (1930), our findings would suggest the possibility of a male bias in breeding populations. This would not be abnormal among avian species, 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 male-biased juvenile sex ratios exist among breeding loons, it is highly unlikely that this factor alone accounts for the significant male bias in ABJ return rates to Seney NWR. In accordance with Greenwood's (1980) hypothesis, it is probable that among Common Loons a significantly greater proportion of adult males than females returns to their natal breeding grounds, while a majority of the latter disperse to other breeding locations. This hypothesis is supported by the results of the ABJ simulation model, which demonstrated that the observed sex-bias in ABJ return rates at Seney NWR is highly unlikely to have resulted solely from

the documented sex-bias in fledging rate ( $P = 0.0113$ ), and by the tertiary sex ratio of dead Lake Huron migrants, which found no male bias among that representative sample of breeding adults. Sex-biased philopatry comparable to that observed at Seney NWR has been documented across several avian orders and may represent an evolutionarily adaptive trait that reduces the probability of mating with a related individual, thus limiting inbreeding within populations and promoting gene flow between populations. Although this rationale is appealing for explaining the male-biased ABJ observations at Seney NWR, further research is needed to evaluate whether and to what extent other factors such as female-biased sub-adult attrition on wintering grounds or during migration may influence sex ratios among adult Common Loons.

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