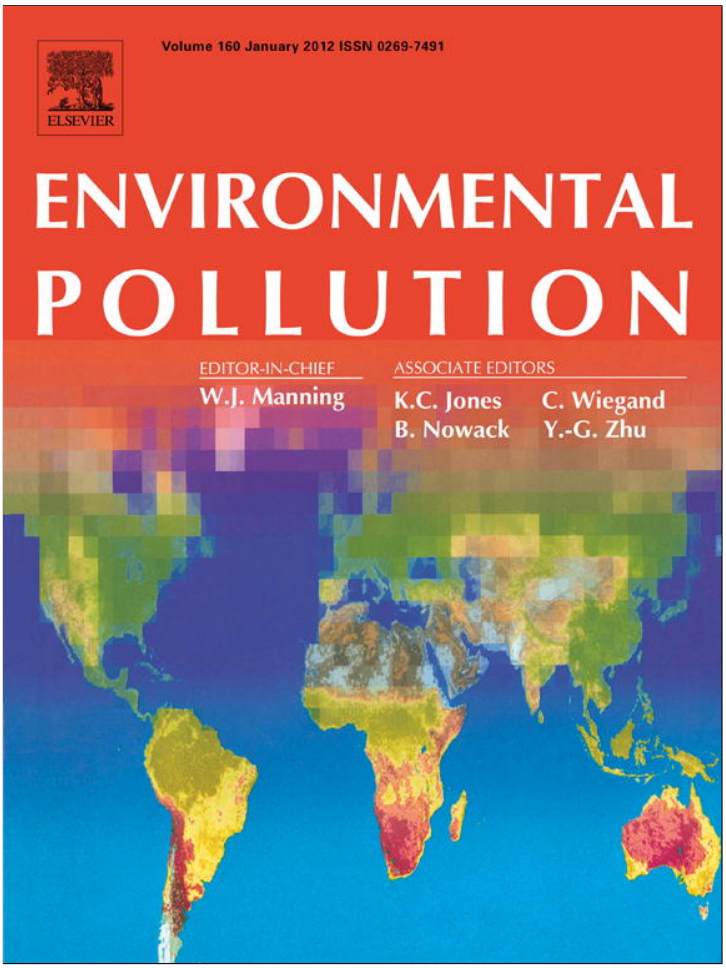

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Effects of dietary selenium on the health and survival of captive wintering lesser scaup

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ABSTRACT

Accumulation of selenium (Se) by lesser and greater scaup (*Aythya affinis, A. marila*) at staging and wintering areas could have contributed to the decline in their continental population. We exposed lesser scaup to background (0.8 μg/g), moderate (8.1 μg/g) and high (20.7 μg/g) levels of dietary Se in captivity and measured survival rates and indices of health in relation to hepatic Se concentrations. There was 100% survival in scaup exposed to Se for 10-weeks (average staging duration at Great Lakes), but ducks in the high treatment group had less lipids. There was 93% survival after 23-weeks (average wintering duration at Great Lakes), but no differences among treatment groups in body composition. There were no effects of Se on oxidative stress and cell-mediated immunity; rather we recorded immuno-stimulatory effects on antibody production. Results from our captive study suggest Se alone did not cause the continental decline in scaup populations.

1. Introduction

The combined population of Greater (*Aythya marila*) and Lesser Scaup (*A. affinis*) (hereafter scaup) decreased substantially between the mid-1980s and late-1990s in North America (Afton and Anderson, 2001). Although the population since has been stable to slightly increasing, in 2011 the estimated population of scaup (4.3 ± 0.3 million) remained 15% below the long-term average and 27% below the North American Waterfowl Management Plan population goal of 6.3 million (U.S. Fish and Wildlife Service, 2009). Several hypotheses have been proposed to explain the decrease in abundance of breeding scaup including loss and degradation of breeding habitat, changes in food resources, and increased contaminant burdens (Austin et al., 2000). The contaminant hypothesis proposed that the decrease in the continental scaup population resulted from increased exposure to environmental contaminants leading to decreased fitness (Austin et al., 2000). Previous studies have identified selenium (Se) as a contaminant of concern for scaup, especially in the Great Lakes region (Custer and Custer, 2000; Custer et al., 2003; Petrie et al., 2007; Anteau et al., 2007; Ware, 2008; Ware et al., 2012). Subsequent research concluded that Se acquired by scaup was not negatively influencing reproduction (Fox et al., 2005; DeVink et al., 2008a; Badzinski et al., 2009). However, it remained unclear whether Se burdens were affecting health or survival of non-breeding scaup at the Great Lakes.

An abundance and diversity of wetlands and open water habitats occur at the lower Great Lakes (LGL) (including lakes Ontario, Erie and St. Clair, as well as, the Niagara, Detroit and St. Clair Rivers) that provide staging and wintering habitat for migratory birds (Prince et al., 1992; Petrie, 1998). Historically, the LGL has been used by up to 500,000 staging (10–12 weeks) and 100,000 wintering (12–24 weeks) scaup (Bellrose, 1980; Dennis et al., 1984). However, with a trend toward annually decreasing ice cover (Assel, 2003), and increased food supply (Custer and Custer, 1996; Petrie and Knapton, 1999), scaup distribution, duration of stay, abundance and diets have changed substantially over the past 20 years (Custer and Custer, 1996; Petrie and Knapton, 1999; Badzinski and Petrie, 2006; Canadian Wildlife Service and Long Point Waterfowl, Unpublished data).

Non-native zebra (*Dreissena polymorpha*) and quagga (*D. bugensis*) mussels (hereafter dreissenid mussels) were inadvertently introduced into the LGL in the 1980s and 1990s, respectively (Neary and Leach, 1992; Kovalak et al., 1993; Leach, 1993).

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Following introduction, scaup shifted from native foods to a diet dominated by dreissenid mussels (Custer and Custer, 1996; Petrie and Knapton, 1999; Badzinski and Petrie, 2006). Because dreissenid mussels filter substantial quantities of water during feeding, bioaccumulated contaminants, such as Se, can be transferred to species that eat them (de Kock and Bowmer, 1993; Mills et al., 1993). Increased abundance of food (i.e., dreissenid mussels) also may have enabled scaup to increase duration of time spent on the LGL, thereby increasing their exposure to contaminants (Petrie and Knapton, 1999). Several chemical forms of Se exist, but most laboratory data used to derive threshold concentrations are based on selenomethionine, the most toxic and available form to wildlife (Heinz, 1996; Hoffman et al., 1996). Based on studies of captive mallards (*Anas platyrhynchos*), hepatic Se concentrations $\geq$10 μg/g (hereafter concentrations are reported as dry tissue weight [dw]) are considered elevated and can cause reproductive impairment (e.g., deformities of embryos and hatching failure), concentrations $\geq$33 μg/g can cause sub-lethal effects (e.g., decreased body weight and histopathological lesions), and concentrations $>$ 60 μg/g can cause adult and juvenile mortality (Heinz et al., 1989; Heinz and Fitzgerald, 1993a,b; Heinz, 1996). However, these thresholds might not apply to scaup because Se tolerances vary among species of waterfowl (Skorupa, 1998; DeVink et al., 2008b).

Elevated concentrations of hepatic Se have been documented in lesser scaup and greater scaup staging and wintering at the LGL (Custer and Custer, 2000; Petrie et al., 2007; Ware et al., 2011). Ware et al. (2012) did not detect a relationship between indices of condition and hepatic Se concentrations in a sample of greater scaup wintering at western Lake Ontario. However, field collections of waterfowl can introduce bias because of over-representation of behaviorally compromised ducks and under-representation of dead ducks (Bain, 1980; Greenwood et al., 1986; Dufour et al., 1993; Pace and Afton, 1999), thus controlled captive studies are needed to corroborate or refute prior findings.

The following questions were addressed regarding effects of Se acquisition on lesser scaup, 1) can hepatic Se concentrations that exceed background concentrations reduce survival of lesser scaup throughout the winter?, 2) do elevated Se concentrations compromise health and body condition?, and 3) is the Se threshold for impaired health/survival derived using mallards, and commonly applied to all waterfowl ($\geq$33 μg/g, Heinz, 1996), appropriate for lesser scaup?

2. Materials & methods

2.1. Study design

This study was conducted at a captive facility 6.8 km south of Aylmer, Ontario (42.7379° N, −81.0104°W), and was approved by the University of Western Ontario (UWO) Animal Care and Use Committee (protocol #2008-044-03) and Canadian Wildlife Service (permit # CWS08-S003). Fifty-four wild-strain, captive reared lesser scaup (female $n = 28$, male $n = 26$) used in this study were housed in three outdoor 30.5 × 30.5 m pens enclosed with chain linked fencing and topped with netting. Sample sizes of males and females differed because of differences in survival of males and females between hatching and the beginning of our experiment.

Each pen was sod-lined, with an in-ground freshwater pond and rain protected food dish. Ducks were randomly assigned to the control or one of two Se enriched diet treatment groups with two exposure durations typical at the LGL (staging 10-weeks and wintering 23-weeks). To obtain desired Se concentrations in each treatment diet, Mazuri Sea Duck Diet pellets were ground into crumbs and L-selenomethionine (molecular weight, 196.11 g/mol) was dissolved in distilled water and sprayed directly onto food. Food was then re-pelleted and dried at the Arkell Feed Mill in Guelph, Ontario. The concentration applied for the high Se treatment diet was 1456.6 mg L-selenomethionine in 0.5 L distilled H^2O per 25 kg bag of food. The moderate treatment group received half the Se applied to the high treatment group.

During a three-week acclimation period, birds were fed untreated Mazuri Sea Duck Diet in pellet form ad libitum. At the beginning of the experiment, scaup were fed Mazuri Sea Duck Diet ad libitum, which was treated with either distilled water (control, female = 10, male = 8) or one of the two Se treatments. Target concentrations in the feed were 11.5 μg/g as Se (moderate, female = 8, male = 9), and 23.0 μg/g (high, female = 10, male = 9). Analyses of Se indicated actual treatment concentrations were 0.84 ± 0.06 μg/g, 8.08 ± 0.92 μg/g, and 20.66 ± 2.92 μg/g for control, moderate and high treatment groups, respectively. The moderate treatment was selected to simulate exposure similar to the maximum reported concentration in zebra mussels from the LGL (i.e., 11.5 μg/g) (U.S. Department of Commerce, 2007), and the high treatment was chosen as an extreme dose (i.e., 23.0 μg/g) to attain sub-lethal hepatic levels (Eisler, 2000). Exposure duration of ducks to treated diets was based on average time scaup spend staging (10 weeks) and maximum time spent wintering (up to 24 weeks) on the LGL. Treatments for both durations began 3 November 2008 and ended 9 January and 9 April 2009, for the staging and wintering durations, respectively.

2.2. Monitoring & sampling

Scaup in treatment groups were observed daily to record daily survival, behavior and to ensure ducks were eating provisioned food. In the captive facility it was not possible to record amount of food eaten by individual ducks, but group intake of each pen was measured as frequency of refills with a 750 g scoop. Food samples were collected and frozen on a weekly basis and analyzed for Se concentration to ensure Se content in the food remained constant over time. All birds were captured with dip nets and weighed to the nearest 0.01 g with a Mettler–Toledo digital scale when treatments started and on weeks 4, 6, 7, 10, 13, 15, 17, 18, 19, 20, 21, 22, and 23.

Ducks were euthanized by cervical dislocation (UWO protocol #2008-044-03), bagged, and frozen on their predetermined endpoints (10-weeks and 23-weeks based on average length of staging and wintering, respectively). Prior to dissection, ducks were partially thawed, and livers collected. To examine Se allocation to reproductive tissues and depuration into feathers during molt, ovaries and 0.25–1.00 g of pin feathers from the breast region were removed from the 23-week duration exposure group. Right and left lobes of liver were separated, bagged and frozen in a standard freezer (−20 °C) for future analysis.

2.3. Dissections and carcass analysis

Before dissection, the following structural measurements were taken using digital calipers (±0.01 mm): 1) culmen length, 2) skull width, 3) head length, 4) bill width, 5) total tarsus length, and 6) internal keel length and body length was measured with a meter stick (±0.01 mm; Dzubin and Cooch, 1992). During dissections, the liver, kidneys, heart, pancreas, and gastrointestinal tract were examined and weighed to the nearest 0.01 g, wet weight (w/w). Lipid and protein content were determined by proximate analysis at the Avian Energetics Laboratory in Port Rowan, Ontario (sensu Badzinski and Petrie, 2006).

2.4. Contaminant analysis

Frozen liver, ovary, feather, and feed samples were analyzed at Laurentian University, Sudbury, Ontario (Belzile et al., 2005). Samples were freeze-dried and ground to a fine powder before digestion. After homogenization, a 0.2-g sample was precisely weighed and digested with 2.0 mL of 30% (w/w) H_2O_2 and 8.0 mL of 15.0 M HNO_3 in a microwave digestion system. A procedure including a three-step pre-heating process was applied and the microwave digestion was done at 210 °C for 10 min. The digest was diluted to appropriate concentration before the determination of total Se by hydride generation-atomic fluorescence spectrometry (HG-AFS; PSA Millennium Excalibur 10.055). The instrument detection limit was 1.00 μg/g for Se. For quality control, the certified reference materials DOLT-2 (dogfish liver) and DORM-2 (dog fish muscle) were used. For every eight samples digested, a reagent blank and a DOLT-2 (6.06 ± 0.49 μg/g) and DORM-2 (1.40 ± 0.09 μg/g) were used as standard reference material for liver, ovary and pinfeather samples. DORM-2 alone was used for feed samples; recovery from all reference materials was within the certified variation range (National Research Council Canada, 2009).

2.5. Immune function

Immune function was assessed using two assays: (1) antibody response to sheep red blood cells (SRBC) and (2) the skin swelling response to phytohemagglutinin-P (PHA-P) (Fairbrother et al., 2004). Two inoculations and three blood samples were completed to quantify baseline (pre-SRBC injection), primary (post SRBC injection #1), and secondary (post SRBC injection #2) antibody response. Scaup were inoculated intravenously (meta-tarsal vein) with 1 mL of 10% SRBC for every 1.00 kg of body weight on day 33, 40, and 59 (10-week duration), and 117, 124, and 138 (23-week duration) (Wayland et al., 2002). Blood was drawn from the brachial vein approximately one week after inoculations with a 23-gauge heparinized needle. It was then centrifuged and the plasma frozen within six hours of collection. Antibodies (IgG and IgM) produced in response to SRBC injections were collected in plasma samples and titrated for hemagglutination; methods followed Wayland et al. (2002). Hemagglutination titers were then grouped by scores (SCORES) based on antibody presence achieved in the highest dilution (Wayland et al., 2002).

On day 66 (10-week duration) and day 144 (23-week duration) the thickness of the right wing web was, 1) measured twice to the nearest 0.01 mm with a pressure sensitive micrometer and averaged and 2) 0.1 mL PHA-P in phosphate buffered

solution (PBS) (1.00 mg/mL) was injected intradermally into the right wing web, while only PBS (0.10 mL) was injected into the left wing web. The wing web was measured 24 h after the last SRBC blood sample, and the response to PHA-P was calculated as the difference between the pre- and post-injection measurements. The response of the test was calculated as follows:

$$(RW_{post-injection} - RW_{pre-injection}) - (LW_{post-injection} - LW_{pre-injection})$$

Where RW and LW refer to PHA-P injected right wing web, PBS injected left wing web (Fairbrother and Fowles, 1990).

2.6. Oxidative stress

Frozen left liver lobes (−20 °C) were sent to the Ecotoxicology Laboratory in the Department of Biological Sciences at the University of Lethbridge to quantify oxidative stress. Extent of oxidative stress was assessed by measuring malondialdehyde (MDA) concentration- a product of lipid peroxidation (LPO), and antioxidant capacity by determining glutathionine (GSH) concentrations. Analysis and determination of MDA and GSH followed Miller et al. (2007).

2.7. Statistical analysis

Statistical analyses were conducted using SAS version 9.2 (SAS Institute, 2009). With the exception of the body mass trend evaluation, all analyses (body composition, organ mass, oxidative stress, immune challenges, Se concentration of biological samples) were separated by exposure duration (10-week vs. 23-week). Therefore, exposure duration was not included in body mass models. Because the study was experimental, results are considered significant at $P \leq 0.05$ for all tests, but we discuss results at $P \leq 0.10$ because not all environmental variables were controllable (Tacha et al., 1982). Throughout, we log-transformed data when appropriate, inspected studentized residuals for normality, and report back-transformed geometric means, Tukey adjusted least-squared means, and 95% confidence limits. When outliers were identified among studentized residuals ($-2.5 \leq$ STDRES ≤ 2.5) we compared outcomes of statistical analyses both with the full dataset and with outliers removed. In all cases, analyses produced similar results with and without outliers removed, so results that follow for all tests are based on analyses of full datasets.

2.7.1. Selenium concentrations in biological samples

Se concentrations in liver and pinfeather samples were compared for each treatment group using Mixed Models (PROC MIXED, SAS Institute, 2009). Analyses were conducted on liver (10-week exposure: n male = 12, female = 15; 23 week exposure: n male = 10, female = 15) and feathers (n male = 10, female = 15) to test if concentrations of Se varied among treatment groups (TRT). Variation attributable to sex was controlled for by including this metric as a covariate. Among-TRT differences were identified using post hoc Tukey's tests. The pattern of among-TRT differences in liver Se concentrations (see results) provided support that differences among TRT in subsequent analyses were from acquired Se burdens. Thus, TRT was used as an independent variable (categorical) in analyses. Because of small sample sizes for ovaries we present only descriptive statistics. Eight females were sub-sampled for analysis of Se in ovaries (n control = 3, moderate = 3, high = 2) representing at least 50% of females in each TRT.

2.7.2. Trends in body mass

Measurements of body mass recorded for each individual throughout the study were analyzed using mixed models (PROC MIXED, SAS Inc. 2009). The model included TRT (control, moderate, high), sample period (categorical [1–15]), and their interaction. Sex was included as a covariate (male or female) to control for sex-specific differences. Individual birds were nested within TRT to account for repeated measurements of mass from the same duck. Individual duck identification numbers were included as a random variable and variance components (VC) was used from a suite of tested covariance structures (i.e., CS, UN, TOEP, AR[1], ARH[1], VC), because VC produced the best fit models (lowest AICc values; Littell et al., 2006). Raw body mass data were not normal, thus body mass data were log-transformed prior to analysis and resultant studentized residuals approximated a normal distribution. Trends in body mass are presented as least-square means of log-transformed data to control for variation attributable to other variables in the model.

2.7.3. Body composition and organ mass

Principal Components Analysis (PCA) was conducted on the correlation matrices of total tarsus length, head length, skull width, culmen length, keel length, and body length (PROC PRINCOMP, SAS Institute, 2009), and PC1 explained variation in structural size (46%) and eigenvectors were 0.33–0.52, suggesting that they described variation in overall body size. Therefore, we included PC1 scores as a covariate in all models to control for structural size differences among individuals. Multiple analysis of covariance (MANCOVA) was used on 10- and 23-week duration organ masses and we included PC1 as covariate with the main effect of TRT to determine if organ masses (log-transformed) varied with Se concentrations. General Linear Models (PROC GLM, SAS Institute, 2009) were used to determine if lipid and protein (log-transformed) data varied with TRT and PC1 was included as a covariate. Among-TRT comparisons for organs, lipid and protein were made using a post-hoc Tukey's test.

2.7.4. Immune challenges & oxidative stress

Least squares regression was used to model the relationship between the response of subcutaneous PHA injections and the hepatic Se concentrations (μg/g) of each bird. Data collected from SRBC hemagglutination titers were analyzed with a Chi-Square test. Scores (SCORE) were grouped categorically into low (2–8), medium (16–32), and high (128–256) antibody presence to increase statistical power (i.e., degrees of freedom) and enable interpretation of results. Data were analyzed separately by exposure duration when blood samples were taken; 1) baseline antibody levels (baseAB), 2) primary (IgM) antibodies, and 3) gauged secondary (IgG) antibodies. Chi-square for each blood sample date was conducted to represent groups of antibodies (Y [antibodies] = TRT × SCORE). Least squares regression was used to model the relationship between endpoints of oxidative stress (MDA and GSH) and the hepatic Se concentrations (μg/g) of each bird.

3. Results

3.1. Selenium concentrations

After the 10-week duration, geometric mean (95% Confidence Limits [CL]; μg/g dry weight) hepatic Se concentrations for control ($\bar{x} = 5.53$ [4.73–6.57]; $n = 9$), moderate ($\bar{x} = 39.00$ [33.96–45.29]; $n = 9$), and high ($\bar{x} = 73.91$ [63.05–87.58]; $n = 9$), TRT were different ($F_{2, 23} = 373.29$, $P < 0.01$), and exhibited a monotonical relationship corresponding to increasing dietary concentrations of Se. Following the 23-week duration, geometric mean (95% CL) hepatic Se concentrations differed ($F_{2, 21} = 1234.31$, $P < 0.01$) among control ($\bar{x} = 4.76$ [4.40–5.16]; $n = 9$), moderate ($\bar{x} = 25.75$ [24.83–27.37]; $n = 9$), and high ($\bar{x} = 60.87$ [51.88–69.18]; $n = 7$). Amount of Se depurated into pin feathers of scaup exposed for 23-weeks differed ($F_{2, 21} = 749.94$, $P < 0.01$), among control ($\bar{x} = 1.39$ [1.28–1.52]; $n = 9$) moderate ($\bar{x} = 24.84$ [22.61–27.33]; $n = 9$), and high ($\bar{x} = 53.67$ [40.12–74.39]; $n = 7$) TRT. Se in ovaries collected from females exposed to dietary Se for 23-weeks increased from control ($\bar{x} = 2.47$ [1.39–3.61]; $n = 3$), moderate ($\bar{x} = 15.42$ [13.23–17.46]; $n = 3$), to high ($\bar{x} = 33.84$ [83.31–15.17]; $n = 2$) TRT.

3.2. Survival

There was a 100% survival of scaup in 10-week exposure duration groups. Two out of nine ducks died in the high Se TRT in the 23-week duration group at week 7 of the experiment (winter survival = 78%). Because these ducks drowned under ice cover at week 7, they did not reach their pre-designated end point and it was not possible to link their mortality directly to Se burdens and, thus, they were excluded from all other analyses. Further, masses of the drowned scaup were similar or greater than mean mass of scaup that survived past week 6 (drowned female = 762 g, surviving females $\bar{x} = 757$ g; drowned male = 806 g, surviving males $\bar{x} = 811$ g). Combined survival of the two exposure durations ($n = 54$) was 96%.

3.3. Trends in body mass

Treatment groups exhibited different patterns of weight change throughout winter (TRT × period; $F_{28, 455} = 6.61$, $P < 0.01$; Fig. 1). In general, TRT had similar mean weights in October, December, March, and April. Differences in body mass among TRT were greatest during January and February. Decreases in mean body mass were greatest between 8 and 30 January and were 54 g, 121 g and 87 g for control, moderate and high TRT, respectively.

3.4. Body composition

For scaup euthanized at the end of the 10-week duration, lipids ($F_{3, 23} = 8.23$, $P < 0.01$), but not protein ($F_{3, 23} = 1.93$, $P = 0.15$)

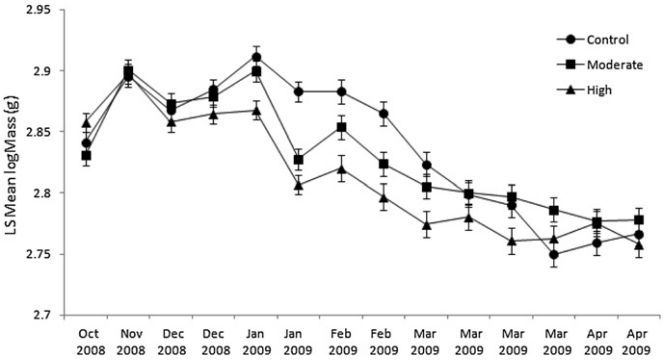

Fig. 1. Least squares mean (±SD) mass of lesser scaup from control, moderate, and high Se treatment groups. Treatment duration was 3 November 2008–9 April 2009.

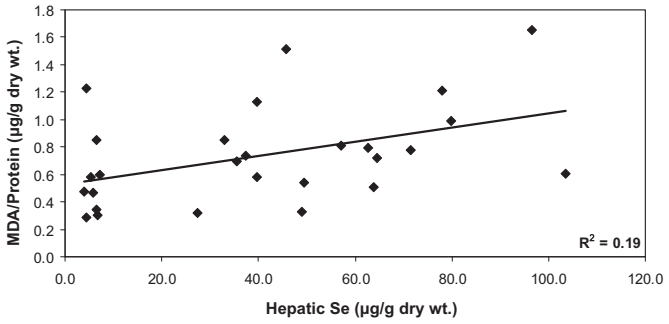

Fig. 2. Relationship between hepatic MDA/protein (µmol/mg) concentrations and increasing hepatic Se concentrations in captive lesser scaup exposed to dietary Se for a 10-week period.

differed among TRT (Table 1). Total lipid reserves in the high treatment group were 34% ($P < 0.01$) and 31% ($P < 0.01$) less than in the control and moderate groups, respectively. Lipid reserves did not differ between control-moderate. For scaup euthanized at the end of the 23-week exposure, lipids ($F_{3,\ 23} = 1.09$, $P = 0.38$) nor protein ($F_{3,\ 23} = 0.29$, $P = 0.83$) differed among TRT (Table 1).

3.5. Organ mass

There was no overall effect of Se concentration on organ mass TRT in the 10-week exposure (Wilks' $\lambda = 0.33$, $F_{2,\ 26} = 1.64$, $P = 0.14$), or 23-week exposure (Wilks' $\lambda = 0.26$, $F_{1,\ 24} = 1.63$, $P = 0.16$).

3.6. Oxidative stress

MDA concentrations ($R^2 = 0.19$, $F_{1,\ 26} = 5.79$, $P = 0.02$; Fig. 2), but not GSH levels ($F_{1,\ 26} = 0.03$, $P = 0.86$), varied positively with hepatic Se concentrations in the 10-week exposure group. In the 23-week exposure group, GSH ($R^2 = 0.17$, $F_{1,\ 24} = 4.71$, $P = 0.04$; Fig. 3), but not MDA ($F_{1,\ 26} = 0.09$, $P = 0.76$), varied positively with hepatic Se concentrations.

3.7. Immune system challenges

T-cell response, as indexed by amount of wing-web swelling after injection of PHA was not influenced by hepatic Se concentrations during the 10-week ($R^2 = 0.02$, $F_{2,\ 26} = 0.48$, $P = 0.23$) or 23-week ($R^2 = 0.01$, $F_{2,\ 24} = 0.278$, $P = 0.60$) exposure periods.

Table 1
Least-squares means (95% confidence interval) of total lipid and total protein of captive lesser scaup exposed to dietary Se for 10 and 23-week periods. Within exposure groups, least-squares means with similar letters are not significantly different ($P > 0.10$).

Exposure period and treatment group (n male and female)	Total lipid	Total protein
10-week exposure		
Control (male = 3, female = 6)	263.03 A (229.09–302.00)	134.90 A (120.23–151.36)
Moderate (male = 4, female = 5)	251.19 A (218.78–199.53)	147.91 A (131.83–165.96)
High (male = 5, female = 4)	173.78 B (154.88–288.40)	147.91 A (131.83–162.18)
23-week exposure		
Control (male = 4, female = 5)	56.23 A (44.67–72.44)	123.03 A (109.65–141.25)
Moderate (male = 4, female = 5)	58.88 A (45.71–74.13)	125.89 A (109.65–144.54)
High (male = 3, female = 4)	44.67 A (33.88–57.54)	123.03 A (107.15–144.54)

There was no difference in amount of baseline ($\chi^2 = 3.06$, df = 4, $P = 0.51$) or secondary (IgG) ($\chi^2 = 2.70$, df = 4, $P = 0.60$) antibodies among TRT in the 10-week exposure, but ducks in the high treatment group had greater primary (IgM) antibody titers than controls ($\chi^2 = 14.22$, df = 4, $P = 0.01$) (Fig. 4). During the 23-week period, no difference was detected in amount of baseline antibodies ($\chi^2 = 4.89$, df = 4, $P = 0.30$), but ducks in the moderate and high treatment groups had greater IgM ($\chi^2 = 8.47$, df = 4, $P = 0.02$) and IgG ($\chi^2 = 13.21$, df = 4, $P = 0.01$) antibodies in response to SRBC inoculations than ducks in control groups (Fig. 5).

4. Discussion

4.1. Hepatic selenium concentrations and survival

Scaup exposed to Se for 10-weeks had 100% survival, despite those in the moderate and high treatment groups having acquired hepatic Se concentrations that exceeded thresholds associated with sub-lethal and lethal effects in mallards (Heinz, 1996). Similarly, DeVink et al. (2008a) reported no mortality in lesser scaup fed 7.5 and 15.0 µg/g Se for 6-weeks. Thus, hepatic Se thresholds associated with sub-lethal and lethal effects can vary interspecifically and caution should be used when mallard thresholds are applied to other species (Skorupa, 1998; Wayland et al., 2002; Franson et al., 2007; DeVink, 2007). Determining species-specific thresholds would aid in understanding the possible effects of Se acquisition in waterfowl, thereby enabling proper identification of potentially problematic levels in these birds.

Mallards fed 20 µg/g Se for 16-weeks experienced 25% mortality (Heinz and Fitzgerald, 1993a), whereas lesser scaup in our study fed 20.7 µg/g for 23-weeks had 22% mortality. However, the 22% mortality was because two scaup became trapped under the ice and

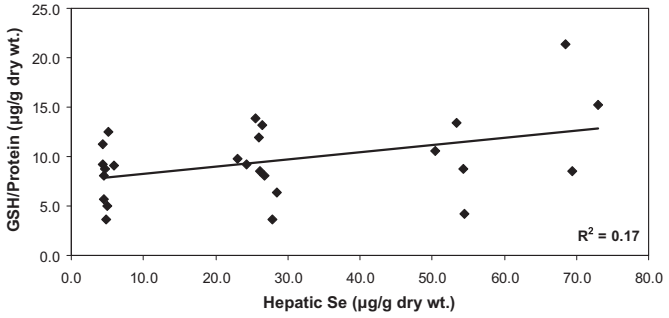

Fig. 3. Relationship between hepatic GSH/protein (µmol/mg) concentrations and increasing hepatic Se concentrations in captive lesser scaup exposed to dietary Se for a 23-week period.

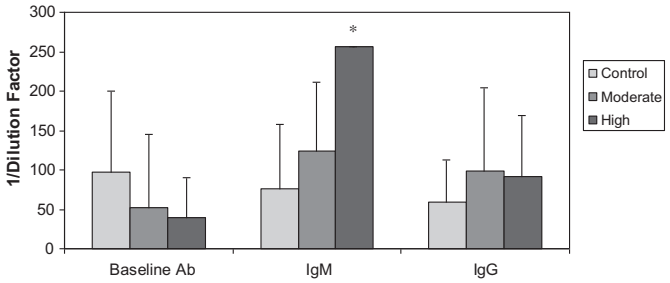

Fig. 4. Mean (±SD) of plasma hemagglutination antibody titers from captive lesser scaup prior to, and 7, and 27 days following SRBC inoculations during a 10-week Se exposure period. *All individuals in treatment group achieved the same level of agglutination. Therefore these categorical variables resulted in no standard error. For all tests and treatment groups, $n = 9$. Dilution ratios were scored by using the inverse of the highest dilution achieved in the titer by each individual (e.g., if an individual had enough antibodies in their plasma to achieve agglutination at a dilution of 1/128, then their antibody score was recorded as 128).

died. Several individuals within the 23-week exposure, high treatment group were noticeably less active relative to birds in both the control and moderate treatment groups (Brady, 2009). Thus, the ducks that became trapped under the ice may have died indirectly from physical or behavioral complications from sub-lethal Se levels. Ducks in the high treatment group were most easily captured for processing. Delay in reaction time to a perceived threat could also result in greater predation/harvest rates for scaup with sub-lethal Se levels in the wild. Changes in behavior related to contaminant exposure might have implications for survival of wild scaup and other waterfowl (Silver and Nudds, 1995), but behavioral changes were not the focus of this study. Overall, results suggest that hepatic Se concentrations equal to those recorded for wild scaup (≤59.7 μg/g; Petrie et al., 2007; Ware et al., 2011) are not directly compromising survival of these ducks staging and wintering at the LGL. We do caution that our findings may be biased by use of captive birds fed an ideal diet (i.e., Mazuri Sea Duck Diet ad libitum) that likely reduced stresses relative to wild scaup and the two mortalities in our study may have indirectly resulted from sub-lethal impacts of Se.

4.2. Seasonal trends in mass and body composition

The influence of elevated Se burdens on the body condition in birds remains unclear because of conflicting results (Yamamoto and Santolo, 2000; Wayland et al., 2002; Anteau et al., 2007; Franson et al., 2007; Ware, 2008; DeVink et al., 2008a; Ware et al., 2012). Lipid reserves varied positively with hepatic Se concentrations in wintering and spring migrating lesser scaup (range 3.7–52.3 μg/g) from the Mississippi flyway (Anteau et al., 2007), whereas no such relationship was detected in greater scaup wintering on the LGL (Ware et al., 2012) or in breeding lesser scaup in the boreal region of North America (DeVink, 2007). In contrast, in this study, scaup in the 10-week exposure group with greater hepatic Se burdens (range 57.0–103.6 μg/g) had less lipid reserves than those with lesser hepatic Se concentrations (range 3.9–49.4 μg/g). However, our study was conducted under relatively controlled conditions, reducing the likelihood of confounding interactions with other environmental contaminants. Further, hepatic Se concentrations in this study were greater than in the aforementioned studies. Simultaneous acquisition of contaminants might have a synergistic or antagonistic affect (Pollock and Machin, 2008; Schummer et al., 2011a; Heinz et al., 2011) and may have confounded previous field studies. Our results suggest a negative influence of Se on lipid levels, but they might not be representative of combinations of environmental contaminants and weather conditions that waterfowl are subject to at the LGL or other staging and wintering locales (for example Schummer et al., 2011b). Thus, we propose that the decreases in lipids we observed may be exacerbated in wild lesser scaup.

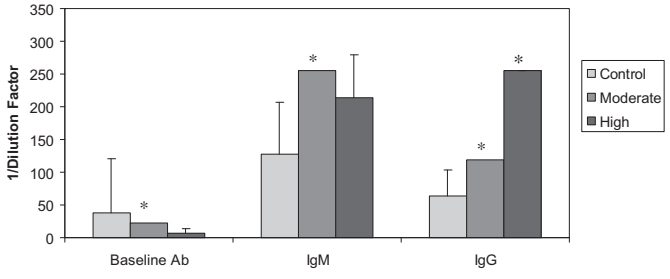

Fig. 5. Mean (±SD) of plasma hemagglutination antibody titers from captive lesser scaup prior to, and 7, and 21 days following SRBC inoculations during a 23-week Se exposure period. *All individuals in treatment group achieved the same level of agglutination. Therefore these categorical variables resulted in no standard error. For all tests and treatment groups, $n = 9$. Dilution ratios were scored by using the inverse of the highest dilution achieved in the titer by each individual (e.g., if an individual had enough antibodies in their plasma to achieve agglutination at a dilution of 1/128, then their antibody score was recorded as 128).

The decrease in body mass in all treatment groups detected throughout this study was typical for waterfowl during winter (King and Farner, 1965; Whyte and Bolen, 1984; Baldassarre and Bolen, 2006), but body mass generally was less in the high than control treatment group during January and February. Following winter, waterfowl forage extensively to increase lipid reserves for migration and reproduction, thus Se-related lipid declines could negatively influence these annual events (Baldassarre and Bolen, 2006). Heinz and Fitzgerald (1993a) suggested decreasing temperatures lowered the Se dietary threshold causing weight loss and mortality in adult mallards. Although hepatic Se concentrations may compromise body mass and lipids during winter, scaup could compensate with increased hyperphagia as spring approaches. In accordance, lipid levels of scaup in this study were similar among treatment groups in the 23-week exposure group and body mass was similar among treatments by April. However, scaup in this study were fed ad libitum which may not accurately reflect forage availability and energy expenditure during late-winter and spring migration (Badzinski et al., 2006; Anteau and Afton, 2008; Schummer et al., 2012). For example, at Long Point Bay, Lake Erie, food stocks available to waterfowl are substantially less during spring than autumn migration (Badzinski et al., 2006). Wild scaup with reduced lipid levels in late-winter that are unable to find adequate food resources during spring may take longer to migrate and have reduced reproductive performance (Anteau and Afton, 2004). Anteau and Afton (2008) reported reduced availability of foods for scaup at staging areas in mid-continent North America. Combined effects of high Se concentrations and lack of adequate food supplies in spring could slow migration by scaup and contribute to reduced reproductive output. However, we cannot directly relate our data to survival of scaup in the wild because detrimental effects of Se could be mediated through potential behavioral changes or there could be synergistic effects of Se burdens and greater energy demands of free-living individuals.

4.3. Oxidative stress

Se is an essential element necessary in relatively small amounts but excess dietary Se can cause oxidative stress in waterfowl (Ohlendorf et al., 1988; Hoffman and Heinz, 1998; Fairbrother and Fowles, 1990; Custer et al., 2000; Franson et al., 2007), but Ware et al. (2012) did not detect a correlation between hepatic Se and MDA concentrations in greater scaup wintering at Lake Ontario. In

this study, a positive relationship between hepatic Se and MDA concentrations in 10-week period ducks suggests that increased Se concentrations in scaup were causing cell damage (i.e., LPO). The lack of a relationship between Se concentrations and GSH levels in 10-week exposure ducks, but positive relationship in 23-week exposure group, suggests that greater exposure time to Se was required for scaup to begin producing significantly more antioxidants (Ware et al., 2012) did not measure GSH levels, but speculated that lack of high MDA concentrations in greater scaup was from the length of time these ducks had been using the Se rich environment of Lake Ontario (i.e., the antioxidant GSH was already being produced and acting as a protectant). The relationship between hepatic Se and GSH levels detected in the 23-week exposure ducks implies that scaup with high Se concentrations were producing greater GSH than other treatment groups, possibly to counteract free radicals produced by excess Se. Further, scaup in this study did not show other signs of oxidative stress, such as suppressed immune response, decreased protein reserves, increase in organ mass, or decreased survival (sensu Hoffman et al., 1991), suggesting that oxidative stress thresholds for Se were not exceeded.

4.4. Immune system challenges

4.4.1. T-cell response

The PHA skin test is considered a reliable indicator of T-cell function in most organisms (Wayland et al., 2002), but results for waterfowl when determining effects of Se have been inconsistent among and within species (Fairbrother and Fowles, 1990; Wayland et al., 2002). Wayland et al. (2002) detected contradictory results in common eiders for the influence of Se on T-cell function, but Franson et al. (2007) documented decreased response to PHA skin test in these ducks fed 60 μg/g Se. Mallards exposed to Se in drinking water exhibited no effect on amount of swelling caused by PHA (Fairbrother and Fowles, 1990). Similarly, T-cell response to PHA injections was unrelated to the hepatic Se burdens acquired by scaup in this study suggesting that Se concentrations in treatment groups never reached the threshold to cause cell-mediated immune response impairment. Nonetheless, disease and other stresses incurred by wild scaup, such as migration, breeding, and food shortages may change the threshold at which dietary Se can disrupt T-cell function.

4.4.2. Antibody response

Production of antibodies against foreign red blood cells is a sensitive indicator of immunotoxicity in birds, but antibody response can vary with environmental stressors (Fairbrother and Fowles, 1990; Grasman and Scanlon, 1995; Svensson et al., 1998; Fair and Ricklefs, 2002). In this study, dietary Se treatments had no effect on baseline antibodies prior to SRBC inoculations. At 10-weeks, high treatment group scaup produced more primary (IgM) antibodies than control and moderate treatment ducks and, at 23-weeks, the high and moderate treatments expressed more IgM and secondary antibodies (IgG) than controls. Thus, Se appeared to continue to act as an immunostimulator for ducks as exposure time increased from 10 to 23-weeks in the moderate and high treatment groups. Overall, scaup with greater Se concentrations needed a longer exposure (>10-weeks) to achieve immunostimulatory effects from Se while scaup with moderate Se concentrations needed longer exposure (i.e., 23-weeks) to stimulate IgM antibodies. Components of the immune system have energetic costs but these costs in adult animals are less than for innate and cell-mediated immunity (Klasing and Leshchinsky, 1999; Lee, 2006). Because Se was acting as an immunostimulator in our sample of adult scaup, we concluded that Se concentrations were not great enough to cause immunosuppression.

4.4.3. Se in feathers and ovaries

Molt in birds is an intense physiological change including synthesis of keratin, increased amino acid metabolism, and daily cycling of body protein (Dolnik and Gavrilov, 1979; Murphy and King, 1991). In this study scaup incorporated Se into new feathers, which supports the hypothesis that, when present, selenomethionine will replace methionine protein structures (Beilstein and Whanger, 1987; Kigawa et al., 2001). Binding of Se into new feathers may allow scaup to depurate excess Se, but specific metabolic pathways and any consequences for feather integrity needs further study. Concentrations of Se in animal tissues vary positively with dietary Se (Heinz et al., 1989; Hoffman et al., 1991; Albers et al., 1996).

In this study, Se was detected in ovaries, but Se was also likely accumulating in other organs (Albers et al., 1996). Specifically, Se can be reduced by GSH or bound by low molecular weight, cysteine-rich proteins called metallothioneins (MTs) synthesized in the liver and kidneys. Both mechanisms can reduce the toxicity and resulting oxidative stress of Se (Brown et al., 1977; Eisler, 2000). Synthesis of MTs is dependent upon an animal's trophic level and the concentration of inorganic contaminants consumed (Braune and Scheuhammer, 2008). Elevated concentrations of Se recorded in feathers and ovaries of moderate and high treatment groups relative to controls suggests that concentrations of Se were at levels too great for reduction by GSH or binding by MTs. Excessive Se binding can impair metabolic function, formation of proteins, and cause other physiological related problems (Eisler, 2000). Because Se was 43× (pinfeathers) and 12× (ovaries) greater in the high treatment than control group, it appears that excess Se was binding to available tissues throughout the body. However, even with relatively excessive increases in Se concentrations in feathers and ovaries, survival and immunity were not compromised. Further, a study of elemental contaminants in ovaries of breeding greater scaup also concluded that Se concentrations they documented were not negatively affecting the study population (Badzinski et al., 2009).

5. Conclusions

Scaup in this study had a range of elevated to sub-lethal Se burdens (4.36–103.63 μg/g) and some negative effects were detected. However, scaup did not exhibit compromised health, such as suppressed immune response, enlarged organs, extreme emaciation or mortality. Thus, health problems and mortality directly related to excessive Se concentrations do not appear to have caused the decline in the population of scaup in North America. Captive waterfowl studies enable experimental manipulation with the advantage of controlling and isolating treatment effects, but often do not account for other factors that individuals experience in the wild. In this study, scaup with greater hepatic Se concentrations did produce more antioxidants and, during mid-winter, had lower lipid levels. Feeding scaup Mazuri Sea Duck Diet ad libitum increases likelihood of synthesis of proteins necessary to detoxify dietary Se and enables accumulation of lipid reserves during spring following winter weight loss, but these nutrient balanced feeding conditions are not encountered by migrating and wintering wild scaup (Anteau and Afton, 2004). Conservation and management of quality wetlands for migrating scaup would help ensure that negative effects of Se acquisition similar to those simulated in this study, are offset by adequate foraging opportunities during spring migration.

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References

Afton, A.D., Anderson, M.G., 2001. Declining scaup populations; a retrospective analysis of long-term population and harvest survey data. Journal of Wildlife Management 65, 781–796.

Albers, P.H., Green, D.E., Sanderson, C.J., 1996. Diagnostic criteria for selenium toxicosis in aquatic birds: dietary exposure, tissue concentrations, and macroscopic effects. Journal of Wildlife Diseases 32, 468–485.

Anteau, M.J., Afton, A.D., 2004. Nutrient reserves of lesser scaup (*Aythya affinis*) during spring migration in the Mississippi Flyway: a test of the spring condition hypothesis. Auk 121, 917–929.

Anteau, M.J., Afton, A.D., Custer, C.M., Custer, T.W., 2007. Relationships of cadmium, mercury, and selenium with nutrient reserves of female lesser scaup (*Aythya affinis*) during winter and spring migration. Environmental Toxicology and Chemistry 26, 515–520.

Anteau, M.J., Afton, A.D., 2008. Amphipod densities and indices of wetland quality across the upper-Midwest, USA. Wetlands 28, 184–196.

Assel, R.A., 2003. Great Lakes Ice Cover, First Ice, Last Ice and Ice Duration: Winters 1973–2002. NOAA Technical Memorandum GLERL-125. Great Lakes Environmental Research Laboratory, Ann Arbor.

Austin, J.E., Afton, A.D., Anderson, M.G., Clark, R.G., Custer, C.M., Lawrence, J.S., Pollard, J.B., Ringleman, J.K., 2000. Declining scaup populations: issues, hypotheses, and research needs. Wildlife Society Bulletin 28, 254–263.

Badzinski, S.S., Ankney, C.D., Petrie, S.A., 2006. Influence of migrant Tundra swans (*Cygnus columbianus*) and Canada geese (*Branta canadensis*) on aquatic vegetation at long point, lake Erie, Ontario. Hydrobiologia 567, 195–211.

Badzinski, S.S., Petrie, S.A., 2006. Lesser scaup spring nutrient reserve dynamics on the lower Great Lakes. Wildlife Society Bulletin 34, 395–407.

Badzinski, S.S., Flint, P.L., Gorman, K.B., Petrie, S.A., 2009. Relationships between hepatic trace element concentrations, reproductive status, and body condition of female greater scaup. Environmental Pollution 157, 1886–1893.

Bain, G.A.C., 1980. The Relationship Between Preferred Habitat, Physical Condition, and Hunting Mortality of Canvasbacks (*Aythya valisineria*) and Redheads (*Aythya americana*) at Long Point, Ontario. M.Sc. thesis, University of Western Ontario, London.

Baldassarre, G.A., Bolen, E.G., 2006. Waterfowl Ecology and Management, second ed. Krieger Publishing Company, Malabar.

Beilstein, M.A., Whanger, P.D., 1987. Metabolism of selenomethionine and effects of interacting compounds by mammalian cells in culture. Journal of Inorganic Biochemistry 29, 137–152.

Bellrose, F.C., 1980. Ducks, Geese, and Swans of North America. Stackpole Books, Harrisburg.

Belzile, N., Chen, Y.-W., Gunn, J.M., Tong, J., Alarie, Y., Delonchamp, T., Lang, C.-Y., 2005. The effect of selenium on mercury assimilation by freshwater organisms. Canadian Journal of Fisheries and Aquatic Science 63, 1–10.

Brady, C.M., 2009. Effects of Dietary Selenium on Health and Survival of Wintering Lesser Scaup. M.Sc. thesis, University of Western Ontario, London.

Braune, B.M., Scheuhammer, A.M., 2008. Trace elements and metallothionein concentrations in seabirds from the Canadian Arctic. Environmental Toxicology and Chemistry 27, 645–651.

Brown, D.A., Bawden, C.A., Chatel, K.W., Parson, T.R., 1977. The wildlife community of Iona Island jetty, Vancouver, B.C. and heavy metal pollution effects. Environmental Conservation 4, 213–216.

Canadian Fish and Wildlife Services, 2007. Long Point Waterfowl and Wetland Research Fund, Lower January Waterfowl Survey Summary, Unpublished Data.

Custer, C.M., Custer, T.W., 1996. Food habits of diving ducks in the Great Lakes after the zebra mussel invasion. Journal of Field Ornithology 67, 86–99.

Custer, C.M., Custer, T.W., 2000. Organochlorine and trace element contamination in wintering and migrating diving ducks in the southern Great Lakes, USA, since the zebra mussel invasion. Environmental Toxicology and Chemistry 19, 2821–2829.

Custer, T.W., Custer, C.M., Hines, R.K., Sparks, D.W., Melancon, M.J., Hoffman, D.J., Bickham, J.W., Wickliffe, J.K., 2000. Mixed-function oxygenases, oxidative stress, and chromosomal damage measured in lesser scaup wintering on the Indiana Harbor canal. Archives of Environmental Contamination and Toxicology 38, 522–529.

Custer, C.M., Custer, T.W., Anteau, M.J., Afton, A.D., Wooten, D.E., 2003. Trace elements in lesser scaup (*Aythya affinis*) from the Mississippi flyway. Ecotoxicology 12, 47–54.

de Kock, W.C., Bowmer, C.T., 1993. Bioaccumulation, biological effects and food chain transfer of contaminants in the zebra mussel (*Dreissena poymorpha*). In: Nalepa, T.F., Schloesser, D.W. (Eds.), Zebra Mussels Biology, Impacts, and Control. Lewis Publishers, Boca Raton, pp. 503–533.

Dennis, D.G., McCullough, G.B., North, N.R., Ross, R.K., 1984. An updated assessment of migrant waterfowl use of Ontario shorelines of the southern Great Lakes. In: Curtis, G., Dennis, D.G., Boyd, H. (Eds.), Waterfowl Studies in Ontario Canadian Wildlife Service Occasional Paper No. 54, pp. 37–42.

DeVink, J.M., 2007. Reproductive Energetics and Selenium Ecotoxicology in Three Boreal-breeding Waterfowl Species. Ph.D. dissertation, University of Saskatchewan, Saskatoon.

DeVink, J.M., Clark, R.G., Slattery, S.M., Wayland, M., 2008a. Is selenium affecting body condition and reproduction in boreal breeding scaup, scoters, and ring-necked ducks? Environmental Pollution 152, 116–122.

DeVink, J.M., Clark, R.G., Slattery, S.M., Scheuhammer, T.M., 2008b. Effects of dietary selenium on reproduction and body mass of captive lesser scaup. Environmental Toxicology and Chemistry 27, 471–477.

Dolnik, V.R., Gavrilov, V.M., 1979. Bioenergetics of molt in the chaffinch (*Fringilla coelebs*). Auk 96, 253–264.

Dufour, K.D., Ankney, C.D., Weatherhead, P.J., 1993. Condition and vulnerability to hunting among Mallards staging at Lake St. Clair, Ontario. Journal of Wildlife Management 57, 209–215.

Dzubin, A., Cooch, E.G., 1992. Measurements of Geese: General Field Methods. California Waterfowl Association, Sacramento.

Eisler, R., 2000. Handbook of Chemical Risk Assessment: Health Hazards to Humans, Plants, and Animals. In: Metalloids, Radiation, Cumulative Index to Chemicals and Species, vol. 3. Lewis, Boca Raton.

Fair, J., Ricklefs, R., 2002. Physiological, growth, and immune responses of Japanese quail chicks to the multiple stressors of immunological challenge and lead shot. Archives of Environmental Contamination and Toxicology 42, 77–87.

Fairbrother, A.J., Fowles, J., 1990. Subchronic effects of sodium selenite and selenomethionine on several immune functions in mallards. Archives of Environmental Contamination and Toxicology 19, 836–844.

Fairbrother, A., Smits, J., Grasman, K.A., 2004. Avian immunotoxicology. Journal of Toxicology and Environmental Health, Part B 7, 105–137.

Fox, G.A., MacCluskie, M.C., Brook, R.W., 2005. Are current contaminant concentrations in eggs and breeding female lesser scaup of concern? Condor 107, 50–61.

Franson, J.C., Hoffman, D.J., Wells-Berlin, A., Perry, M.C., Shearn-Bochsler, V., Finley, D.L., Flint, P.L., Hollmen, T., 2007. Effects of dietary selenium on tissue concentrations, pathology, oxidative stress, and immune function in common eiders (*Somateria mollissima*). Journal of Toxicology and Environmental Health, Part A 70, 861–874.

Grasman, K.A., Scanlon, P.F., 1995. Effects of acute lead ingestion and diet on antibody and T-cell mediated immunity in Japanese quail. Archives of Environmental Contamination and Toxicology 28, 161–167.

Greenwood, H., Clark, R.G., Weatherhead, P.J., 1986. Condition bias of hunter-shot Mallards. Canadian Journal of Zoology 64, 599–601.

Heinz, G.H., Hoffman, D.J., Gold, L.G., 1989. Impaired reproduction of mallards fed an organic form of selenium. Journal of Wildlife Management 53, 418–428.

Heinz, G.H., Fitzgerald, M.A., 1993a. Overwinter survival of mallards fed selenium. Archives of Environmental Contamination and Toxicology 25, 90–94.

Heinz, G.H., Fitzgerald, M.A., 1993b. Reproduction of mallards following overwinter exposure to selenium. Environmental Pollution 81, 117–122.

Heinz, G.H., 1996. Selenium in birds. In: Beyer, W.N., Heinz, G.H., Redmond-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations Society of Environmental Toxicology and Chemistry Special Publication. CRC Press Inc., Boca Raton, pp. 447–458.

Heinz, G.H., Hoffman, D.J., Klimstra, J.D., Stebbins, K.R., 2011. A comparison of the teratogenicity of methylmercury and selenomethionine injected into bird eggs. Archives of Environmental Contamination and Toxicology. http://dx.doi.org/10.1007/s00244-011-9717-4 (online early).

Hoffman, D.J., Heinz, G.H., LeCaptain, L.J., Bunck, C.M., Gree, D.E., 1991. Subchronic hepatotoxicity of selenomethionine ingestion in mallard ducks. Journal of Toxicology and Environmental Health 32, 449–464.

Hoffman, D.J., Heinz, G.H., LeCaptain, L.J., Eisemann, J.D., Pendleton, G.W., 1996. Toxicity and oxidative stress of different forms of organic selenium and dietary protein in mallard ducklings. Archives of Environmental Contamination and Toxicology 31, 120–127.

Hoffman, D.J., Heinz, G.H., 1998. Effects of mercury and selenium on glutathione metabolism and oxidative stress in mallard ducks. Environmental Toxicology and Chemistry 17, 161–166.

Kigawa, T., Yamaguchi-Nunokawa, E., Kodama, K., Matsuda, T., Yabuki, T., Matsuda, N., Ishitani, R., Nureki, O., Yokoyama, S., 2001. Selenomethionine incorporation into a protein by cell-free synthesis. Journal of Structural and Functional Genomics 2, 29–35.

King, J.R., Farner, D.S., 1965. Studies of fat deposition in migratory birds. Annals of the New York Academy of Science 131, 422–440.

Klasing, K.C., Leshchinsky, T.V., 1999. Functions, costs, and benefits of the immune system during development and growth. Ostrich 69, 2817–2832.

Kovalak, W.P., Longton, G.D., Smithee, R.D., 1993. Infestation of power plant water systems by the zebra mussel (*Dreissena polymorpha*). In: Nalepa, T.F., Schloesser, D.W. (Eds.), Zebra Mussels Biology, Impacts, and Control. Lewis Publishers, Boca Raton, pp. 359–380.

Leach, J.H., 1993. Impacts of the zebra mussel (*Dreisenna polymopha*) on water quality and fish spawning reefs in the western Lake Erie. In: Nalepa, T.F., Schloesser, D.W. (Eds.), Zebra Mussels Biology, Impacts, and Control. Lewis Publishers, Boca Raton, pp. 381–397.

Lee, K., 2006. Linking immune defenses and life history at the levels of the individual and the species. Integrative and Comparative Biology 6, 1000–1015.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O., 2006. SAS for Mixed Models, second ed. SAS Institute, Inc, Cary, North Carolina.

Miller, L.L., Wang, F., Palace, V.P., Hontela, A., 2007. Effects of acute and subchronic exposures to waterborne selenite on the physiological stress response and oxidative stress indicators in juvenile rainbow trout. Aquatic Toxicology 8, 263–271.
Mills, E.L., Dermott, R.M., Roseman, E.F., Dustin, D., Mellina, E., Conn, D.M., Spidle, A.P., 1993. Colonization, ecology, and population structure of the "quagga" mussel (Bivalvia: dreissenidae) in the lower great lakes. Canadian Journal of Fisheries and Aquatic Science 50, 2305–2314.
Murphy, M.E., King, J.R., 1991. Nutritional aspects of avian molt. Acta Congressional International Ornithology 1, 2186–2194.
National Research Council Canada, 2009. Certified Reference Materials. http://www.nrc-cnrc.gc.ca/eng/services/inms/reference-materials.html.
Neary, B.P., Leach, J.H., 1992. Mapping the potential spread of the zebra mussel (*Dreissena polymorpha*) in Ontario. Canadian Journal of Fisheries and Aquatic Science 49, 406–415.
Ohlendorf, H.M., Kilness, A.W., Simmons, J.L., Stroud, R.K., Hoffman, D.J., Moore, J.F., 1988. Selenium toxicosis in wild aquatic birds. Journal of Toxicology and Environmental Health 24, 67–92.
Pace III, R.M., Afton, A.D., 1999. Direct recovery rates of lesser scaup banded in northwest Minnesota: sources of heterogeneity. Journal of Wildlife Management 63, 389–395.
Petrie, S.A., Badzinski, S.S., Drouillard, K.G., 2007. Contaminants in lesser and greater scaup staging on the lower Great Lakes. Archives of Environmental Contamination and Toxicology 52, 580–589.
Petrie, S.A., Knapton, R.W., 1999. Rapid increase and subsequent decline of zebra and quagga mussels in Long Point Bay, Lake Erie; possible influence of waterfowl predation. Journal of Great Lakes Research 25, 772–782.
Petrie, S.A., 1998. Waterfowl and Wetlands of Long Point and Old Norfolk County: Present Conditions and Future Options for Conservation. Unpublished Norfolk Land Stewardship Council Report. Long Point Waterfowl and Wetlands Research Fund, Port Rowan.
Pollock, B., Machin, K.L., 2008. Effects of cadmium, mercury, and selenium on reproductive indices in male lesser scaup (*Aythya affinis*) in the western boreal forest. Archives of Environmental Contamination and Toxicology 54, 730–739.
Prince, H.H., Padding, P.I., Knapton, R.W., 1992. Waterfowl use of the Laurentian Great Lakes. Journal of Great Lakes Research 18, 673–699.
SAS Institute, 2009. SAS/STAT User's Guide. SAS Institute, Cary, North Carolina.
Schummer, M.L., Petrie, S.A., Badzinski, S.S., Chen, Y.-W., Belzile, N., 2011a. Hepatic concentrations of inorganic contaminants and their relationships with nutrient reserves in autumn-migrant common loons at Lake Erie. Archives of Environmental Contamination and Toxicology 62, 704–713.
Schummer, M.L., Petrie, S.A., Badzinski, S.S., Deming, M., Chen, Y.-W., Belzile, N., 2011b. Elemental contaminants in livers of mute swans on Lakes Erie and St. Clair. Archives of Environmental Contamination and Toxicology 61, 677–687.
Schummer, M.L., Petrie, S.A., Bailey, R.C., Badzinski, S.S., 2012. Factors affecting lipid reserves and foraging activity of bufflehead, common goldeneye, and long-tail ducks during winter at Lake Ontario. Condor 114, 62–74.
Silver, T.M., Nudds, T.D., 1995. Influence of low-level cadmium and reduced calcium intake on tissue Cd concentrations and behaviour of American black ducks. Environmental Pollution 90, 153–161.
Skorupa, J.P., 1998. Selenium poisoning of fish and wildlife in nature: lessons from twelve real-world examples. In: Frankenberger, W.T., Engberg, R.A. (Eds.), Environmental Chemistry of Selenium. Marcel Dekker, New York, pp. 315–354.
Svensson, E., Raberg, L., Koch, C., Hasselquist, D., 1998. Energetic stress, immunosuppression and the costs of an antibody response. Functional Ecology 12, 912–919.
Tacha, T.C., Warde, W.D., Burnham, E.P., 1982. Use and interpretation of statistics in wildlife journals. Wildlife Society Bulletin 10, 355–362.
U.S. Department of Commerce, 2007. Mussel Watch in National Centers for Coastal Ocean Science. http://egisws02.nos.noaa.gov/nsandt/index.html#.
U.S. Fish and Wildlife Service, 2009. Waterfowl Population Status. http://www.fws.gov/migratorybirds/NewReportsPublications/PopulationStatus/Waterfowl/StatusReport2009_Final.pdf.
Ware, L.L., 2008. Selenium Uptake and Effects in Greater Scaup (*Aythya marila*) Wintering on Western Lake Ontario. M.Sc. thesis, University of Western Ontario, London, Ontario.
Ware, L.L., Petrie, S.A., Badzinski, S.S., Bailey, R.C., 2011. Selenium concentrations in greater scaup and dreissenid mussels during winter on western Lake Ontario. Archives of Environmental Toxicology 61, 292–299.
Ware, L.L., Petrie, S.A., Bailey, R.C., Badzinski, S.S., Schummer, M.L., Chen, Y.-W., Belzile, N., 2012. Effects of elevated selenium on body condition, oxidative stress, and organ health in greater scaup wintering at Lake Ontario. Wildlife Society Bulletin 36, 506–511.
Wayland, M., Gilchrist, H.G., Marchant, T., Keating, J., Smits, J.E., 2002. Immune function, stress response, and body condition in arctic-breeding common eiders in relation to cadmium, mercury, and selenium concentrations. Environmental Research, Section A 90, 47–60.
Whyte, R.J., Bolen, E.G., 1984. Variation in winter fat depots and condition indices of mallards. Journal of Wildlife Management 48, 1370–1373.
Yamamoto, J.T., Santolo, G.M., 2000. Body condition effects in American kestrels fed selenomethionine. Journal of Wildlife Diseases 36, 646–652.