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Research Paper

Selenium levels in Lesser Scaup (*Aythya affinis*) experimentally infected with introduced trematodes

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ABSTRACT. Lesser Scaup (*Aythya affinis*) have declined nearly 60% over the past five decades and remain below the continental population objective. Since the early 2000s, epizootic intestinal trematodiasis caused by introduced trematodes (*Cyathocotyle bushiensis*, *Sphaeridiotrema globulus*, and *Sphaeridiotrema pseudoglobulus*) have been documented as a proximate cause of scaup mortalities while the consequences of sub-lethal infections on individuals remain unknown. Trace elements including lead, cadmium, and selenium may also have deleterious effects on scaup by reducing the immune system function, but cumulative effects in combination with trematodiasis have not been previously investigated. We evaluated the potential interactive relationships of heavy metal contaminants and trematode infections in wild-caught and captive-reared scaup in captivity. Across three experimental trials, 60 scaup received a single, sub-lethal dose of metacercariae and were subsequently assessed for hepatic lead, cadmium, and selenium concentrations relative to gross lesions and trematode counts at necropsy. No relationship was detected between lead or cadmium liver concentrations and trematode infections. We found a negative relationship between trematode infections and selenium liver concentrations. Selenium is an essential nutrient for animals specifically to protect the host body from cellular damage during an immune response. Trematodiasis in free ranging scaup may contribute to selenium deficiencies, which could deleteriously affect survival and body condition. Selenium deficiency from trematodiasis could be a contributing factor to health of Lesser Scaup, but we did not find interactive effects across contaminants and trematodiasis that would suggest substantial population-level issues.

Les niveaux de sélénium chez les fuligules à tête noire (*Aythya affinis*) infectés expérimentalement par des trématodes introduits

RÉSUMÉ. La population de Fuligule à tête noire (*Aythya affinis*) a diminué de près de 60 % au cours des cinq dernières décennies et reste en deçà de l'objectif de population continental. Depuis le début des années 2000, des trématodoses intestinales épizootiques causées par des trématodes introduits (*Cyathocotyle bushiensis*, *Sphaeridiotrema globulus*, et *Sphaeridiotrema pseudoglobulus*) ont été documentées comme l'une des causes immédiates de la mortalité des fuligules, alors que les effets des infections sublétales sur les individus restent inconnus. Les oligoéléments, tels que le plomb, le cadmium et le sélénium, ont également des effets délétères sur le Fuligule et réduisent la fonction du système immunitaire. Toutefois, les effets cumulatifs en combinaison avec la trématodiase n'ont jamais été étudiés. Nous avons évalué les interactions potentielles entre les contaminants métalliques lourds et les infections par les trématodes chez les fuligules capturés dans la nature et élevés en captivité. Au cours de trois essais expérimentaux, 60 fuligules ont reçu une seule dose sublétale de métacercaires et ont ensuite été évalués pour déterminer les concentrations hépatiques de plomb, de cadmium et de sélénium par rapport aux lésions macroscopiques et au nombre de trématodes lors de l'autopsie. Aucune relation n'a été observée entre les concentrations hépatiques de plomb ou de cadmium et les infections par les trématodes. Nous avons trouvé une relation négative entre les infections de trématodes et les concentrations de sélénium dans le foie. Le sélénium est un nutriment essentiel chez les animaux, notamment pour protéger l'organisme hôte des dommages cellulaires lors d'une réponse immunitaire. Chez les fuligules en liberté, la trématodiase peut causer une carence en sélénium, entraînant des effets délétères sur la survie et l'état corporel. Cette même carence en sélénium due à la trématodiase pourrait être un facteur impactant la santé du Petit Fuligule, mais nous n'avons pas trouvé d'effets interactifs entre les contaminants et la trématodiase qui suggéreraient des problèmes importants au niveau de la population.

Key Words: *Aythya affinis; cadmium; Cyathocotyle bushiensis; heavy metal; lead; Lesser Scaup; selenium; Sphaeridiotrema pseudoglobulus; trematode; waterfowl*

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INTRODUCTION

Since the early 2000s, recurring spring and fall mortality events have been observed for migrating waterfowl throughout the Upper Midwest, USA (Cole and Franson 2006, Sauer et al. 2007). These epizootic events are caused by introduced trematode species, *Cyathocotyle bushiensis*, *Sphaeridiotrema globulus*, and *Sphaeridiotrema pseudoglobulus* (Cole and Franson 2006, Sauer et al. 2007). *C. bushiensis* and *Sphaeridiotrema* spp. have a three-host life cycle in which they use the faucet snail (*Bithynia tentaculata*) as their first and second intermediate host and waterfowl as their final host (Kahn 1962, Macy and Ford 1964). *C. bushiensis* and *Sphaeridiotrema* spp. have the potential to cause severe morbidity and mortality depending on the host species, the parasite species, and the intensity of infection (Price 1934, Gibson et al. 1972, Cole and Franson 2006).

The faucet snail is native to Europe and was introduced to North America in the late 1800s (Mills et al. 1993). The snail is presumed to have spread east to west across the Great Lakes ecosystem as indicated by natural history records (Kipp et al. 2025) and was detected in the Upper Mississippi River in the early 2000s following the onset of recurring waterfowl mortality events (Cole and Franson 2006, Sauer et al. 2007). Faucet snails infected with larval trematodes are consumed by waterfowl, where *C. bushiensis* and *Sphaeridiotrema* spp. mature, reproduce, and complete their life cycle in the intestines of their avian host (Szidat 1937, Kahn 1962, McLaughlin et al. 1993). The faucet snail has continued to expand its range and has been detected as far south as Navigational Pool 13 at river mile 522.5 of the Mississippi River near Fulton, Illinois, USA. (Kipp et al. 2025).

Wetland degradation often comes in the form of sedimentation, nutrification, and pollution and the amount of pollution on the landscape has increased, including heavy metal contamination (Hoffman et al. 2003). Waterfowl can experience negative effects from heavy metal exposure, including metabolism disruption and reduced efficacy of the immune system (Furness 1996, Heinz 1996, Pain et al. 2019). Austin et al. (2000) identified trace elements including lead, cadmium, and selenium, as contaminants that may have deleterious effects on waterfowl survival, reproduction, and recruitment. Trematodiasis in waterfowl often occurs at times of these energetically costly activities (i.e., spring migration and the pre-breeding season) and may work in tandem with environmental factors to deplete nutrient reserves required for successful breeding efforts (Sauer et al. 2007). Individuals that survive trematode infection are assumed to be in poorer condition, which may contribute to reduced reproductive success and breeding propensity (England et al. 2018). Trematodiasis has been proposed as a contributing factor of Lesser Scaup (*Aythya affinis*) population declines (Sauer et al. 2007, Herrmann and Sorensen 2011). Although effects of trematode related fatalities on scaup population dynamics are currently unknown, biologists are concerned that faucet snails will continue to move further south in the Mississippi River and colonize additional important scaup migration stopover sites in the Upper Mississippi River (Austin 2010).

Our objectives were to (1) evaluate the difference in lead, selenium, and cadmium concentrations between wild-caught and captive-reared captive scaup; and (2) evaluate the interaction between lead, selenium, and cadmium hepatic concentrations and trematode infections. We predicted that individuals with heavier contaminant loads (i.e., wild-caught scaup) would be more susceptible to trematode infections.

METHODS

Study location

Our work was conducted at Forbes Biological Station (Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign) near Havana, Illinois (40°21′12.5″ N, 90°01′17.1″ W) between March 2019 and October 2020.

Husbandry

Lesser Scaup were uniquely marked by affixing numbered leg bands. Birds were maintained in captivity on un-medicated, pelletized ration (e.g., Sportsman's Choice Floating Pond & Catfish Food; Cargill, Inc. Minneapolis, Minnesota, 32% protein, 4% fat, 7.5% fiber; Checkett et al. 2002) that contained 0.25 ug/g of selenium in the form of selenite from the manufacturer. The selenium is within the limits of adequate for nutritional requirements range (0.30–1.1 ug/g) and lower than the toxic range (> 5.0 ug/g) for birds (Ohlendorf and Heinz 2011).

Faucet snails

We collected faucet snails to obtain larval trematode metacercarial stage (encysted late larval stage of trematode that is infectious) for the infection treatments given to scaup. Faucet snails were collected on 1–2 June 2019, 22 September 2019, and 21 September 2020, by hand, using long-handled nets, or with Ponar dredges from Navigation Pools 7 and 8 of the Mississippi River near La Crosse, Wisconsin (43°48′00.0″ N, 91°15′00.0″ W). The U.S. Fish and Wildlife Service has reported yearly avian mortalities due to intestinal trematodiasis caused by *C. bushiensis* and *Sphaeridiotrema* spp. in these pools since 2000 (USFWS 2017). Previous snail collections yielded an average of 5 metacercariae per snail (R. A. Cole, *unpublished data*). We wanted each treatment dose to have 200–400 metacercariae, so collected 80 snails for each treatment dose. This target was based on total trematode abundance of *C. bushiensis* and *Sphaeridiotrema* spp. in free-ranging scaup that were lethally collected and appeared to be in healthy condition (England et al. 2018). Snails were held in aquaria at Western Illinois University for removal of trematode metacercariae for infection trials. Because faucet snails are not endemic to this area of Illinois, biosecurity measures were taken to prevent snails from escaping including housing aquaria in a laboratory without floor drains and properly disinfecting all equipment and trash before removing from rooms (e.g., boiled at 100 °C for a minimum of 2 minutes, frozen for a minimum of 1 week, or treated with undiluted bleach for a minimum of 4 hr; Mitchell and Cole 2008).

Cyathocotyle bushiensis and *Sphaeridiotrema* spp. metacercariae

C. bushiensis and *Sphaeridiotrema* spp. metacercariae have limited viability (< 72 hr) after dissection from snail hosts (Gagnon et al. 1993), therefore, handling time was < 48 hr. For each infection dose, snails were pulverized using a mortar and pestle, and artificially digested (2 g pepsin and 3 ml of 12 M HCL in 200 ml distilled water) at 37 °C for 15 minutes using a magnetic stirrer (Gagnon et al. 1993). The remaining sediment was washed

through a 125-µm sieve with tap water to remove digest solution (Hoeve and Scott 1988). One week prior to each infection experiment, we used a dissecting microscope to identify metacercariae by morphological characters as reported by Kahn (1962) and Szidat (1937), quantified the number of snails required to obtain 200–400 metacercariae, and calculated a mean dose of *C. bushiensis* and *Sphaeridiotrema* spp. Because *Sphaeridiotrema* spp. metacercariae cannot be identified morphologically we verified the identification of *S. pseudoglobulus* by sequencing a portion of the cytochrome oxidase subunit 1 (CO1; Van Steenkiste et al. 2015) from metacercariae and a subsequent sub sample of adult worms recovered at necropsy. Samples used to establish the mean were disposed of after quantification and were not administered to birds. Metacercariae doses were stored separately in Locke's solution at 4 °C until the date of quantification or infection (no more than three days post digestion; Gagnon et al. 1993).

Infection trials

In all trials, each bird was assigned to one of three groups (i.e., baseline control, trial control, or infection). Baseline control birds were euthanized on day 0 of the trial. Trial controls were not given a parasite dose and were euthanized on day 10. Birds in the infection group were given a mixture of *C. bushiensis* and *S. pseudoglobulus* metacercariae via oral gavage on day 0 and euthanized on day 10.

2019 Wild-caught birds: Trial 1

In March 2019, we captured female, wild Lesser Scaup (n = 37) in the Illinois River floodplain near Havana, Illinois using baited swim-in traps modified for diving ducks (Haramis et al. 1982). Only female scaup were used because our interest in Trial 1 was in the effect of the introduced trematodes on female body condition. In July 2019, following an acclimatization period in pens (Beach et al. 2024), we assigned birds into three groups (i.e., baseline control, trial control, and infection) using a stratified-random design, with stratification based on a mass/morphological body condition index score utilizing using wing chord (Johnson et al. 1985). We had eight baseline controls and eight trial controls, with the remaining 21 birds in the infection group.

Captive-reared birds: Trial 2

In June 2019, we collected Lesser Scaup eggs (n = 108) during early stages of incubation from natural nests located near Cando, North Dakota, USA (48°29′17.0″ N, 99°12′54.7″ W) and transferred them to Forbes Biological Station to complete incubation using methods in Ward and Batt (1973). We hatched and reared 24 ducklings in captivity. In December 2019, birds became fully flighted, at which time we assigned them to one of three groups (i.e., baseline control, trial control, and infection) using a stratified random design based on sex to ensure that a nearly even amount of male and female birds were represented in each experimental group. We had four baseline control birds (n = 2 male and n = 2 female), four trial control birds, (n = 2 male and n = 2 female), and 16 infection birds (n = 9 male and n = 7 female).

2020 Wild-caught birds: Trial 3

In the spring of 2020, we captured male and female, wild scaup (n = 35) in the Illinois River floodplain near Havana, Illinois, USA using baited swim-in traps modified for diving ducks (Haramis et al. 1982). In October 2020, following an acclimatization period in pens (Beach et al. 2024), we assigned birds into three groups (i.e., baseline control, trial control, and infection) using a stratified random design based on sex to ensure that a nearly even amount of male and female birds were represented in each experimental group. There were six baseline control birds (n = 3 male and n = 3 female), six trial control birds, (n = 3 male and n = 3 female), and 23 infection birds (n = 10 male and n = 13 female).

Tissue collection and carcass processing

On day 0 of each trial, baseline control birds were fasted for at least 2 hr to reduce digesta for the ease of counting and identifying parasites in the intestines, whereafter they were euthanized and necropsied (Hoeve and Scott 1988). We euthanized and necropsied all remaining birds (infection and trial control) on day 10 of the trial following the same procedure as baseline control birds. Day 10 post-infection was chosen as the termination to maximize potential for disease because resolution of lesions and parasite death have been reported between days 8 and 10 post infection in Mallard (*Anas platyrhynchos*) ducklings fed 200 metacercariae of *S. globulus* (Mucha and Huffman 1991). In addition, Gagnon et al. (1993) reported that by day 9 post-infection, adult helminths had diminished between 54.0% and 1.4% depending on initial dose in 14-day old Pekin ducklings.

During the necropsy, the abdominal cavity was exposed by peeling away the skin and cutting the ribcage enabling it to be pushed aside (England et al. 2018). The right liver lobe was removed from each bird and stored at -29 °C in a bag marked with each unique bird identification number after which instruments were rinsed. Liver samples were analyzed for (mg/kg) lead, cadmium, and selenium by inductively coupled plasma mass spectrometry at the Eurofins Frontier Global Services Analytical Laboratory (Bothell, Washington, USA; Levengood 2003). Although other organs could be collected to examine contaminant concentrations, we chose the liver because it has been widely used in previous work in scaup, allowing us to compare our results to previous work (Custer et al. 2003, Osborn et al. 2016).

To collect adult *C. bushiensis* and *S. pseudoglobulus*, we divided and removed sections of the gastrointestinal tract as described below. We sectioned the gastrointestinal tract using a ligature of butcher's twine. The esophagus was tied at the most anterior section and just posterior to the gizzard. The small intestine from the gizzard to the cecal bifurcation was ligatured, divided into five equal length sections and ligatured (Bush and Holmes 1986). Each ceca was tied off separately. Finally, the anterior section of colon to the end of the cloaca was closed with twine. All sections of the intestinal tract were removed and frozen in 70% non-denatured ethanol until examination. For examination, ceca were thawed, placed in separate dishes, opened longitudinally, and flushed with tap water. Using a dissection microscope, *C. bushiensis* and *S. pseudoglobulus* adults were counted, removed, and preserved in 70% non-denatured ethanol. The same procedure was conducted on each section of the small intestine and colon-cloacal section. Following the removal of parasites, all cecal plaques and cores from each ceca were removed, dried for 24–48 hr in a drying oven and weighed separately (Hoeve and Scott 1988). Plaques attached to the cecal mucosa are caseous secretions in response to damage from *C. bushiensis* attachment and feeding. Cores form in the cecal lumen composed of fibrin and blood from parasite damaged mucosa. Both plaques and cores serve as proxy for tissue damage due to parasite infection (Hoeve and Scott 1988).

Table 1. Descriptive statistics of cadmium (Cd), lead (Pb), and selenium (Se) liver concentrations (mg/kg) detected in wild-caught and captive-reared Lesser Scaup (*Aythya affinis*) after experimental trematode infection trials.

Trial	Source	Treatment	Baseline control			Trial control			Infection		
		Element	Cd mg/kg	Pb mg/kg	Se mg/kg	Cd mg/kg	Pb mg/kg	Se mg/kg	Cd mg/kg	Pb mg/kg	Se mg/kg
1	Wild-caught 2019	Mean	0.47	0.08	1.45	0.69	0.08	1.41	0.83	0.13	1.52
		SE	0.14	0.03	0.06	0.27	0.04	0.09	0.13	0.06	0.06
		Min.	0.06	0.02	1.19	0.04	0	0.92	0.19	0	0.7
		Max.	1.27	0.29	1.68	2.43	0.35	1.69	2.21	1.23	2.02
2	Captive-reared	Mean	0.07	0.02	1.38	0.07	0.02	1.48	0.1	0.03	1.39
		SE	0.02	0.01	0.03	0.01	0.01	0.09	0.01	0.01	0.04
		Min.	0.04	0	1.28	0.05	0	1.23	0.05	0	1.07
		Max.	0.12	0.05	1.41	0.09	0.02	1.64	0.15	0.07	1.59
3	Wild-caught 2020	Mean	0.19	0.04	1.3	0.08	0.07	1.34	0.23	0.03	1.17
		SE	0.07	0.02	0.04	0.02	0.04	0.13	0.06	0.01	0.07
		Min.	0.05	0	1.18	0.04	0.02	1.1	0.13	0	1.15
		Max.	0.47	0.14	1.5	0.11	0.16	1.55	0.91	0.15	1.69

Statistical analysis

We implemented a multi-model framework using the second-order form of Akaike's Information Criterion (AIC_c) to rank models relative to the null. The null model contained no predictor variables. Final candidate models were considered if they were within 2 ΔAIC_c and informative parameters by having beta-coefficients and 85% confidence limits that did not overlap zero (Arnold 2010). We present all our results with 85% and 95% confidence limits. All statistical analyses were completed in R (Version 4.4.1, R Core Team 2024). For the lead, cadmium, and selenium models, we did not check for correlation as all predictors were categorical, but we did verify there were data in all combinations of categorical variables levels. For the remaining four model sets, we checked for correlation among the continuous predictor variables, and all correlations were < 0.45. We checked for distribution of continuous data across categorical variables and retained all variables. We have data from all combinations of categorical variables, except male and Trial 1, because Trial 1 only contained female birds.

Heavy metals

To evaluate the difference between lead, cadmium, and selenium concentrations (response variable) in different groups, we developed a priori a set of generalized linear models with a Gaussian distribution (Appendices 1, 2, 3).

Total trematode abundance

To evaluate the effect of heavy metals on total trematode abundance (parasite number across all hosts exposed to parasites; Bush et al 1997; total number of adult *S. pseudoglobulus* and *C. bushiensis,* response variable), we developed a priori a set of generalized linear models for consideration with a Poisson distribution (Appendix 4). To evaluate the effect of heavy metals on *S. pseudoglobulus* and *C. bushiensis* infection intensity (number of parasites in single host; Bush et al 1997; response variable), we developed a priori a set of generalized linear models with a Gaussian distribution (Appendices 5, 6). To evaluate the effect of heavy metals on total plaque and cecal mass (dry weight, response variable), we developed a priori a set of generalized linear models with a Gaussian distribution (Appendix 7).

RESULTS

Heavy metals

Lead levels were below the levels for sub-clinical poisoning (Pain et al. 2019; Table 1). The null model was the top model (Appendix 1). Cadmium levels were well below the liver threshold for cadmium poisoning (40 ug/g; Kanstrup et al. 2019; Table 1). Cadmium concentration was best explained by a combination of trial and infection (w_i = 0.97; Table 2, Appendix 2). Captive-reared (Trial 2) and 2020 wild-caught birds (Trial 3) had 0.64 ± 0.1 and 0.53 ± 0.1, respectively, less cadmium (mg/kg) in their livers compared to 2019 wild-caught birds (baseline intercept [Trial 1]; Table 2). Birds in the infection group had 0.16 ± 0.1 greater cadmium concentrations (mg/kg) in their livers compared to the baseline intercept group (Table 2). Trial control birds and baseline control birds had similar cadmium concentrations (Table 2).

Selenium concentration was best explained by a combination of trial and treatment group (w_i = 0.92; Table 2, Appendix 3). The 2020 wild-caught birds (Trial 3) had 0.28 ± 0.1 less selenium (mg/kg) in their livers compared to 2019 wild-caught birds (Trial 1; Table 2). Selenium values did not differ among treatment groups. We had 3 mortalities on trial, and those individuals had selenium concentrations 2 times lower than all other birds in our study (1.4 ± > 0.0 mg/kg).

Total trematode abundance

Total trematode abundance was best explained by a combination of selenium concentration, sex, and trial (w_i = 1.00, Appendix 4). Total trematode abundance decreased 1.54 ± 0.1 for every mg/kg increase in selenium (Table 3). Male birds had 0.8 ± 0.1 fewer trematodes than females (Table 3). Captive-reared birds (Trial 2) contained 0.8 ± 0.2 fewer trematodes than 2019 wild-caught birds (Trial 1, Table 3). The 2019 wild-caught birds (Trial 1) and 2020 wild-caught birds (Trial 3) contained similar numbers of trematodes (Table 3).

The top models for *S. pseudoglobulus* infection intensity included three parameters, lead, selenium concentration, and sex ($w_i \geq 0.13$, Appendix 5). Confidence intervals for beta values of lead overlapped 0, so we used the Selenium + Sex model as our best model (w_i = 0.35, Appendix 5). *S. pseudoglobulus* infection

Table 2. Coefficient estimates for top models for two model sets with standard error and 85% and 95% confidence limits used to evaluate the potential interactive relationships of heavy metal contaminants and trematode infections in wild-caught and captive-reared Lesser Scaup (*Aythya affinis*) under experimental setting. The intercept value in the cadmium and selenium models is based on cadmium and selenium concentrations in the livers of 2019 wild-caught Lesser Scaup (Trial 1) and serves as the baseline control group. The values for Trial 2 (captive-reared) and 3 (2020 wild-caught Lesser Scaup), and for trial control and infection, are relative to the Trial 1 intercept value.

Model set	Coefficient	Estimate	Standard error	85% lower	85% upper	95% lower	95% upper
Cadmium	Baseline control (intercept)	0.613	0.103	0.465	0.762	0.412	0.815
	Trial 2	-0.647	0.103	-0.795	-0.499	-0.848	-0.446
	Trial 3	-0.538	0.094	-0.673	-0.404	-0.722	-0.355
	Trial control	0.071	0.137	-0.126	0.268	-0.197	0.339
	Infection	0.167	0.105	0.016	0.318	-0.038	0.373
Selenium	Baseline control (intercept)	1.497	0.067	1.400	1.593	1.365	1.628
	Trial 2	-0.072	0.067	-0.169	0.024	-0.204	0.059
	Trial 3	-0.287	0.061	-0.375	-0.199	-0.406	-0.167
	Trial control	-0.007	0.089	-0.135	0.122	-0.182	0.169
	Infection	-0.028	0.068	-0.126	0.071	-0.162	0.106

intensity decreased by 9.3 ± 5.6 individual parasites for every mg/kg increase in selenium (Table 3). Male birds contained 15.15 ± 4.3 fewer *S. pseudoglobulus* than females (Table 3).

C. bushiensis infection intensity was best explained by selenium concentration (w_i = 0.74, Appendix 6). *C. bushiensis* infection intensity decreased by 24.60 ± 6.6 for every mg/kg increase in selenium (Table 3).

Selenium concentration, sex, and trial were included in the top models ($w_i \geq 0.17$) predicting cecal core mass, but confidence intervals for sex overlapped 0, so we considered selenium and trial to be informative parameters and provide the estimates from the Selenium + Trial model (w_i = 0.46, Appendix 7). Cecal core mass decreased by 0.39 ± 0.13 grams for every mg/kg increase in selenium (Table 3). The 2020 wild-caught birds (Trial 3) contained 0.21 ± 0.08 more cecal core mass compared to 2019 wild-caught birds (Trial 1, Table 3). Captive-reared birds (Trial 2) and 2019 wild-caught birds (Trial 1) had similar cecal core masses (Table 3).

DISCUSSION

We found concentrations of selenium were associated with trematode infection metrics across trials, although effect sizes were low. Although selenium is an essential trace element, much previous research has explored selenosis caused by excess selenium, especially related to stopover sites in the Great Lakes Region contaminated by industry (Custer et al. 2003, Anteau et al. 2007, DeVink et al. 2008). In our study, selenium levels were lower (~1.4 mg/kg) than experimentally collected birds in the wild (~14.6 mg/kg; Osborn et al. 2016; < 10 mg/kg; Ohlendorf and Heinz 2011) suggesting a potential different mechanism associated with trematodiasis. Selenium levels were lower in birds with higher intensity and abundance of *C. bushiensis* and *S. pseudoglobulus*. Selenium was also lower in birds with higher cecal core weights, a proxy for *C. bushiensis* tissue damage. Lower selenium levels during parasitic infections could result in an additional negative effect to the bird beyond the damage caused by the parasite, because selenium is an essential element.

Our selenium results could be due to intestinal damage and inflammation caused by the parasite limiting selenium absorption, which then could have negative effects on the individual in the short and long term. Selenium is physiologically necessary and plays a key role in making several selenoproteins that are vital for normal physiology such as antioxidant defense, immune function, production of thyroid hormones, DNA synthesis, fertility, and reproduction in vertebrates (Combs and Combs 1986, Arthur et al 2003, Rivera et al. 2003, Pilarczyk et al. 2008, Mehdi et al. 2013, Dalgaard et al. 2018, Dkhil et al. 2019). Vertebrates store selenium and selenoproteins in most organs and tissues of the body with large stores in the lymph nodes, liver, and spleen (Mehdi et al. 2013). Selenium's antioxidative activity occurs extracellularly, in the cytosol and in close contact with cell membranes, especially enterocytes, to influence the immune response (Arthur et al. 2003). Invertebrates, such as parasitic worms, utilize selenium in their own defense against internal oxidative stress from their metabolism and external oxidative stress from the host inflammatory response (Salinas et al. 2006, Bonilla et al. 2008, Sagerup et al. 2009, Provencher et al. 2014, Rashidi et al. 2022). Sagerup et al. (2009) found a positive relationship, but overall had high selenium levels across individuals that could have resulted in an advantage to the parasites in their competition for macronutrients, while our study had overall low selenium levels across individuals. Provencher et al. (2014) found positive relationships between presence of gut parasites and selenium but did not put forward a predicted cause. Selenium supplementation through the diet in mice and humans (Pilarczyk et al. 2008, Alcolea and Pérez-Silanes 2020) or through selenium nanoparticles have been explored to reduce parasite burdens or lessen parasitic disease processes in mice (Dkhil et al. 2019).

Cyathocotyle bushiensis and *S. pseudoglobulus* attach to cecal and duodenal enterocytes, respectively, releasing proteolytic enzymes causing hemorrhage and cell rupture, triggering an inflammatory response in their host (Hoeve and Scott 1988, Huffman and Roscoe 1989). The attachment and resulting inflammation and destruction of cells compromises active transport of selenium in the area of gut that is critical in absorption from food items (Mehdi et al. 2013). Our results suggest *C. bushiensis* and *S. pseudoglobulus* infections even with a one-time sublethal exposure may lower selenium via interference with absorption from food items and competition with the host for selenium, which subsequently lowers tissue storage of selenium (Sagerup et al. 2009). Wayland et al. (2002) found that female Common Eiders (*Somateria mollissima borealis*) with higher selenium concentrations had better immune response and could decrease the effects of their stress response more efficiently compared to female eiders with lower selenium concentrations. Selenium-deficiency prior to infection could exacerbate the effects of trematode infections by increased tissue damage, blood loss, and selenium-deficiency because of the loss of mucosal epithelial cells.

The spring condition hypothesis, which suggests habitat in the Upper Midwest region has degraded to such a large degree that there is not sufficient food for migratory Lesser Scaup to maintain

Table 3. Coefficient estimates for top models for four model sets with standard error, 85% and 95% confidence limits. The intercept represents the first level of all factors and serves as the baseline control group. For total trematode abundance, the intercept includes Trial 1 (2019 wild-caught scaup) and female. For *Sphaeridiotrema pseudoglobulus* intensity the intercept includes female. For cecal core mass the intercept includes Trial 1. The values reported for the later levels of each factor are relative to that intercept. For cecal core mass the value for Trial 2 (captive-reared) is relative to Trial 1 (the intercept).

Model set	Coefficient	Estimate	Standard error	85% lower	85% upper	95% lower	95% upper
Total trematode abundance	Baseline control (intercept)	4.60	0.17	4.36	4.84	4.27	4.92
	Selenium	-1.55	0.11	-1.71	-1.39	-1.77	-1.33
	Trial 2	-0.81	0.15	-1.03	-0.58	-1.11	-0.50
	Trial 3	0.13	0.10	-0.02	0.27	-0.07	0.32
	Sex male	-0.80	0.11	-0.96	-0.65	-1.02	-0.59
Sphaeridiotrema pseudoglobulus intensity	Baseline control (intercept)	29.84	7.32	19.30	40.38	15.49	44.19
	Selenium	-9.30	5.60	-17.36	-1.25	-20.28	1.67
	Sex male	-15.15	4.34	-21.40	-8.91	-23.65	-6.65
Cyathocotyle bushiensis intensity	Baseline control (intercept)	37.83	8.44	25.68	49.97	21.29	54.36
	Selenium	-24.60	6.66	-34.19	-15.00	-37.66	-11.54
Cecal core mass	Baseline control (intercept)	0.66	0.19	0.39	0.94	0.29	1.04
	Selenium	-0.39	0.13	-0.57	-0.20	-0.63	-0.14
	Trial 2	0.07	0.08	-0.04	0.19	-0.09	0.24
	Trial 3	0.21	0.08	0.09	0.32	0.04	0.37

body condition (Austin et al. 2000, Afton and Anderson 2001), was postulated before the widespread presence of these trematodes. Therefore, the hypothesis does not account for the potential for synergism between declining food abundance and quality and pathogenic trematode infections simultaneously decreasing body condition of birds (England et al. 2018). Our work suggests selenium deficiencies could interact with parasitic infections to have a negative effect on body condition, which could be contributing to the overall reduction in body condition during spring migration put forward by the spring condition hypothesis. Migrating scaup are not exposed to a one-time infection but most likely experience repeated infections as they migrate through pools 1–13 of the Mississippi River. Scaup that survive trematode infections may be subjected to additional cross-seasonal effects through reduced body condition, which could contribute to reduced reproductive success and breeding propensity (England et al. 2018).

Interestingly, DeVink et al. (2008) examined the potential for high selenium loads encountered during spring migration in scaup to decrease body mass and reproduction and found that birds could overcome high selenium loads relatively quickly because of short selenium half-lives. They noted selenium in food items from wintering areas was higher than in boreal areas, so replenishing selenium stores once birds have survived an infection could take additional time during spring migration or after arrival in boreal areas for the breeding season. Future studies on understanding the interplay of habitat quantity and quality, trematodiasis, and selenium on scaup reproductive success and population dynamics could help support the management of a healthy and sustainable scaup population across the continent. The potential of management of food resources at migratory stopover areas, especially those without *C. bushiensis* and *S. pseudoglobulus* and breeding locations to favor selenium rich food items could be examined as a way to address selenium depressions in infected birds.

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ETHICS STATEMENT

All animal handling, captive holding, and euthanasia protocols were approved by the Institutional Animal Care and Use Committee at University of Illinois at Urbana-Champaign and Western Illinois University (Protocol #18128 and #009-20, respectively), the U.S. Fish and Wildlife Service (Permit #MB145466-3, #MB145466-4, and #MB145466-6), Illinois Department of Natural Resources (Permit #W19.6079 and #W20.6079A), and North Dakota Game and Fish Department (Permit #GNF04969331).

Data Availability:

Data are available from Auriel Fournier (auriel@illinois.edu) upon request.

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Appendix 1. Akaike Information Criterion for models examining Lesser Scaup (*Aythya affinis*) during captive experimental trials, comparing the effect of trial and treatment group on lead concentrations. K is the number of parameters in the model. AICc is Akaike Information Criterion corrected for small sample size. Delta_AICc is the difference between a given models AICc and the top models AICc. ModelLik is the relative likelihood of the model given the data. AICcWt is the model weight. LL is the Log-likelihood. Cum.Wt is the Cumulative model weight of that model and all higher models.

Modnames	K	AICc	Delta_AICc	ModelLik	AICcWt	LL	Cum.Wt
Null	2	-87.93	0.00	1.00	0.61	46.03	0.61
Trial + Treatment	6	-86.60	1.32	0.52	0.31	49.77	0.92
Treatment	4	-83.69	4.24	0.12	0.07	46.06	0.99
Trial*Treatment	10	-78.40	9.53	0.01	0.01	50.49	1.00

Appendix 2. Akaike Information Criterion for models examining Lesser Scaup (*Aythya affinis*) during captive experimental trials, comparing the effect of trial and treatment group on cadmium concentrations. K is the number of parameters in the model. AICc is Akaike Information Criterion corrected for small sample size. Delta_AICc is the difference between a given models AICc and the top models AICc. ModelLik is the relative likelihood of the model given the data. AICcWt is the model weight. LL is the Log-likelihood. Cum.Wt is the Cumulative model weight of that model and all higher models.

Modnames	**K**	**AICc**	**Delta_AICc**	**ModelLik**	**AICcWt**	**LL**	**Cum.Wt**
Trial + Treatment	6	99.65	0.00	1.00	0.97	-43.35	0.97
Trial*Treatment	10	106.57	6.92	0.03	0.03	-41.99	1.00
Null	2	134.05	34.40	0.00	0.00	-64.96	1.00
Treatment	4	137.63	37.98	0.00	0.00	-64.60	1.00

Appendix 3. Akaike Information Criterion for models examining Lesser Scaup (*Aythya affinis*) during captive experimental trials, comparing the effect of trial and treatment group on selenium concentrations. K is the number of parameters in the model. AICc is Akaike Information Criterion corrected for small sample size. Delta_AICc is the difference between a given models AICc and the top models AICc. ModelLik is the relative likelihood of the model given the data. AICcWt is the model weight. LL is the Log-likelihood. Cum.Wt is the Cumulative model weight of that model and all higher models.

Modnames	K	AICc	Delta_AICc	ModelLik	AICcWt	LL	Cum.Wt
Trial + Treatment	6	17.82	0.00	1.00	0.92	-2.44	0.92
Trial*Treatment	10	22.74	4.92	0.09	0.08	-0.08	1.00
Null	2	32.14	14.32	0.00	0.00	-14.01	1.00
Treatment	4	35.17	17.35	0.00	0.00	-13.36	1.00

Appendix 4. Akaike Information Criterion for models examining Lesser Scaup (*Aythya affinis*) during captive experimental trials, comparing the effect of lead (Pb), cadmium (Cd), selenium (Se), sex, and trial on Total Trematode Abundance.

Modnames	K	AICc	Delta_AICc	ModelLik	AICcWt	LL	Cum.Wt
Se + Trial + Sex	5	963.15	0.00	1.00	1.00	-476.05	1.00
Se + Sex	3	1006.18	43.03	0.00	0.00	-499.89	1.00
Se +Trial	4	1021.24	58.09	0.00	0.00	-506.27	1.00
Se	2	1103.99	140.85	0.00	0.00	-549.90	1.00
Pb + Trial + Sex	5	1130.17	167.02	0.00	0.00	-559.56	1.00
Cd + Trial + Sex	5	1146.82	183.67	0.00	0.00	-567.88	1.00
Pb + Trial	4	1241.51	278.36	0.00	0.00	-616.41	1.00
Trial	3	1252.88	289.74	0.00	0.00	-623.24	1.00
Cd + Trial	4	1255.07	291.92	0.00	0.00	-623.19	1.00
Pb + Sex	3	1308.12	344.97	0.00	0.00	-650.86	1.00
Cd + Sex	3	1331.06	367.91	0.00	0.00	-662.32	1.00
Sex	2	1338.35	375.21	0.00	0.00	-667.08	1.00
Pb	2	1427.06	463.91	0.00	0.00	-711.43	1.00
Null	1	1442.14	478.99	0.00	0.00	-720.04	1.00
Cd	2	1444.13	480.98	0.00	0.00	-719.96	1.00

Appendix 5. Akaike Information Criterion for models examining Lesser Scaup (*Aythya affinis*) during captive experimental trials, comparing the effect of lead (Pb), cadmium (Cd), selenium (Se), sex, and trial on *Sphaeridiotrema pseudoglobulus* intensity.

Modnames	K	AICc	Delta_AICc	ModelLik	AICcWt	LL	Cum.Wt
Se + Sex	4	301.31	0.00	1.00	0.35	-146.05	0.35
Sex	3	301.69	0.38	0.83	0.29	-147.49	0.63
Pb + Sex	4	303.25	1.94	0.38	0.13	-147.02	0.77
Cd + Sex	4	303.67	2.36	0.31	0.11	-147.23	0.87
Se + Trial + Sex	6	305.31	4.01	0.13	0.05	-145.30	0.92
Pb + Trial + Sex	6	306.31	5.00	0.08	0.03	-145.80	0.95
Cd + Trial + Sex	6	306.64	5.33	0.07	0.02	-145.96	0.97
Null	2	309.73	8.42	0.01	0.01	-152.69	0.98
Cd	3	309.75	8.44	0.01	0.01	-151.52	0.98
Trial	4	310.12	8.81	0.01	0.00	-150.45	0.99
Se + Trial	5	310.17	8.86	0.01	0.00	-149.15	0.99
Se	3	310.17	8.86	0.01	0.00	-151.73	1.00
Cd + Trial	5	311.98	10.67	0.00	0.00	-150.05	1.00
Pb	3	311.99	10.69	0.00	0.00	-152.64	1.00
Pb + Trial	5	312.28	10.97	0.00	0.00	-150.20	1.00

Appendix 6. Akaike Information Criterion for models examining Lesser Scaup (*Aythya affinis*) during captive experimental trials, comparing the effect of lead (Pb), cadmium (Cd), selenium (Se), sex, and trial on *Cyathocotyle bushiensis* intensity.

Modnames	K	AICc	Delta_AICc	ModelLik	AICcWt	LL	Cum.Wt
Se	3	193.90	0.00	1.00	0.74	-93.38	0.74
Se + Sex	4	196.71	2.80	0.25	0.18	-93.35	0.92
Se + Trial	5	199.51	5.60	0.06	0.04	-93.17	0.97
Null	2	202.94	9.03	0.01	0.01	-99.20	0.97
Se + Trial + Sex	6	203.01	9.11	0.01	0.01	-93.17	0.98
Trial	4	203.73	9.83	0.01	0.01	-96.87	0.99
Pb	3	204.81	10.90	0.00	0.00	-98.83	0.99
Cd	3	205.35	11.44	0.00	0.00	-99.10	0.99
Sex	3	205.36	11.46	0.00	0.00	-99.11	0.99
Pb + Trial	5	206.40	12.49	0.00	0.00	-96.62	1.00
Cd + Trial	5	206.63	12.73	0.00	0.00	-96.74	1.00
Pb + Sex	4	207.16	13.25	0.00	0.00	-98.58	1.00
Cd + Sex	4	207.80	13.89	0.00	0.00	-98.90	1.00
Pb + Trial + Sex	6	209.22	15.32	0.00	0.00	-96.28	1.00
Cd + Trial + Sex	6	209.55	15.65	0.00	0.00	-96.44	1.00

Appendix 7. Akaike Information Criterion for models examining Lesser Scaup (*Aythya affinis*) during captive experimental trials, comparing the effect of lead (Pb), cadmium (Cd), selenium (Se), sex, and trial on Cecal Core Weight.

Modnames	K	AICc	Delta_AICc	ModelLik	AICcWt	LL	Cum.Wt
Se + Trial	5	48.07	0	1	0.46	-18.68	0.46
Se + Trial + Sex	6	49.15	1.08	0.58	0.26	-18.07	0.73
Se	3	50.01	1.94	0.37	0.17	-21.86	0.90
Se + Sex	4	52.20	4.12	0.12	0.05	-21.86	0.96
Trial	4	55.07	6.99	0.03	0.01	-23.30	0.97
Cd + Trial + Sex	6	56.91	8.84	0.01	0.005	-21.95	0.98
Pb + Trial + Sex	6	56.95	8.88	0.01	0.005	-21.97	0.98
Cd + Trial	5	57.00	8.92	0.01	0.005	-23.14	0.99
Pb + Trial	5	57.13	9.05	0.01	0.004	-23.21	0.99
Null	2	66.32	18.25	0.0001	5.02E-05	-31.09	0.99
Pb	3	67.21	19.13	6.98E-05	3.23E-05	-30.46	0.99
Cd	3	67.29	19.21	6.71E-05	3.10E-05	-30.50	0.99
Sex	3	68.46	20.39	3.73E-05	1.72E-05	-31.09	0.99
Cd + Sex	4	69.35	21.27	2.40E-05	1.11E-05	-30.44	0.99
Pb + Sex	4	69.38	21.31	2.35E-05	1.09E-05	-30.45	1