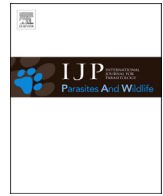




Contents lists available at ScienceDirect

# International Journal for Parasitology: Parasites and Wildlife

journal homepage: [www.elsevier.com/locate/ijppaw](http://www.elsevier.com/locate/ijppaw)

## Spatio-temporal variation in prevalence and intensity of trematodes responsible for waterfowl die-offs in faucet snail-infested waterbodies of Minnesota, USA

Charlotte L. Roy<sup>a,\*,1</sup>, Véronique St-Louis<sup>b</sup><sup>a</sup> Wetland Wildlife Populations and Research Group, Minnesota Department of Natural Resources, 102 23rd St NE, Bemidji, MN 56601, USA<sup>b</sup> Wildlife Biometrics Unit, Minnesota Department of Natural Resources, 5463-C West Broadway Avenue, Forest Lake, MN 55025, USA

### ARTICLE INFO

#### Article history:

Received 12 May 2017  
 Received in revised form  
 11 July 2017  
 Accepted 13 July 2017

#### Keywords:

*Bithynia tentaculata*  
*Cyathocotyle bushiensis*  
 Faucet snail  
*Sphaeriodiotrema* spp.  
 Trematodiasis  
 Waterfowl die-offs

### ABSTRACT

Several non-native trematodes hosted by the invasive Eurasian faucet snail, *Bithynia tentaculata*, have been causing die-offs of waterfowl in the Midwestern United States and Canada for several decades. Because of the potential implications of these die-offs on waterfowl in non-native settings, it is necessary to better understand the trematodes that cause the die-offs. Here, we studied the spatio-temporal dynamics of two trematodes, *Cyathocotyle bushiensis* and *Sphaeriodiotrema* spp., known to infect waterfowl in northern Minnesota, USA, via their intermediate host, the faucet snail (*Bithynia tentaculata*). We studied prevalence (% of snails infected within a sample) and intensity (mean number of parasites per infected snail within a sample) of faucet snail infection with these two trematodes in small lakes, large lakes, ponds, and rivers in northern Minnesota in the spring, summer, and fall of 2011–2013. We tested whether parasite prevalence and infection intensity could be explained spatially (as a function of the abundance of faucet snails, average snail size, water depth, and proximity to known waterfowl groups) and temporally (across years and seasons) using generalized estimating equation models. The spatial and temporal patterns we observed varied within and among waterbodies. For both parasite species, parasite prevalence and intensity of infection were consistently higher in samples with larger snails and in deeper portions of the waterbodies. In Lake Winnibigoshish, prevalence was lower farther from the large waterfowl groups we observed, but the abundance of snails in a sample had no effect on prevalence or intensity of infection. Our findings help improve understanding of this multi-species system, but also illustrate the complexity of modeling the spatial and temporal dynamics of infections in waterbodies that are so variable in size, shape, waterfowl use, and function.

© 2017 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### 1. Introduction

Since 2002, more than 135,000 waterbirds, primarily Lesser scaup (*Aythya affinis*) and American coots (*Fulica americana*), have died in the Midwestern United States from trematodiasis caused by the intestinal trematodes *Cyathocotyle bushiensis*, *Sphaeriodiotrema* spp., and *Leyogonimus polyoon* (USGS National Wildlife Health Center, J. Chipault, Unpublished results). The faucet snail (*Bithynia tentaculata*), a non-native species from Europe that serves as host to

these trematodes, was first discovered in the Upper Mississippi River in 2002 (Sauer et al., 2007). The faucet snail is the only known first intermediate host of these trematodes in the Midwestern United States and also serves as the second host for both *C. bushiensis* and *Sphaeriodiotrema* spp. (Khan, 1962; Gibson et al., 1972; Huffman and Fried, 1983; Ménard, 1986; Lepitzki et al., 1994). Although other snail species have been shown to be susceptible to infection of *Sphaeriodiotrema* spp. (Lepitzki, 1993), the faucet snail is still thought to be the principal host of the metacercarial stage infectious to waterfowl. Thus large waterfowl die-offs to trematodiasis have been found on waterbodies with faucet snail infestations (USGS National Wildlife Health Center, J. Chipault, unpubl. data), and noticeably large die-offs often provide the first indication that a waterbody is infested with faucet snails. For example, the faucet snail was discovered in Lake Winnibigoshish in

\* Corresponding author.

E-mail address: [charlotte.roy@state.mn.us](mailto:charlotte.roy@state.mn.us) (C.L. Roy).

<sup>1</sup> Present address: Forest Wildlife Population and Research Group, Minnesota Department of Natural Resources, 1201 East Highway 2, Grand Rapids, MN USA 55744.

north-central Minnesota after a die-off affecting thousands of Lesser scaup and hundreds of American coots in the fall of 2007 (Minnesota Department of Natural Resources, Unpublished results). Since then, waterfowl die-offs seem to have lessened over time, but the factors governing the severity of the die-offs remain unknown. To better understand these dynamics, our goal was to study spatio-temporal patterns in the prevalence and intensity of trematode infections within faucet snail-infested waterbodies in Minnesota.

The life cycle of *C. bushiensis*, *Sphaeridiotrema* spp., and *L. polyoon* is complex and involves two intermediate hosts and a definitive waterfowl host. The life cycle begins with the production of eggs by adult trematodes that are deposited in the gut of an infected waterfowl (Khan, 1962; Huffman et al., 1984). Infected waterfowl defecate parasite eggs (Khan, 1962; Huffman et al., 1984), which hatch after 3–5 weeks depending on water temperature (i.e., the warmer the faster the maturation; Erasmus, 1972; Ménard and Scott, 1987a; McKindsey and McLaughlin, 1993). Eggs hatch into miracidia that persist less than 24 h (8.8 h for *C. bushiensis* (Ménard and Scott, 1987a) or 14–22 h for *Sphaeridiotrema* spp. (McKindsey and McLaughlin, 1993)) before entering the first intermediate host, the faucet snail. Inside the faucet snail, they transform into sporocysts or rediae and proliferate asexually to produce large numbers of cercariae for what is thought to be the remainder of the snail's life (Herrmann, 2007). These cercariae are shed and exist outside the host briefly before infecting the same snail or another host snail (Dailey, 1996). They subsequently encyst into metacercariae and remain until the snail is consumed by the definitive waterfowl host or the snail dies (Dailey, 1996). The trematodes mature into adults in the gut of waterfowl, sexually reproduce, and then the eggs are defecated into the water. In heavy infections, which can be acquired in 24 h, waterfowl die within 3–10 days (Hoeve and Scott, 1988; Huffman and Roscoe, 1989; Mucha and Huffman, 1991). In milder infections, however, waterfowl may recover because the adult trematode life span is short (7–12 days in *C. bushiensis* (Khan, 1962), 10–16 days for *Sphaeridiotrema* spp. (Berntzen and Macy, 1969; Mucha and Huffman, 1991)) and waterfowl can acquire resistance that persists at least two weeks (Huffman and Roscoe, 1986, 1989; Hoeve and Scott, 1988).

Snails play a key role in transmitting trematode infections by shedding large numbers of cercariae (hundreds to thousands per day, Haseeb and Fried, 1997) and hosting metacercariae infective to waterfowl (Dailey, 1996). The number of snails at a given point in time and space therefore influences the number of potential first and second intermediate hosts, and the distances that must be traversed by miracidia or cercariae to reach hosts (Ménard and Scott, 1987a; Lepitzki et al., 1994), as well as the availability of snails infective to waterfowl. The abundance of snail hosts where trematode eggs are deposited is expected to be an important predictor of parasite prevalence and intensity in snails, because higher snail abundance should improve trematode success infecting hosts. However, in the summer, the addition of young, uninfected snails to the population reduces parasite prevalence in snails (Ménard and Scott, 1987b; Richter, 2001; Herrmann and Sorensen, 2009). The average infection intensity in a sample would be expected to decline temporarily as well, as young snails acquire infections of initially low intensity. Metacercarial abundance has been documented to decline in summer (Ménard and Scott, 1987b; Herrmann and Sorensen, 2009), and has been attributed to this new cohort of uninfected snails.

The mortality rates of infected and uninfected snails might also influence parasite prevalence and intensity. If infected snails are less able to cope with stress and have higher mortality rates than uninfected snails, prevalence would be expected to decline in the absence of new infections and as a result of stresses such as overwintering and reproduction (Ménard and Scott, 1987b). Likewise, if

snail mortality rates increase with infection intensity, then snails with more intense infections would be lost from the population faster than those with less intense infections. Differences in overwinter mortality rates were reported in Central Europe (Richter, 2001), but were not found in the Midwestern USA (Herrmann and Sorensen, 2009). Richter (2001) reported that almost all of the heavily parasitized older females died between May and July. Parasite prevalence and intensity of infection might thus decline in adult snails after the demands of summer reproduction. Consistent with this, Herrmann and Sorensen (2009) reported a reduction in the adult snail population after reproduction, which may result in reduced parasite prevalence and intensity after reproduction, but could not determine whether it occurred through movement or mortality.

Waterfowl are key for distributing trematode infections (Haseeb and Fried, 1997) through the defecation of parasite eggs because neither miracidia (from hatched eggs) nor cercariae are able to move very far (Ménard and Scott, 1987a; Dailey, 1996). If waterfowl consume infected and uninfected snails indiscriminately, directional impacts of foraging on parasite prevalence or intensity would not be expected. However, waterfowl defecation of parasite eggs should increase both prevalence and intensity of sporocyst and rediae infection in snails, and eventually (i.e., 3–4 months later after eggs hatch and cercariae begin shedding) metacercarial infections would be expected to increase. Furthermore, because warmer water temperatures favor faster maturation of eggs and a high proportion of eggs fail to hatch in cold water in the fall (Ménard and Scott, 1987a; McKindsey and McLaughlin, 1993), the influence of waterfowl defecating eggs would be expected to be greater after spring migration than after fall migration because conditions are better for parasite egg hatching in the spring and summer than in the fall (Herrmann and Sorensen, 2009).

Faucet snail distribution and abundance, waterfowl use, season, and perhaps local environmental conditions (e.g., current, depth) are all likely to influence the spatio-temporal distribution and abundance of trematode infections. Our goal was to evaluate how parasite prevalence and intensity varies spatially and temporally in faucet snail-infested waterbodies of north-central Minnesota, USA, to better understand what might affect the availability of trematodes to waterfowl. The waterbodies we selected include important stopover sites for waterfowl, as well as sites that receive little waterfowl use and where faucet snail abundance and distribution varies (Roy et al., 2016). We examined trematode prevalence and intensity in waterbodies of different sizes and function, including two large lakes, two small lakes, two rivers, and five ponds. We collected data for nine seasons over three years, as part of a larger study that also examined spatio-temporal patterns in faucet snail abundance and distribution (Roy et al., 2016).

We examined the following predictions:

- P1 Prevalence and intensity of trematode infections are positively related to local snail abundance within a waterbody because trematodes are more likely to encounter and infect faucet snail hosts where they are abundant. Water depth is expected to be related to infection intensity and prevalence of snails because of its strong correlation with snail abundance (Roy et al., 2016).
- P2 Prevalence and intensity are greater in the fall after snails and parasites reproduce than in the previous spring (same calendar year). Prevalence and intensity are lower in spring than the previous fall (different calendar years) after overwinter mortality of infected snails occurs.
- P3 Prevalence and intensity of all trematode stages are higher in waterbodies with more waterfowl use. On waterbodies where waterfowl provide a seasonal source of new

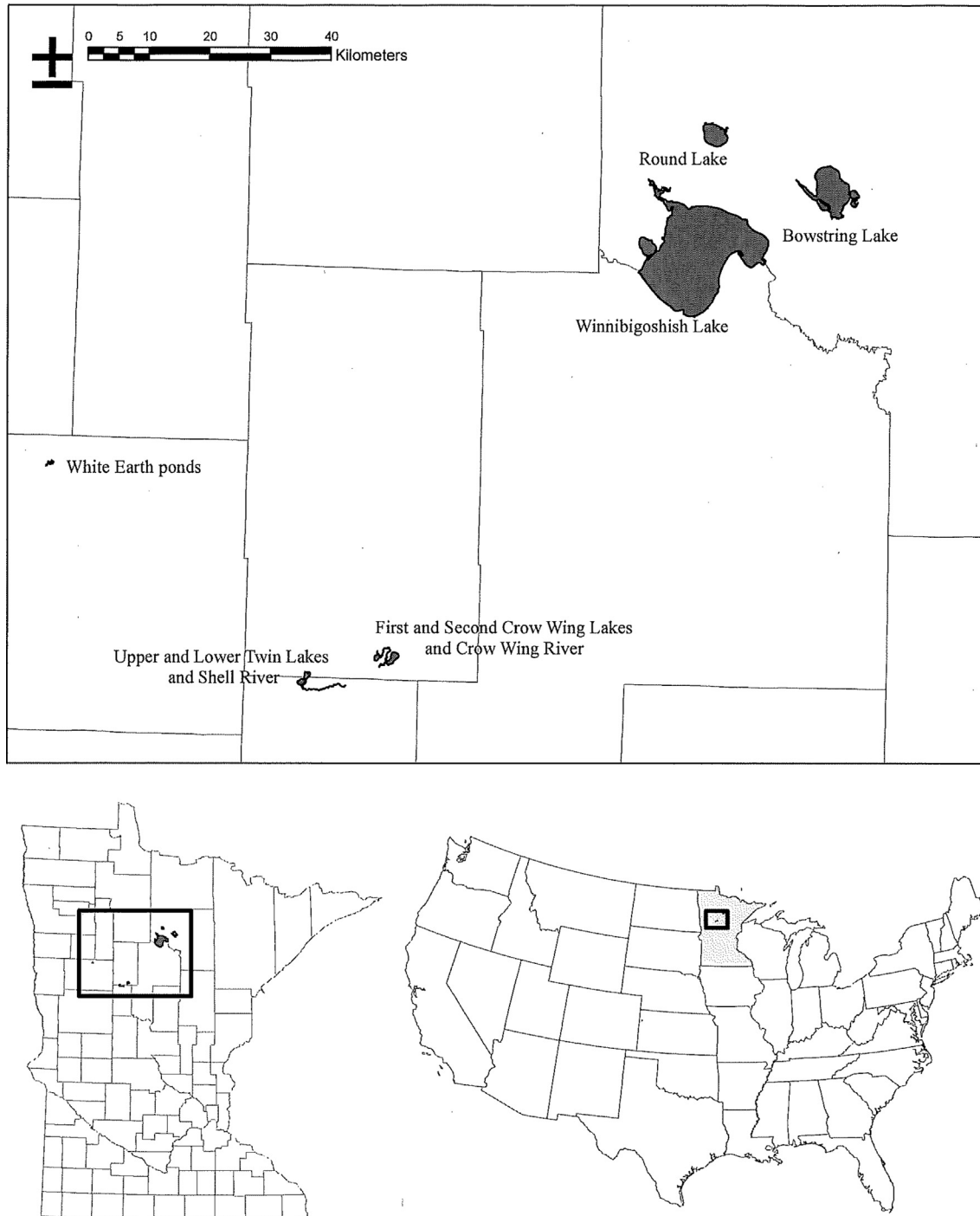
infections, prevalence will increase over time. Additionally, parasite prevalence and intensity within a waterbody is lower in samples collected farther away from known locations of large scaup groups within a waterbody.

**2. Materials and methods**

**2.1. Study area**

Our study area included all the waterbodies within Minnesota that were either known to be infested with faucet snails in 2011 or

where new infestations were discovered by the Minnesota Department of Natural Resources (MNDNR) Invasive Species Program (Roy et al., 2016) during the course of our three year study (2011–2013). We sampled Lake Winnibigoshish, Upper and Lower Twin lakes, and the Shell River during spring, summer, and fall in all three years of the study (Fig. 1; Table 1). The First and Second Crow Wing lakes, the Crow Wing River, and five ponds located on the White Earth Nation were added to the study later when these infestations were discovered, and then were sampled in all three seasons. An infestation was also discovered at Bowstring Lake in 2012, but we lacked resources to add another large waterbody to



**Fig. 1.** Map of study area in northcentral Minnesota depicting the study lakes with county boundaries, within the state and USA.

**Table 1**

Seasonal faucet snail sampling at the same locations in infested northern Minnesota waterbodies during spring, summer, and fall. Samples collected refers to the number of samples collected across all sampling seasons and years at repeatedly sampled snail points. Prevalence and intensity were estimated at points with  $\geq 40$  snails.

Location	Years sampled	Seasons sampled (No. visits/yr) <sup>a</sup>	Samples collected, no birds present	Points sampled under birds	Points with $\geq 40$ snails
East Winnibigoshish	2011–13	3 (11–12)	623	6	197
West Winnibigoshish	2011–13	3 (13)	628	28	110
Upper Twin Lake	2011–13	3 (6–7)	195	0	4
Lower Twin Lake	2011–13	3 (6–10)	347	0	58
First Crow Wing Lake	Fall 2011–2013	3 (7)	217	0	0
Second Crow Wing Lake	2012–13	3 (4)	96	0	0
Crow Wing River	Fall 2011–2013	3 (5–9)	107	1	27
White Earth Ponds	Fall 2012–2013	3 (3)	76	0	33
Shell River	2011–13	3 (3–6)	180	0	59
Bowstring Lake	Summer 2013	0 (2–4) <sup>b</sup>	20	36	2
Round Lake	2013	0 (1–6) <sup>b</sup>	NA	39	1

<sup>a</sup> When no season is specified, sampling occurred in the spring, summer, and fall.

<sup>b</sup> We sampled under rafts of scaup in spring 2012 and falls of 2011, 2012 and 2013 at Bowstring and Round lakes but we did not conduct snail sampling at established points.

the study and sampled near the known location of infestation only. Faucet snails were widely distributed and abundant at Lake Winnibigoshish, the Shell River, and White Earth ponds, and were more localized in their distribution at Upper and Lower Twin lakes, First and Second Crow Wing lakes, and the Crow Wing River (Roy et al., 2016).

## 2.2. Snail and trematode sampling

Our approach to sampling varied at each waterbody based on the area and whether the waterbody was a lake, river, or small pond (Roy et al., 2016). At Lake Winnibigoshish (22,853 ha), our largest lake, we sampled two index areas because of the size of the lake, one on the southwest side near the mouth of the Mississippi River (hereafter West Winnibigoshish) and one in the northeast, just south of Cut Foot Sioux (hereafter East Winnibigoshish). These two index areas were chosen because they were near boat accesses where faucet snails would likely be introduced and well established, yet they were on opposite sides of the lake. Each index area was 5–6 km along the longest dimension and approximately 2 km in width. We stratified points by depth using a Generalized Random Tessellation Stratified (GRTS) spatially-balanced design for sampling (Stevens and Olsen, 1999) based on basin bathymetry, and sampled seven depth increments of 5 feet (152 cm) up to 35 feet (1067 cm). In small lakes (<500 ha), we sampled points along transects spaced  $\geq 100$  m apart that traversed the entire length of the lake, and as a result included a range of depths. In rivers, we sampled points every 500 m along infested segments (as determined by the Invasive Species Program of the MNDNR) up to 10 km. In small ponds ranging in size from 2 to 8 ha, sample locations were separated by  $\sim 100$  m and determined in such a way as to maximize the number of sampling points in each pond (diameter 75–320 m). We sampled faucet snails during spring (Apr–May, but late ice-off delayed sampling in 2013 until May–mid Jun), summer (Jul and Aug, and early Sep 2011), and fall (Oct and Nov) at the same locations within lakes, rivers, and ponds in each of three years, or as new infestations of faucet snails were discovered by the Invasive Species Program of the MNDNR (Table 1), or while we were sampling foods under scaup rafts.

We used a benthic sled to sample snails because of its versatility at a range of depths and substrates. Depth was recorded at each point with the use of a depth finder or by dropping a line demarcated with distance increments of 25 cm. We dragged the sled 1.2 m at each point to quantify snail abundance. For samples possessing 10–49 *B. tentaculata*, we collected additional snails from the same location so that we could determine prevalence and intensity with a sample of approximately 50 snails (R. Cole, USGS

National Wildlife Health Center, pers. comm.). These additional snails were excluded from snail abundance estimates.

Snails were stored in the refrigerator until processed. We measured the size of faucet snails along the central axis from the apex with calipers. The proportion of snails that were infected (hereafter prevalence) was determined for all samples with at least 40 faucet snails. Snails were crushed, and the species of trematodes (*C. bushiensis*, *Sphaeriodiotrema* spp., *L. polyoon*) and stages present (sporocysts, rediae, cercariae or metacercariae) were determined with a microscope (Burns, 1961; Macy and Ford, 1964; Yamaguti, 1975). The average number of parasites of a given species and stage within the sample of infected hosts, thus excluding individuals that were not infected, was also calculated (infection intensity) for these same samples.

## 2.3. Scaup-level data collection

We observed Lesser scaup, Greater scaup (*Aythya marila*), and American coots foraging and resting on our focal waterbodies from shore or from a boat during our sampling visits (Table 1). The location of rafts of birds was determined with a compass from 2 to 3 observation points and plotted in ArcMap version 10 (Environmental Systems Research Institute, Inc., Redlands, California) in the field. We collected samples with a benthic sled at 100 m intervals along a transect passing through the entire area occupied by the birds. We sampled under as many groups of birds as possible during our site visits, but recognize that other groups of birds might have used these waterbodies but gone unobserved. Additional detail on waterfowl use is provided in Roy et al. (2016). For each of the waypoints for which we could determine parasite prevalence and infection intensity, the distance to scaup groups was determined by calculating the minimum Euclidean distance between a given waypoint and the nearest point sampled under a raft of scaup in either the same season, or up to two seasons prior in that same year. If a waypoint was sampled in the fall of 2011, for example, we calculated the minimum distance between that location and all locations sampled underneath scaup rafts in either the spring, summer, or fall of 2011. We determined minimum distances to scaup groups for Winnibigoshish only, because waterfowl use was more consistent compared to other waterbodies and snails were also widespread there (Roy et al., 2016).

We also collected a small sample of sick or dead Lesser scaup, Greater scaup, and American coot at study lakes and sent them to the National Wildlife Health Center (United States Geological Survey) in Madison, Wisconsin for confirmation of trematodiasis. During 2011–2013, 22 Lesser scaup, 7 Greater scaup, and 5 American coot were submitted from Winnibigoshish. Additionally,

Bowstring and Round lakes are known as stopover sites for migrating scaup and trematodiasis has been detected in birds from these locations. We sent 5 sick birds including 3 Lesser scaup, 1 Mallard (*Anas platyrhynchos*), and 1 Ring-necked duck (*Aythya collaris*) from Bowstring Lake for diagnosis as well.

#### 2.4. Data analysis

We modeled parasite prevalence and intensity as a function of faucet snail size (mm), abundance (log), water depth (cm), minimum distance to observed scaup groups, and year. Distance to observed scaup groups was only included in models for Lake Winnibigoshish because observations of waterfowl groups at other waterbodies were too limited to justify inclusion in models. We used generalized estimating equation (GEE) models (Hardin and Hilbe, 2013) to model parasite prevalence and intensity while accounting for the repeated nature of the data (i.e., each sampling point was repeated up to nine times during the study) (Table 2), because we were more interested in population responses than point-specific (subject-specific) responses (Fieberg et al., 2009). We used an exchangeable correlation structure so as not to infer a temporal pattern in the data. We calculated 95% bootstrap confidence intervals to evaluate if the coefficient estimates from the models were different from 0 at the 95% level. Specifically, we followed the nonparametric residual bootstrap approach proposed by Thai et al. (2013) and (1) fit a given model to the data, (2) extracted the fitted values and residuals, (3) resampled the residuals with replacement, (4) added up the “new” residuals to the fitted values to reconstruct the response ( $Y_{new}$ ), (5) re-ran the model using  $Y_{new}$  as new bootstrap responses with the original data as covariates, and (6) extracted and stored the coefficient estimates in a matrix. We repeated the procedure described above 1000 times, and looked at the 0.025 and 0.975 quantiles to construct 95% confidence intervals around the estimated coefficient for each of the covariates.

We fit the models described above for all waterbodies for which we were able to evaluate parasite prevalence across years and seasons (Table 1). Thus, our analysis included East Winnibigoshish and West Winnibigoshish, Lower Twin Lake, Shell River, Crow Wing River, and the five White Earth ponds (the latter combined into a single dataset). Note that prevalence could be determined for only 27 sampling points in the Crow Wing River, so results for this waterbody should be considered cautiously.

We used linear models to examine changes in parasite prevalence and intensity overwinter within a waterbody. Specifically, we compared prevalence and intensity of trematode infections in snails between fall 2011 and spring 2012, and also fall 2012 and spring 2013. We did not pair waypoints for this analysis, but rather looked for an overall decrease or increase in prevalence or intensity after the winter season in samples with at least 40 snails.

Additionally, we tested whether parasite prevalence and

infection intensity was significantly different between summer and spring, and between fall and summer of any given year using a linear model for each of the 3 years and pairs of the aforementioned, consecutive seasons. We used the first season as a reference (e.g., spring would be used as a reference in the spring vs. summer comparison), and repeated the analysis on 1000 bootstrap samples to calculate 95% bootstrap quantile intervals for the regression coefficients, i.e., following the same approach as outlined above.

All analyses were conducted in R (R Core Team, 2016). We used the geepack package (Yan, 2002; Yan and Fine, 2004; Højsgaard et al., 2006), specifically the geeglm function (Liang and Zeger, 1986; Prentice and Zhao, 1991) for fitting GEE models.

### 3. Results

#### 3.1. Parasite infections in faucet snails

We detected *C. bushiensis* and *Sphaeriodotrema* spp. in faucet snails from Lake Winnibigoshish, Lower Twin Lake, the Shell River, the Crow Wing River, Bowstring Lake, and the White Earth ponds. Only *C. bushiensis* was detected at Upper Twin Lake, but few samples ( $n = 4$ ) had enough faucet snails to determine prevalence and intensity (Table 1). We also detected *L. polyoon* infections in 4 snails from the Shell River, and at East Winnibigoshish, 15 snails were infected with unknown parasite species. Sporocyst and rediae infections were uncommon in all of the waterbodies we sampled (<2% of snails sampled, except Bowstring, Table 2), but metacercarial infections were common (Fig. 2a and b). Thus, we focused on metacercarial prevalence and intensity in our models.

Prevalence of *C. bushiensis* metacercariae was generally higher than that of *Sphaeriodotrema* spp. within a waterbody (Fig. 2a and b), but intensity of *Sphaeriodotrema* spp. metacercariae was often higher than *C. bushiensis* and individual snails could have >500 *Sphaeriodotrema* spp. metacercariae compared to typically <5 for *C. bushiensis* (Fig. 3a and b). Across all seasons, prevalence of *C. bushiensis* metacercariae was highest at the Shell River and the White Earth ponds, followed by West Winnibigoshish, East Winnibigoshish, Lower Twin, and Crow Wing River. Prevalence of *Sphaeriodotrema* spp. metacercariae was highest at West Winnibigoshish and then the Shell River, followed by East Winnibigoshish, Lower Twin, the White Earth ponds, and then the Crow Wing River. Intensity patterns among waterbodies were related to prevalence but exhibited more variability among waterbodies (Fig. 3a and b).

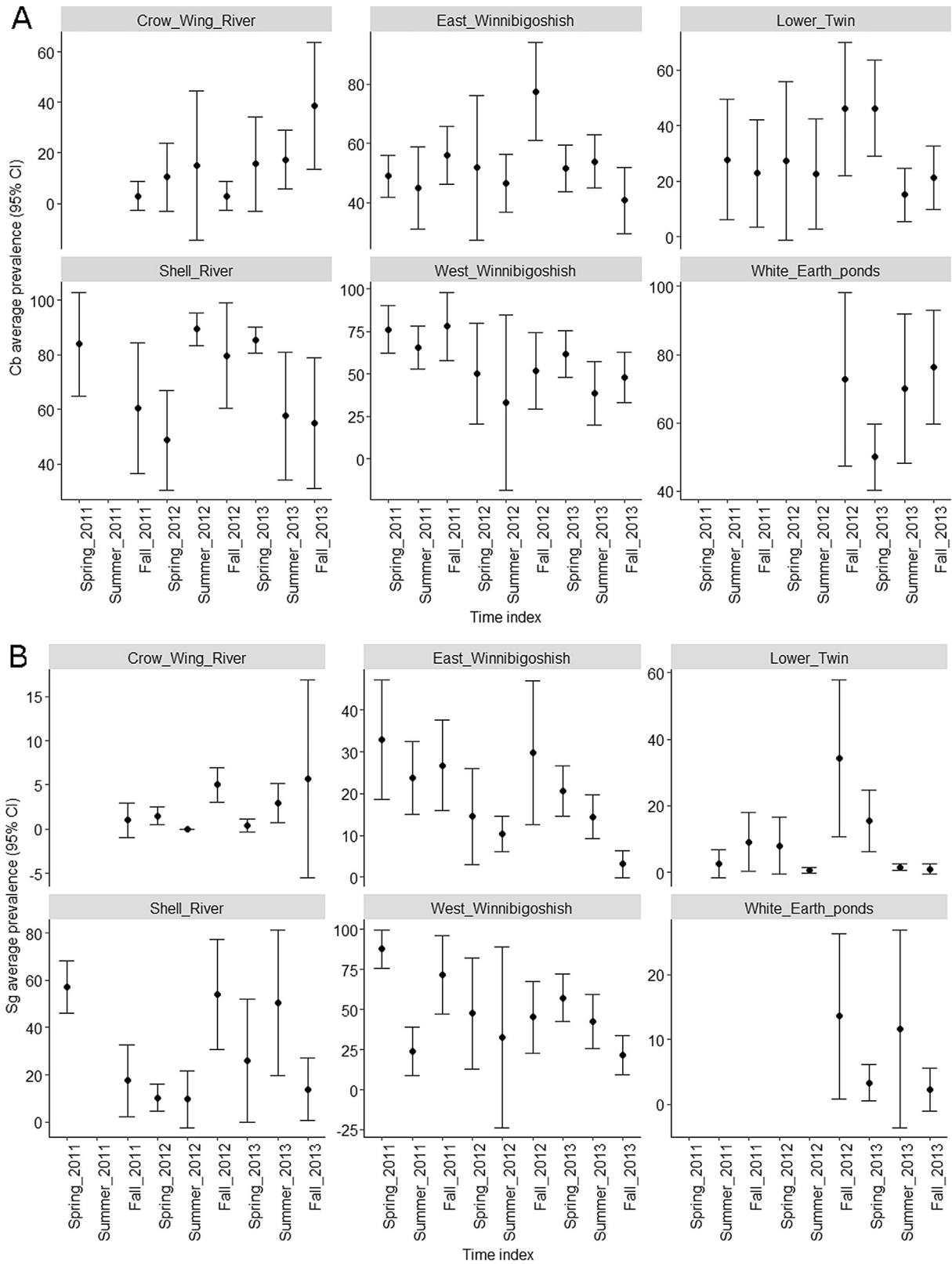
#### 3.2. Spatio-temporal patterns of metacercarial infections in faucet snails

Metacercarial prevalence (Fig. 2a and b) and intensity (Fig. 3a and b) varied among seasons in most waterbodies, but patterns differed within and among waterbodies both spatially and temporally (Fig. 4a–f). Prevalence of *Sphaeriodotrema* spp. and

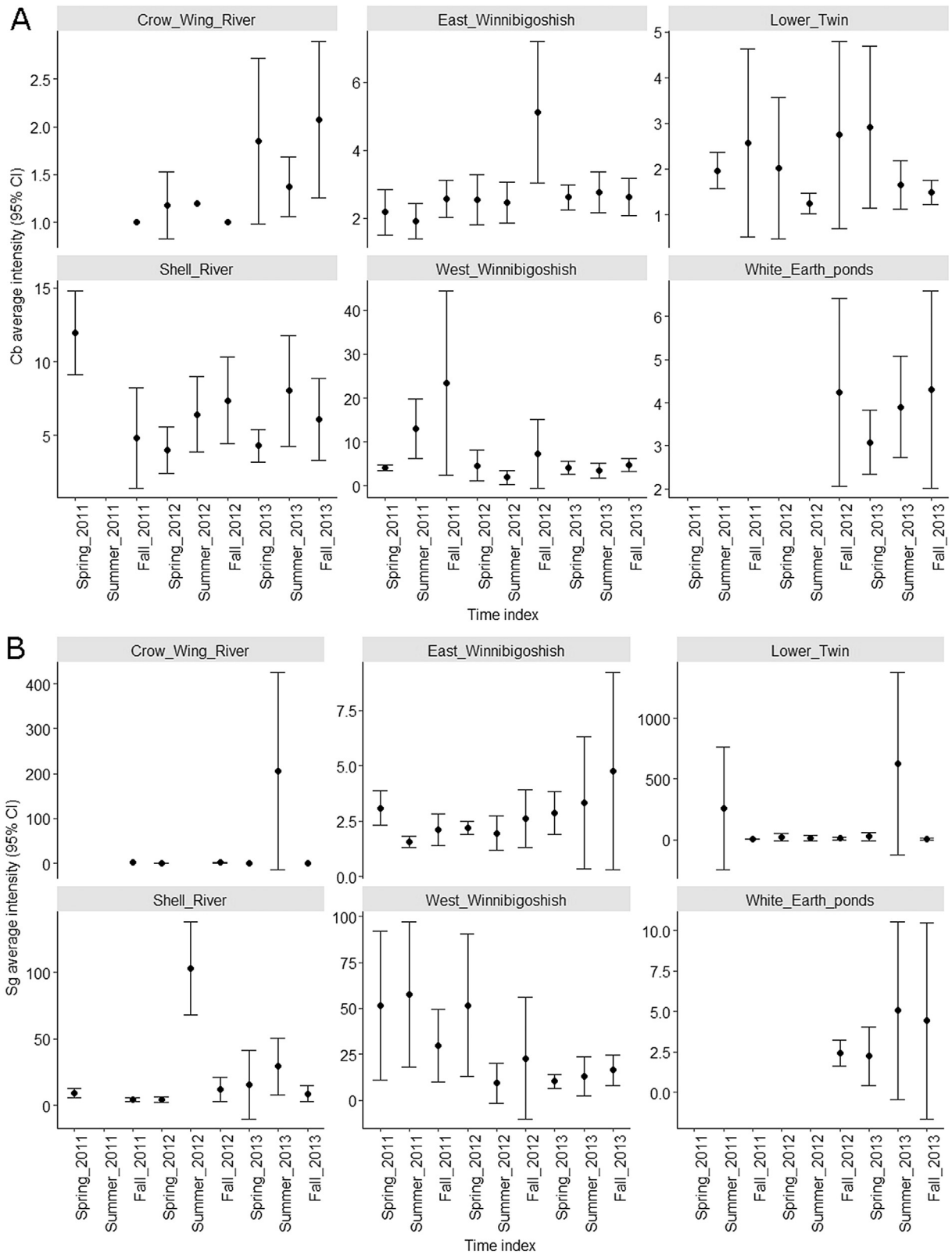
**Table 2**

Number (and percentage) of snails infected with rediae, sporocysts and/or cercariae of *Cyathocotyle bushiensis* (*Cb*) or *Sphaeriodotrema* spp. (*Sg*) in samples with enough snails to determine parasite prevalence and intensity of infection (i.e.,  $\geq 40$  snails) at each waterbody.

Lake	<i>Cb</i> infection	<i>Sg</i> infection	Total number of snails examined
East Winnibigoshish	0	5 (0.05%)	9848
West Winnibigoshish	16 (0.28%)	64 (1.14%)	5637
Lower Twin	0	1 (0.03%)	3040
Upper Twin	0	0	200
Shell River	3 (0.10%)	18 (0.59%)	3034
Crow Wing River	0	0	1339
White Earth ponds	0	0	1626
Bowstring	5 (5.05%)	1 (1.01%)	99



**Fig. 2.** Average prevalence of (a) *Cyathocotyle bushiensis* (Cb) and (b) *Sphaeriodiotrema* spp. (Sg) metacercariae in each of the waterbodies studied in northcentral Minnesota during nine seasons in 2011–2013 with 95% confidence intervals.



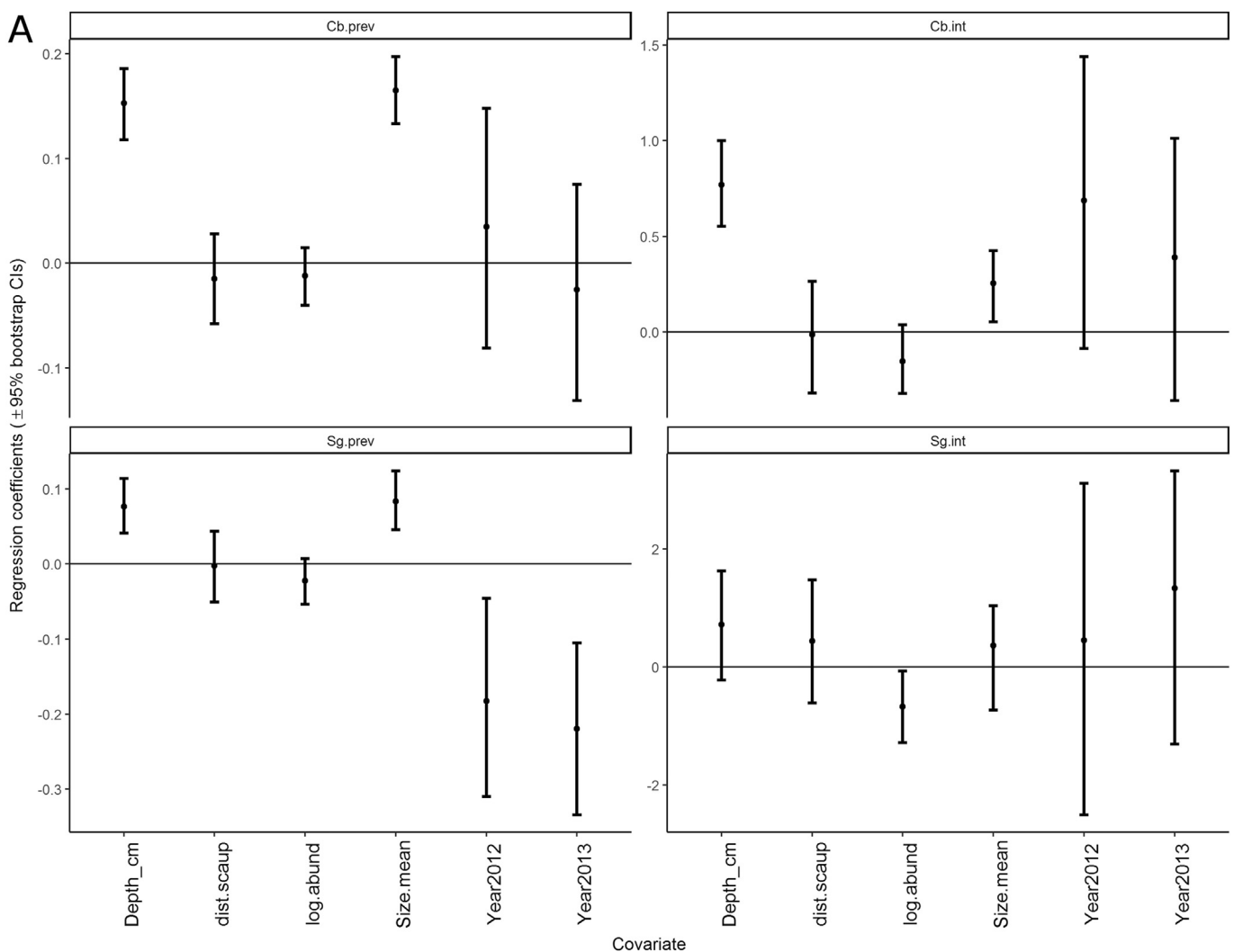
**Fig. 3.** Average intensity of (a) *Cyathocotyle bushiensis* (Cb) and (b) *Sphaeridiotrema* spp. (Sg) metacercariae in each of the waterbodies studied in northcentral Minnesota during nine seasons in 2011–2013 with 95% confidence intervals. Note that waterbody specific y-axis scales are used to highlight differences within a waterbody.

*C. bushiensis* metacercariae in fall samples was not consistently higher or lower than those collected in spring, but was most frequently greater than summer when significant (Table 3). At Winnibigoshish, all significant comparisons that included summer, had the lowest prevalence and intensity in summer (Fig. 2a and b; Table 3). Pairwise seasonal comparisons suggest that there were no consistent patterns in the prevalence and intensity of metacercarial infections in either parasite species on Winnibigoshish (Fig. 2a and b), although when detected, intensity was most frequently highest during fall for *C. bushiensis* and during spring for *Sphaeridiotrema* spp. (Table 3).

Metacercarial intensity and prevalence was highest in deep areas of Lake Winnibigoshish for both trematode species (Fig. 4a–b). Prevalence was also highest near observed scaup groups at the west index area of Lake Winnibigoshish (Fig. 4a–b) where we observed more birds (Roy et al., 2016). Other waterbodies had too few observations of waterfowl to allow testing the effect of proximity to waterfowl group statistically. Snail abundance did not

have strong patterns with metacercarial prevalence and intensity in either trematode species and at any of the waterbodies (Fig. 4a–f). Prevalence of both parasites was generally higher in samples where snails were larger (significant in 9 of 12 comparisons), but patterns were not as strong for intensity (significant in 5 of 12 comparisons), where sample sizes were lower due to the omission of uninfected samples in intensity estimates, especially where prevalence was low (e.g., Crow Wing River).

Examination of changes in prevalence over the winter indicated that in the cases where differences were significant, prevalence was always lower in spring than fall. This occurred for *C. bushiensis* at East Winnibigoshish during 2012–2013 ( $F_{1,51} = 11.9$ ,  $P < 0.005$ ) and in the White Earth ponds during 2012–2013 ( $F_{1,18} = 4.8$ ,  $P < 0.05$ ). Changes in *Sphaeridiotrema* spp. prevalence occurred overwinter at the Crow Wing River during 2012–2013 ( $F_{1,5} = 29.1$ ,  $P < 0.01$ ) and the White Earth ponds during 2012–2013 ( $F_{1,18} = 5.6$ ,  $P < 0.05$ ).



**Fig. 4.** Models of *Cyathocotyle bushiensis* (Cb) and *Sphaeridiotrema* spp. (Sg) metacercarial prevalence (prev) and intensity (int) in the waterbodies we studied in northern Minnesota during 2011–2013; a) East Winnibigoshish index area, b) West Winnibigoshish index area, c) Lower Twin Lake, d) Crow Wing River, e) White Earth ponds, f) Shell River. Depth\_cm is water depth at the sampling location. Dist.scaup is the minimum Euclidean distance between a given waypoint and the nearest point sampled under a raft of scaup in either the same season, or up to two seasons prior in that same year. Log.abund is the log transformed snail abundance at a sampling point. Size.mean is the mean snail size at a sampling point. Year2012 and Year2013 are comparisons between samples collected in 2011 vs 2012 and 2011 vs 2013, respectively.



3.3. Waterfowl

Large rafts of scaup (100–9000 birds) were located at Lake Winnibigoshish during our sampling activities in springs 2011, 2012, 2013 and falls 2011 and 2013, especially on the west side where we sampled snails. We observed large rafts of scaup (250–30,000 birds) at Bowstring Lake during springs 2011 and 2012 and falls 2011, 2012, and 2013. Small groups of scaup (<100) were located in the spring during our sampling on Lower Twin Lake, Crow Wing River, and First Crow Wing Lake, but birds often moved before their precise location could be determined. Large groups (350–800) of coots were observed once at the Twin Lakes and once at Lake Winnibigoshish during the fall of 2013. We sampled 110 points below 33 scaup rafts for faucet snails (Table 1, see also Roy et al., 2016).

All scaup submitted for confirmation of trematodiasis were infected with *Sphaeridiotrema* spp. and most (i.e., 73% of Lesser scaup and 86% of Greater scaup) were infected with *C. bushiensis*. The average intensity of infection with *C. bushiensis* and *Sphaeridiotrema* spp. (defined similarly as for snails) was 45 (95% bootstrap CI [16, 83]) and 6609 (95% bootstrap CI [4,344, 8926]) in Lesser scaup and 12 (95% bootstrap CI [7, 15]) and 4473 (95% bootstrap CI [2,579, 6568]) in Greater scaup, respectively. The coots

all tested positive for *C. bushiensis* (mean intensity 32, 95% bootstrap CI [8, 57]), negative for *Sphaeridiotrema* spp. and positive for *L. polyoon* (mean intensity 472, 95% CI [134, 920]). One American coot was submitted from Upper Twin Lake and died from infection with 100 *C. bushiensis*. The mallard and ring-necked duck tested positive for other parasites, but not *C. bushiensis* or *Sphaeridiotrema* spp.

4. Discussion

Our primary goal was to describe spatio-temporal patterns in trematode prevalence and intensity in faucet snail-infested waterbodies in Minnesota, with the ultimate goal of understanding what limits the availability of trematodes to waterfowl. Our results suggest that seasonal patterns of prevalence and intensity in faucet snails vary among waterbodies of different sizes and types, but also within a waterbody among years and seasons. This explains how disparate results arise from different studies, and it emphasizes the complexity of this system. Birds and snails vary in abundance and distributions over time, contributing to the high spatial and temporal variability in parasites as well. Furthermore, birds move on and off waterbodies, snails move within waterbodies, and early parasite stages can move in a river current or

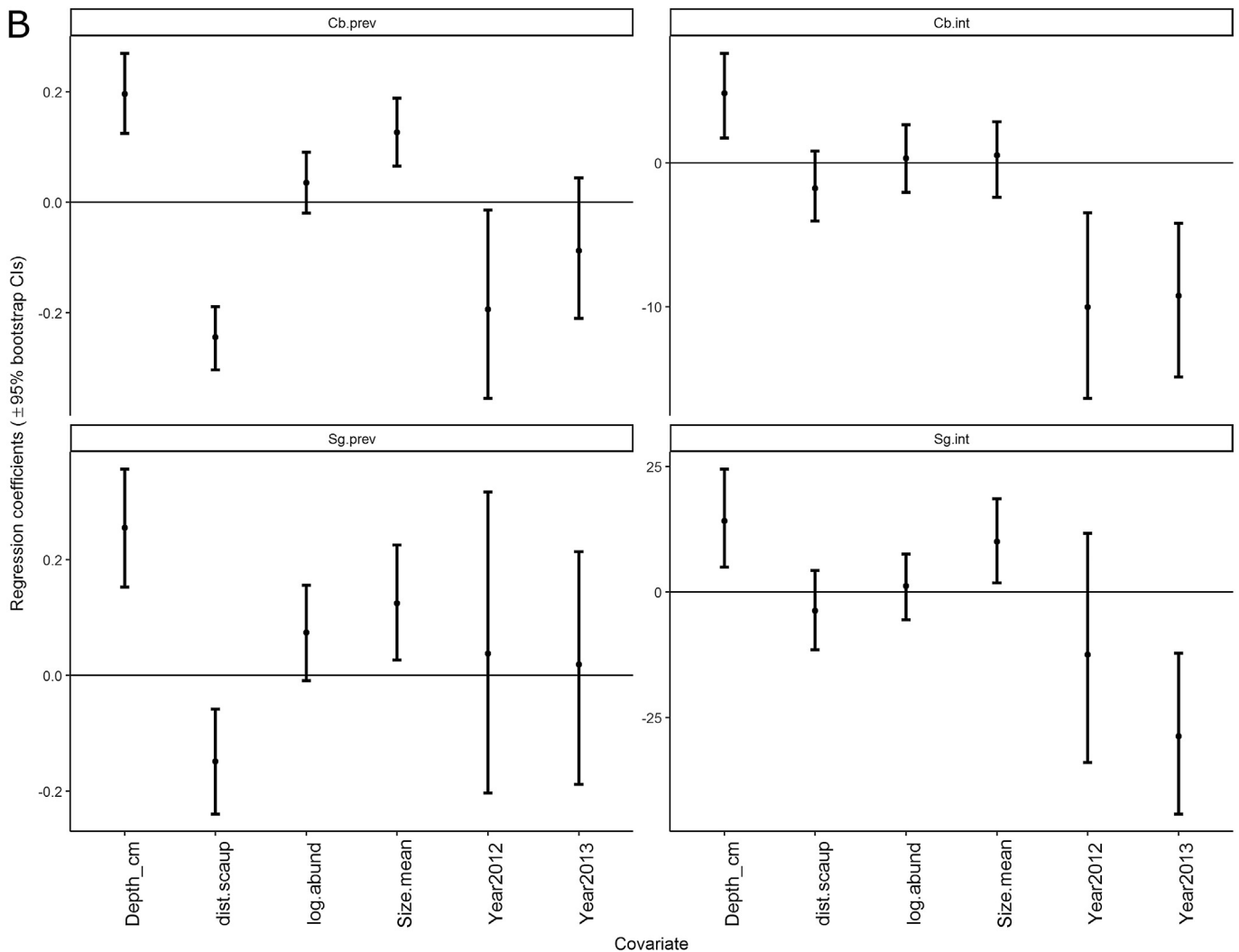


Fig. 4. (continued).

during a strong wind in shallow areas. As a result, generalizing and predicting patterns of trematode infections is quite difficult, yet the more we study these complex dynamics the closer we are to understanding large waterfowl die-offs.

Two patterns were fairly robust among waterbodies and index areas at Lake Winnibigoshish; prevalence and intensity of both trematodes was positively related to average snail size and water depth. Snail size has been demonstrated to be positively related to trematode infection in other studies (Ménard and Scott, 1987b; Lepitzki, 1993) because metacercarial infections persist for the lifetime of the host, and larger, older snails have more time to acquire infections. Infected snails also grow to a larger size, a phenomenon termed gigantism (Sandland et al., 2012). Greater surface area of larger snails might also provide a larger target for parasite encounters but this has not been demonstrated (Lepitzki et al., 1994). However, a positive relationship between infection metrics and snail size was expected and is consistent with findings from previous studies.

In contrast, the positive relationship we found between metacercarial prevalence and intensity with depth differs from the negative relationship observed in an intermittent stream in Ontario with negligible current (Lepitzki et al., 1994). These authors

attributed the negative relationship observed in sentinel snails to a possible dilution of cercariae in deeper water, or a possible preference for shallow water by snails shedding cercariae (Lepitzki et al., 1994). Yet depths in this sentinel snail study ranged 22–92 cm, which represents a narrow portion of the range of depths we examined (5–1186 cm across the whole study area). We found faucet snails to be more abundant in deep water at Lake Winnibigoshish (Roy et al., 2016) and thus expected that parasite prevalence and intensity would be higher in deeper areas as a result of a higher abundance of snails. Yet, we tested for and found that even after accounting for variability in snail average size and abundance (results not shown) the pattern we observed between parasite infections and depth still held. We suggest that the positive relationship between prevalence and intensity with depth might arise in three ways: 1) from spatial overlap of snails and infected waterfowl resting away from the shoreline in the fall, 2) through the action of wind-generated currents to move parasite eggs, miracidia, and/or shedding cercariae into deeper areas, or 3) through the movement of infected snails into deeper water.

We observed sick waterfowl among large groups of scaup resting away from the shoreline in the fall, presumably to avoid hunters (Shirkey, 2012). Sick and dying waterfowl generally occupy

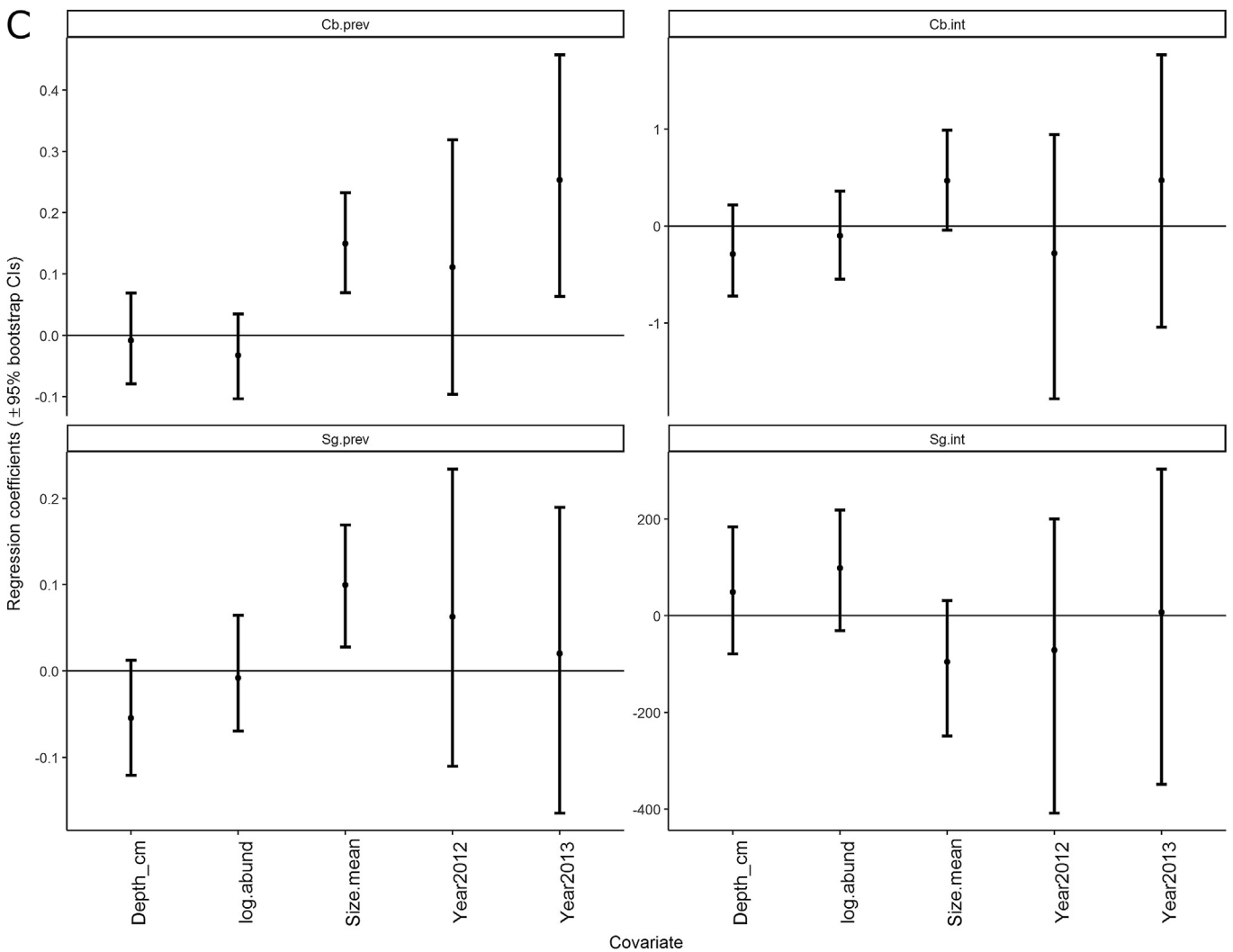


Fig. 4. (continued).

shallow areas when they are no longer able to dive or hold a position on the water in the wind (Roscoe and Huffman, 1982; Huffman and Roscoe, 1989; Mucha and Huffman, 1991; Herrmann and Sorensen, 2011), but in the fall, hunting pressure along the shoreline may deter use of shallow areas during the day (Shirkey, 2012). Some of the birds we observed off-shore were also unable to dive or fly and remained behind when the rest of the raft took flight. These birds may have been less infected than those near shore, but they nevertheless likely defecated trematode eggs in the deep waters where they were resting. This finding might also suggest that waterfowl hunting could influence the distribution of trematodes on large waterbodies by directly impacting waterfowl use patterns, and displacing egg deposition to areas where waterfowl rest but are unlikely to forage. Scaup indeed prefer to forage at shallower depths (Cottam, 1939; Cronan, 1957; White and James, 1978; Mulholland, 1985) than those where the most infected snails occurred (Roy et al., 2016). This pattern of prevalence and intensity with depth could persist in other seasons because of the limited movements of faucet snails (2.28 m/day, MacRae and Lepitzki, 1994) relative to the distance of our deepest points from the shore ( $\leq 2$  km).

Alternatively, or perhaps in conjunction, trematode eggs

deposited in shallow areas might be moved by wind-generated currents and gravity, as might miracidia and cercariae which are not capable of moving very far on their own (Lepitzki et al., 1994). The prevailing wind direction at Lake Winnibigoshish was out of the northwest, which would push trematodes east and south of our index areas into deeper areas. However, the patterns we observed could also result from movements of the most infected snails into deeper areas to avoid waterfowl predation or for other reasons (Roy et al., 2016). Infected snails may become unavailable to foraging waterfowl through the use of deep water habitats (Jones and Drobney, 1986; Mitchell, 1992; Roy et al., 2016), and other areas that are difficult or costly for waterfowl to forage (Lovvorn and Jones, 1991; Lovvorn et al., 1991; Beauchamp et al., 1992; de Leeuw and van Eerden, 1992; Lovvorn, 1994) such as the underside of rocks (Covich and Knežević, 1978; Brönmark, 1985; see review in Covich, 2010 for predator refugia). Less spatio-temporal overlap between waterfowl and the most infected snails, or reduced accessibility to snails, would reduce the magnitude of waterfowl die-offs.

Waterfowl use varied considerably among years, even within a waterbody. In some years, few birds became sick. When only a small number of waterfowl become infected, few eggs are

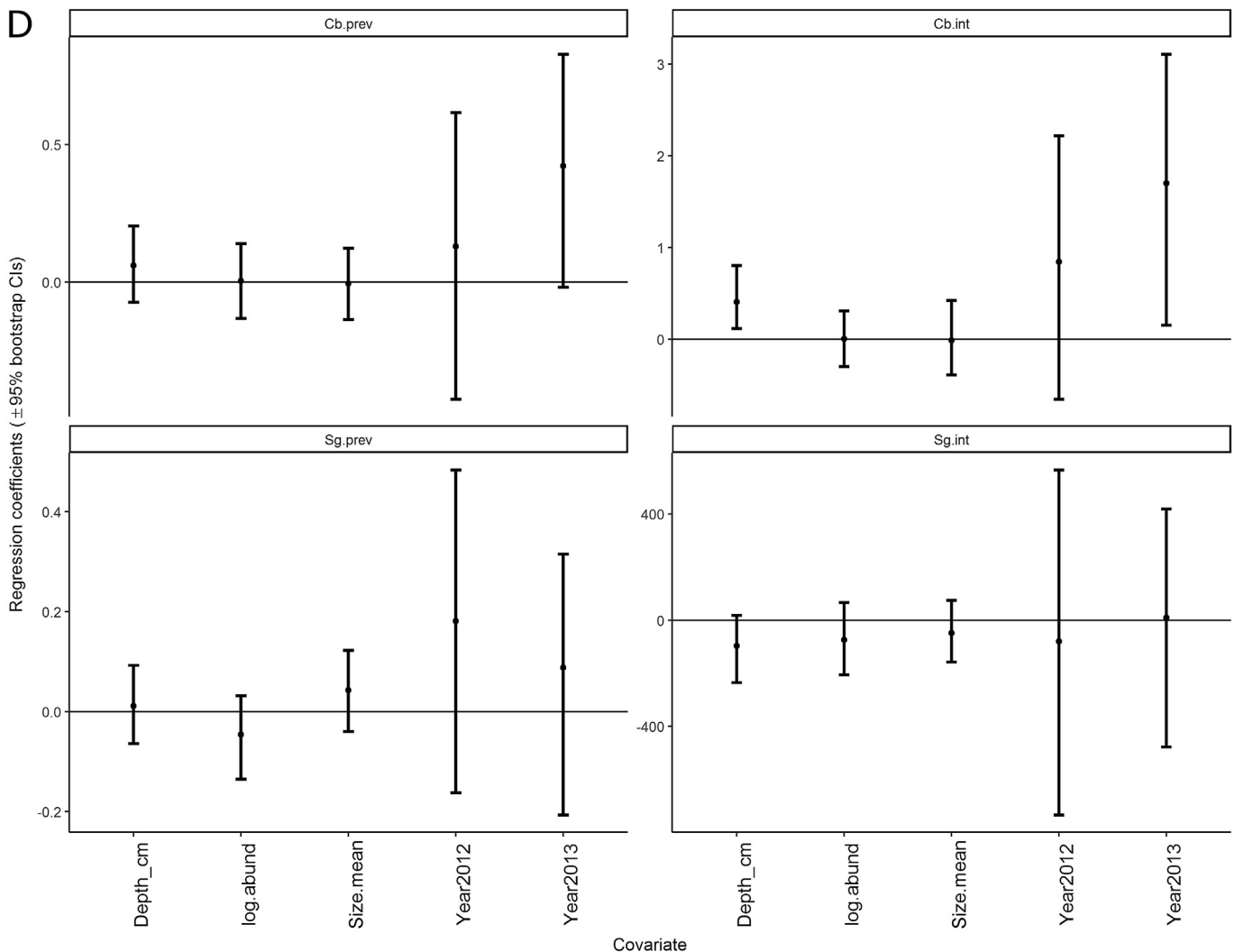


Fig. 4. (continued).

defecated and provide a limited source of new infections. We found low levels of infections with rediae or sporocysts compared to other studies. Prevalence of *Sphaeridiotrema* spp. rediae and sporocysts in snails in our study was lower, for example, than that reported for Upper Mississippi River (12.3–15.8%; Herrmann and Sorensen, 2009) and Golden Hill Creek in NY (12.3%, Karatayev et al., 2012). Prevalence of *C. bushiensis* rediae and sporocyst was also lower than reported for the Upper Mississippi River (2.1–2.2%) and more similar to those reported in Québec (0.5%; Gibson et al., 1972; 0.05%; Ménard and Scott, 1987b). This low level of sporocyst and rediae infection might indicate that the supply of eggs from infected waterfowl limits the life cycle of these parasite stages in the waterbodies we studied. However, snails infected with these early parasite stages shed hundreds to thousands of cercariae each day (Haseeb and Fried, 1997), which may result in an exponential increase in the number of metacercarial infections and explain the patterns we observed. Metacercarial infections in Winnibigoshish were high compared to other waterbodies in Minnesota with less waterfowl use and were more comparable to those of other studies, which is consistent with waterfowl dissemination of parasite eggs and subsequent amplification of cercarial stages to produce infections with metacercariae. *C. bushiensis* metacercarial prevalence at an impoundment lake of the Upper Mississippi River was

~40–90% and 33.3, 60.0, and 6.1% at Golden Hill Creek, Lake Oneida, and the Niagara River in NY in July, respectively (Herrmann and Sorensen, 2009; Karatayev et al., 2012). Similarly, *Sphaeridiotrema* spp. metacercarial prevalence at the impoundment lake of the Upper Mississippi River was 81.2–100% at two sites. Metacercarial infections are thought to persist for the life of the snail (three years; Dailey, 1996), but without waterfowl, will be lost slowly as infected snails die and are replaced with uninfected snails.

We found no relationship between snail abundance and the intensity of metacercarial infections. We had predicted a positive relationship because a local abundance of snails would provide more possible hosts for trematodes and thus be expected to produce more successful infections (i.e., fewer trematodes would fail to encounter a host). Miracidia and cercariae exist only briefly outside the snail host and are relatively immobile (Ménard and Scott, 1987a; Dailey, 1996), so we reasoned that many snails in a small area would increase infection success. Hundreds to thousands of cercariae are shed daily per primary intermediate host (Haseeb and Fried, 1997), we therefore did not anticipate that infections would be limited by the number of cercariae, but rather by the proximity to snail hosts. Lepitzki et al. (1994) reported that infection rates varied substantially in sentinel snails within meters of each other and that the spatial scale at which trematode stages move in the

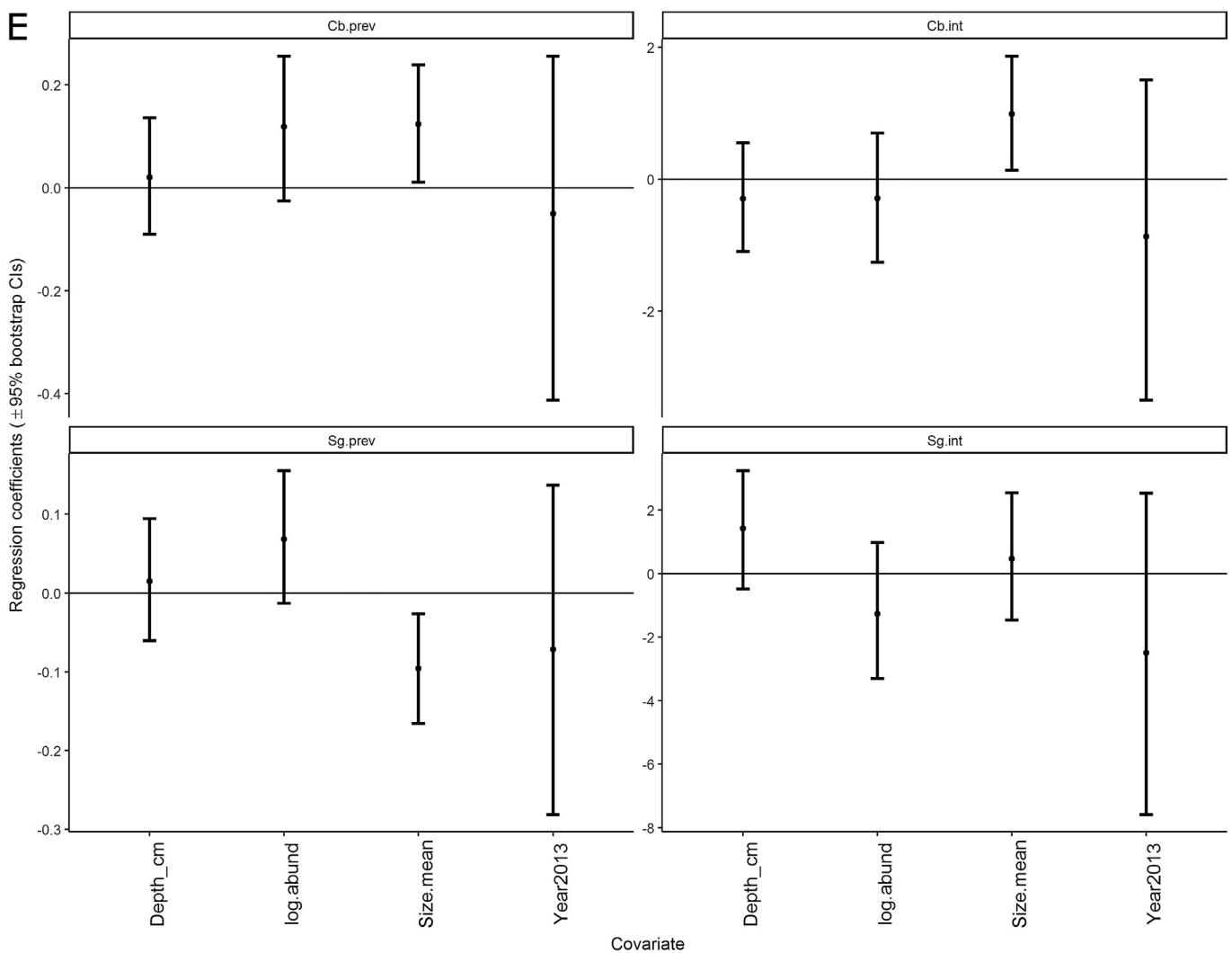


Fig. 4. (continued).

**Table 3**

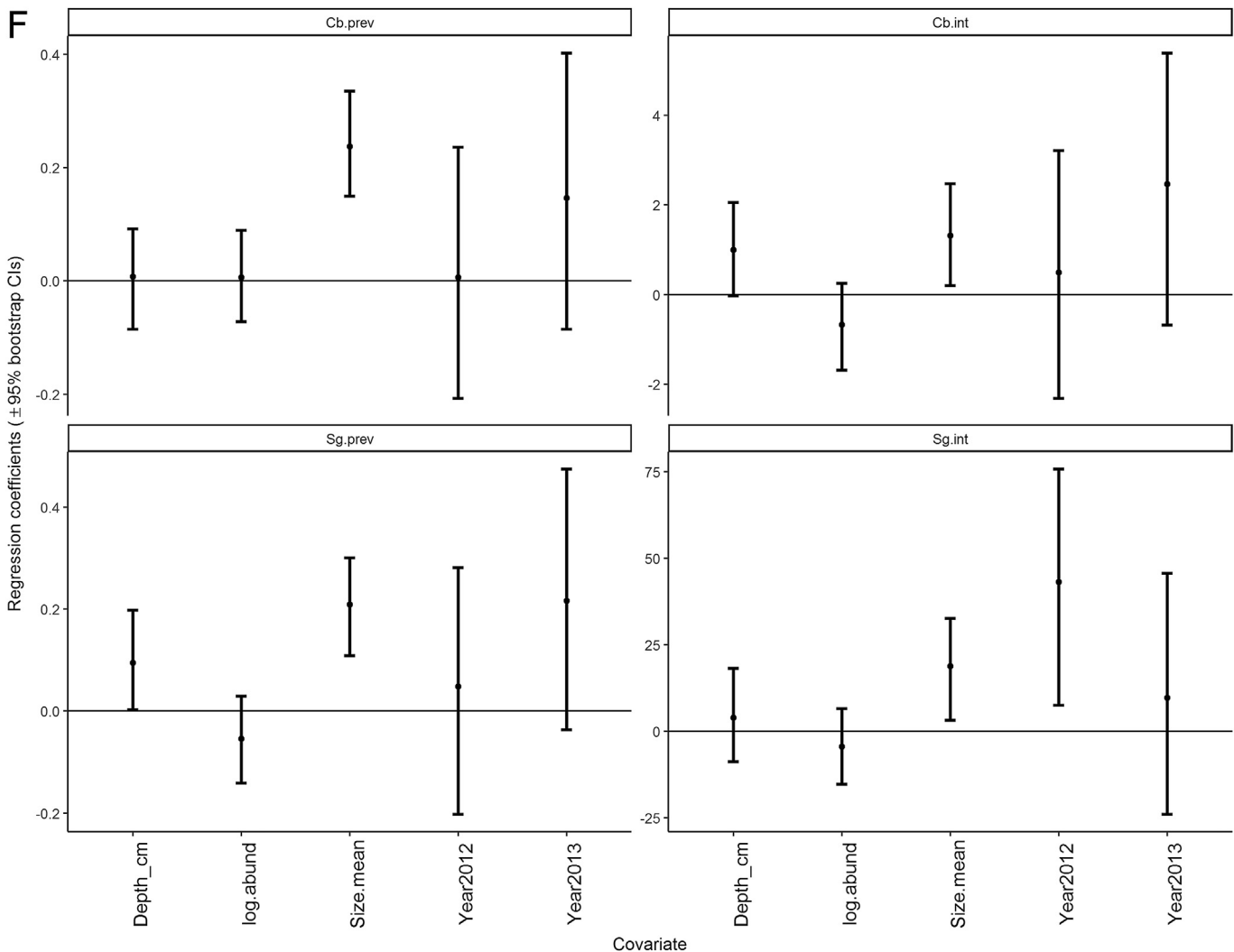
Statistical seasonal differences ( $\alpha = 0.05$ ) in linear models of trematode metacercarial stages in faucet snails (*Bithynia tentaculata*) after bootstrapping confidence intervals. Parasite infection metrics were determined for samples collected from Lake Winnibigoshish when containing  $\geq 40$  snails. Both prevalence (percent of snails infected) and infection intensity (average number of metacercariae in infected faucet snails) were considered for *Cyathocotyle bushiensis* (Cb) and *Sphaeridiotrema* spp. (Sg). Comparisons were made between spring (SP), summer (SU) and fall (FA) within a calendar year.

Waterbody	Cb prevalence	Cb intensity	Sg prevalence	Sg intensity
E. Winnibigoshish				
2011				SP > SU
2012	SU < FA	SP < FA FA > SU	SU < FA	
2013			SP > FA SU > FA	
W. Winnibigoshish				
2011		SP < FA SU > SP	SP > SU FA > SU	
2012	SU < FA		SU < FA	SP > SU
2013			SP > FA	

water was extremely localized. Host-directed orientation by miracidia and cercariae (Haas and Haberl, 1997) may result in snails nearest the source of infective stages receiving the majority of

trematodes, with nearby snails remaining uninfected. A higher number of possible hosts in a small area would then produce a smaller proportion of the available hosts becoming infected, but those infected would have more intense infections. However, if localized to this degree, it is unlikely that current or wind-generated movement of trematodes can explain the patterns we observed in relation to water depth.

Within a calendar year, we expected fall to have higher prevalence and intensity, following the reproduction of trematodes and snails in spring and early summer, which would lower prevalence and intensity through the addition of young, uninfected snails. Higher prevalence and intensity in the fall are consistent with parasite transmission, new infections, and asexual reproduction of early parasite stages during warmer months (Schell, 1985). Such a pattern has been observed in other studies (Herrmann and Sorensen, 2009). We also observed this, although prevalence and intensity during fall was sometimes lower than that observed during the previous spring, more similar to findings by Ménard and Scott (1987b) in Québec, Canada. The lowest prevalence and intensity has been found pretty consistently in summer (Ménard and Scott, 1987b; Herrmann and Sorensen, 2009), and that was the most common pattern we observed as well. We suggest that the addition of new snails to the population outpaced the addition of



**Fig. 4.** (continued).

new trematode infections in our study, where infections with sporocysts were uncommon like in Québec (Gibson et al., 1972; Ménard and Scott, 1987b). We suggest that in the impoundment lake of the Upper Mississippi River, where sporocyst and rediae infections are more prevalent (Herrmann and Sorensen, 2009), high waterfowl use increases the availability of trematode stages relative to snail abundance.

We compared the change in prevalence and intensity between fall and spring in samples collected from the same waterbody to examine overwinter mortality. Most often, we observed declines in prevalence over winter, with declines occurring during the 2012–2013 time period in numerous waterbodies. This would suggest that declines may be related to overwinter severity, or another factor external to the waterbody itself. Overwinter mortality may be related to winter temperatures or to the length of the winter (Richter, 2001). Spring of 2013 was very late in Minnesota, so late that ice-off of lakes occurred after scaup departed and birds could only find open water on rivers with flowing water. The impact of ice cover or water temperature on waterfowl access to infected faucet snails could explain differences between studies by Herrmann and Sorensen (2009) and Richter (2001). Differences in winter severity among years could even produce temporal variation within a waterbody but would be expected to have a correlated effect across waterbodies in the same geographic region. Annual differences in overwinter mortality might also be related to the intensity of infections within snails in a waterbody, with more intense infections producing greater mortality rates than less intense infections under stressful conditions. Yet under low stress conditions, trematode infection might have little impact on snail mortality, and then higher overwinter mortality of smaller snails could produce small increases in prevalence and intensity through loss of smaller, less infected snails. Small snails generally have fewer and less intense trematode infections but also have higher overwinter mortality in general (Richter, 2001).

We suggest that the complexity and stochasticity of the system will preclude, at least at this point, the reliable prediction of waterfowl die-offs in these waterbodies. Factors like wind strength and direction, water volume, current or flow, availability of snail refugia from waterfowl, and the number of susceptible waterfowl stopping during migration adds stochasticity to the system. Waterfowl maintain and perpetuate the life cycle of the trematodes we studied in each waterbody. Moreover, the availability of snail hosts does not appear to be limiting in the systems we studied. As long as waterfowl stopover frequently enough (e.g., every few years), the snail population will keep getting inoculated with trematode eggs, and infections will persist in the snail population. The high variability in use of waterbodies by waterfowl in our study creates a fluctuating pattern in trematode prevalence and intensity that does not appear to have a regular periodicity from the sample of waterbodies that we observed.

## 5. Conclusions

Our study suggests that, in the waterbodies we examined, water depth and distance to waterfowl groups can be important factors related to trematode prevalence and intensity within faucet snail populations. We also provide further evidence that trematode prevalence and infection intensity is higher in samples with larger snails on average, but that trematode infection in the waterbodies we studied was not related to the abundance of snails in a sample. High variability in this host-parasite system necessitates the consideration of various parasite stages, intermediate hosts, and definitive hosts in the same waterbody, as well as consideration of waterbodies of different sizes, types and functions, to fully capture its complexity. Continuing research in this area will complement

existing results and broaden our understanding of this system and potential applications to new systems.

## Acknowledgements

**Funding:** This work was supported by the U.S. Fish and Wildlife Service Migratory Bird Conservation Grant Program; with additional funding in the final year provided by MNDNR. Waterfowl die-offs were monitored by P. Loegering, M. Broschart, K. Carlisle, and M. Spoden. Trematodiasis in waterfowl was confirmed by L. White of the USGS National Wildlife Health Center. Water chemistry analysis was overseen by M. Briggs. Instruction on snail sampling and trematode identification was provided by G. Sandland, R. Haro, and R. Cole. The Wetland Wildlife Populations Group of the MNDNR provided input into study design and helped in the field. Statistical advice was provided by J. Fieberg and J. Giudice. J. House helped train interns and technicians, which included S. Adams, E. Butler, A. Fotjik, S. Shearen, M. Weegman, D. Ingison, K. Kuechle, N. Tourville, J. Norenberg, and N. Huls.

## References

- Beauchamp, G., Guillemette, M., Ydenberg, R., 1992. Prey selection while diving in common eiders, *Somateria mollissima*. *Anim. Behav.* 44, 417–426.
- Berntzen, A.K., Macy, R.W., 1969. In vitro cultivation of the digenetic trematode *Sphaeriodotremata globulus* (Rudolphi) from the metacercarial stage to egg production. *J. Parasitol.* 55, 136–139.
- Brönmark, C., 1985. Freshwater snail diversity: effects of pond area, habitat heterogeneity and isolation. *Oecologia* 67, 127–131.
- Burns, W.C., 1961. The life history of *Sphaeriodotremata spinoacetabulum* sp. n. (Trematoda: Psilostomidae) from the ceca of ducks. *J. Parasitol.* 47, 933–938.
- Cottam, C., 1939. Food habits of North American diving ducks. U. S. Dep. Agric. Tech. Bull. 643.
- Covich, A.P., Knežević, B., 1978. Size-selective predation by fish on thin-shelled gastropods (Lymnaea): the significance of floating vegetation as a physical refuge. *Verh. Intern. Ver. Limnol.* 20, 2172–2177.
- Covich, A.P., 2010. Winning the biodiversity arms race among freshwater gastropods: competition and coexistence through shell variability and predator avoidance. *Hydrobiologia* 653, 191–215.
- Cronan, J.M., 1957. Food and feeding habits of the scaups in Connecticut waters. *Auk* 74, 459–468.
- Dailey, M.D., 1996. Meyer, Olsen, and Schmidt's Essentials of Parasitology, sixth ed. McGraw Hill Companies, Inc., USA.
- de Leeuw, J.J., van Eerden, M.R., 1992. Size selection in diving tufted ducks *Aythya fuligula* explained by differential handling of small and large mussels *Dreissena polymorpha*. *Ardea* 80, 353–362.
- Erasmus, D.A., 1972. The Biology of Trematodes. Edward Arnold, London.
- Fieberg, J., Rieger, R.H., Zicus, M.C., Schildcrout, J.S., 2009. Regression modelling of correlated data in ecology: subject-specific and population averaged response patterns. *J. Appl. Ecol.* 46, 1018–1025.
- Gibson, G.C., Broughton, E., Choquette, L.P.E., 1972. Waterfowl mortality caused by *Cyathocotyle bushiensis* Khan, 1962 (Trematoda: Cyathocotylidae), St. Lawrence River, Québec. *Can. J. Zool.* 50, 1351–1356.
- Haas, W., Habertl, B., 1997. Host recognition by trematode miracidia and cercariae. In: Fried, B., Graczyk, T.K. (Eds.), *Advances in Trematode Biology*. CRC Press, New York, pp. 197–227.
- Hardin, J.W., Hilbe, J.M., 2013. Generalized Estimating Equations, second ed. Chapman & Hall/CRC Press, Boca Raton FL.
- Haseeb, M.A., Fried, B., 1997. Modes of transmission of trematode infections and their control. In: Fried, B., Graczyk, T.K. (Eds.), *Advances in Trematode Biology*. CRC Press, New York, pp. 31–56.
- Herrmann, K.K., 2007. Transmission Dynamics of *Cyathocotyle bushiensis* and *Sphaeriodotremata globulus* in Pool 7 of the Upper Mississippi River National Wildlife and Fish Refuge. Thesis. Minnesota State University, Mankato, 90 pp.
- Herrmann, K.K., Sorensen, R.E., 2009. Seasonal dynamics of two mortality-related trematodes using an introduced snail. *J. Parasitol.* 95, 823–828.
- Herrmann, K.K., Sorensen, R.E., 2011. Differences in natural infections of two mortality-related trematodes in lesser scaup and American coot. *J. Parasitol.* 97, 555–558.
- Hoeve, J., Scott, M.E., 1988. Ecological studies on *Cyathocotyle bushiensis* (Digenea) and *Sphaeriodotremata globulus* (Digenea), possible pathogens of dabbling ducks in southern Québec. *J. Wildl. Dis.* 24, 407–421.
- Højsgaard, S., Halekoh, U., Yan, J., 2006. The R package geepack for generalized estimating equations. *J. Stat. Softw.* 15, 1–11.
- Huffman, J.E., Fried, B., 1983. Trematodes from *Goniobasis virginica* (Gastropoda: Pleuroceridae) in Lake Musconetcong, New Jersey. *J. Parasitol.* 69, 429.
- Huffman, J.E., Fried, B., Roscoe, D.E., Cali, A., 1984. Comparative pathologic features and development of *Sphaeriodotremata globulus* (Trematoda) infections in mute

- swan and domestic chicken and chicken chorioallantois. *Am. J. Vet. Res.* 45, 387–391.
- Huffman, J.E., Roscoe, D.E., 1986. Acquired resistance in mallard ducks (*Anas platyrhynchos*) to infection with *Sphaeridiotrema globulus* (Trematoda). *J. Parasitol.* 72, 958–959.
- Huffman, J.E., Roscoe, D.E., 1989. Experimental infections of waterfowl with *Sphaeridiotrema globulus* (Digenea). *J. Wildl. Dis.* 25, 143–146.
- Jones, J.J., Drobney, R.D., 1986. Winter feeding ecology of scaup and common goldeneye in Michigan. *J. Wildl. Manage.* 50, 446–452.
- Karatayev, A.Y., Mastitsky, S.E., Burlakova, L.E., Karatayev, V.A., Hajduk, M.M., Conn, D.B., 2012. Exotic molluscs in the Great Lakes host epizootically important trematodes. *J. Shellfish Res.* 31, 885–894.
- Khan, D., 1962. Studies in larval trematodes infecting freshwater snails in London (U.K.) and some adjoining areas. Part VI. The cercariae of the “Vivax” group and the life history of *Cercaria bushiensis* n. sp. (= *Cyathocotyle bushiensis* n.sp.). *J. Helminthol.* 36, 67–94.
- Lepitzki, D.A.W., 1993. Epizootiology and Transmission of Snail-inhabiting Metacercariae of the Duck Digeneans *Cyathocotyle bushiensis* and *Sphaeridiotrema globulus*. Dissertation. Institute of Parasitology, McGill University.
- Lepitzki, D.A.W., Scott, M.E., McLaughlin, J.D., 1994. Assessing cercarial transmission of *Cyathocotyle bushiensis* and *Sphaeridiotrema pseudoglobulus* by use of sentinel snails. *Can. J. Zool.* 72, 885–891.
- Liang, K.Y., Zeger, S.L., 1986. Longitudinal data analysis using generalized linear models. *Biometrika* 73, 13–22.
- Lovvorn, J.R., Jones, D.R., 1991. Effects of body size, body fat, and change in pressure with depth on buoyancy and costs of diving in ducks. *Can. J. Zool.* 69, 2879–2887.
- Lovvorn, J.R., Jones, D.R., Blake, R.W., 1991. Mechanics of underwater locomotion in diving ducks: drag, buoyancy and acceleration in a size gradient of species. *J. Exp. Biol.* 159, 89–108.
- Lovvorn, J.R., 1994. Biomechanics and foraging profitability: an approach to assessing trophic needs and impacts of diving ducks. *Hydrobiologia* 279/280, 223–233.
- MacRae, M., Lepitzki, D.A., 1994. Population estimation of the snail *Bithynia tentaculata* (Gastropoda: Prosobranchia) using mark-recapture and the examination of snail movement in pools. *Can. Field Nat.* 108, 58–66.
- Macy, R.W., Ford, J.R., 1964. The psilostome trematode *Sphaeridiotrema globulus* (Rud.) in Oregon. *J. Parasitol.* 128, 7–14.
- Ménard, L., 1986. Aspects of the Ecology of the Larval Stages of *Cyathocotyle bushiensis* Khan 1962 (Digenea) Thesis. Institute of Parasitology, McGill University.
- Ménard, L., Scott, M.E., 1987a. Hatching and survival characteristics of *Cyathocotyle bushiensis* Khan 1962 (Digenea) eggs and miracidia. *Parasitol.* 95, 355–362.
- Ménard, L., Scott, M.E., 1987b. Seasonal occurrence of *Cyathocotyle bushiensis* Khan 1962 (Digenea: Cyathocotylidae) metacercariae in the intermediate host *Bithynia tentaculata* L. (Gastropoda: Prosobranchia). *Can. J. Zool.* 65, 2980–2992.
- McKindsey, C.W., McLaughlin, J.D., 1993. The viability of *Sphaeridiotrema pseudoglobulus* (Digenea) eggs following cold water storage as a possible overwintering strategy. *Parasitol.* 107, 441–447.
- Mitchell, C.A., 1992. Water depth predicts redhead distribution in the lower laguna madre, Texas. *Wildl. Soc. Bull.* 20, 420–424.
- Mucha, K.H., Huffman, J.E., 1991. Inflammatory cell stimulation and wound healing in *Sphaeridiotrema globulus* experimentally infected mallard ducks (*Anas platyrhynchos*). *J. Wildl. Dis.* 27, 428–434.
- Mulholland, R., 1985. Habitat suitability index model: lesser scaup (wintering). *U. S. Fish Wildl. Serv. Biol. Rep* 82 (10.91), 15.
- Prentice, R.L., Zhao, L.P., 1991. Estimating equations for parameters in means and covariances of multivariate discrete and continuous responses. *Biometrics* 47, 825–839.
- R Core Team, 2016. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>.
- Richter, D.T., 2001. Reproductive Biology and Life History Strategy of *Bithynia tentaculata* (Linnaeus, 1758) and *Bithynia leachii* (Sheppard, 1823). Dissertation. Vom Fachbereich Biologie der Universität Hannover, 176 pp.
- Roscoe, D.E., Huffman, J.E., 1982. Trematode (*Sphaeridiotrema globulus*)-induced ulcerative hemorrhagic enteritis in wild mute swans (*Cygnus olor*). *Avian Dis.* 26, 214–224.
- Roy, C.L., St-Louis, V., House, J., 2016. Seasonal distribution of the invasive faucet snail, *Bithynia tentaculata*, within infested waterbodies in Minnesota, USA, including waterfowl migration. *Biol. Invasions* 18, 2923–2941. <http://dx.doi.org/10.1007/s10530-016-1183-5>.
- Sauer, J.S., Cole, R.A., Nissen, J.M., 2007. Finding the exotic faucet snail (*Bithynia tentaculata*): Investigation of water bird die-offs on the Upper Mississippi River National Wildlife and Fish Refuge. U.S. Geological Survey Open-File Report 2007–1065, 3 p.
- Sandland, G.J., Houk, S., Walker, B., Haro, R.J., Gillis, R., 2012. Differential patterns of infection and life-history expression in native and invasive hosts exposed to a trematode parasite. *Hydrobiologia* 701, 89–98.
- Schell, S.C., 1985. Trematodes of North America, North of Mexico. University Press of Idaho, Moscow, Idaho, 263 pp.
- Shirkey, B.T., 2012. Diving Duck Abundance and Distribution on Lake St. Clair and Western Lake Erie. Thesis. Michigan State University, 116pp.
- Stevens, D.L., Olsen, A.R., 1999. Spatially restricted surveys over time for aquatic resources. *J. Agric. Biol. Environ. Stat.* 4, 415–428.
- Thai, H.T., Mentré, F., Holford, N.H.G., Veyrat-Follet, C., Comets, E., 2013. A comparison of bootstrap approaches for estimating uncertainty of parameters in linear mixed-effects models. *Pharm. Stat.* 12, 129–140.
- White, D.H., James, D., 1978. Differential use of freshwater environments by wintering waterfowl of coastal Texas. *Wilson Bull.* 90, 99–111.
- Yamaguti, S., 1975. A Synoptical Review of Life Histories of Digenetic Trematodes of Vertebrates: with Special Reference to the Morphology of Their Larval Forms. Keigaku Publishing Co, Tokyo.
- Yan, J., 2002. Geepack: Yet Another Package for Generalized Estimating Equations R-news, 2/3, pp. 12–14.
- Yan, J., Fine, J.P., 2004. Estimating equations for association structures. *Stat. Med.* 23, 859–880.