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Environmental and ecological factors driving trematode parasite community assembly in central Alberta lakes

Michelle A. Gordy [a], Janet Koprivnikar [b], Brooke McPhail [a], Patrick C. Hanington [a,*]

[a] School of Public Health, University of Alberta, Edmonton, AB, Canada
[b] Department of Chemistry & Biology, Ryerson University, Toronto, ON, Canada

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ABSTRACT

Parasites have been neglected from most biodiversity surveys even though they are an essential component of ecosystems and intimately associated with the free-living communities within them. Parasites with complex life cycles, such as digenean trematode flatworms, utilize at least two host species within an ecosystem for their development and transmission, taking advantage of species networks to complete their life cycles. Despite this knowledge, our understanding of the processes that contribute to parasite community assembly, and which limit their geographic distributions, are rudimentary, including the importance of host diversity. Utilizing recent advancements in the identification of cryptic trematode species through molecular barcoding, we examined patterns of community assembly involving 79 species in six Alberta lakes over three years. Specifically, we focused on spatiotemporal variation in trematode diversity within their snail first intermediate hosts (component communities), how this might relate to host diversity through the specificity of host-parasite relationships, and the role of certain environmental factors in structuring these communities. We found substantial natural fluctuations of trematode communities through space and time within these lakes. Trematode communities were diverse, showing an overall positive relationship with snail diversity, but were often dominated by a few common species. We found that ecoregion and lake trophic status were key predictors for the presence of these trematode species. Such information is key for understanding how biodiversity alterations may affect parasite community composition, as well as our ability to formulate predictive models, by considering how this could influence both species richness and evenness.

1. Introduction

Historically, parasites have been largely overlooked in studies considering the structure and biodiversity of ecological communities; yet their presence or absence may tell us more about ecosystem dynamics than only considering their larger and more visible free-living hosts (Hatcher and Dunn, 2011; Marcogliese, 2005; Sures et al., 2017). This is particularly true for parasites with complex, multi-host life cycles that involve diverse taxa. For instance, digenean trematodes are parasitic flatworms that typically utilize snails as their first intermediate host for larval development, and a vertebrate as the definitive host for adult worms, with trophic transmission via consumption of an infected second intermediate host (Esch et al., 2002). As such, trematodes take advantage of food webs, incorporating vertebrates, invertebrates, and even plants as vehicles for the transmission of infective stages. Trematodes, therefore, have close ties with many other species within ecological communities, not only functioning as parasites, but also as competitors, and even as prey (Johnson et al., 2010; Hatcher and Dunn, 2011; Soldánová et al., 2012).

Snail populations in aquatic ecosystems often host rich trematode component communities (see Bush et al., 1997), i.e. a diverse array of species (Gordy et al., 2016; Schwelm et al., 2018). However, trematode transmission success between the definitive and intermediate hosts can be shaped by several, and perhaps correlated, factors, such as intermediate host density, diversity, competence, and community assembly (Johnson et al., 2019). Trematode transmission success can thus be considered from the perspective of parasite community composition and evenness within snail hosts, along with patterns in their community assembly and disassembly. As such, here we sought to identify key environmental influences on local trematode component community structure within snails found in temperate lake ecosystems in Alberta, aiming to characterize variation through space and time, and also to

* Corresponding author. School of Public Health, University of Alberta, 357F South Academic Building, Edmonton, AB, T6G 2G7, Canada.
E-mail address: pch1@ualberta.ca (P.C. Hanington).

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examine the relationship between snail and trematode communities at different scales.

While other studies have broadly reported positive associations between host and parasite diversity (e.g., Kamiya et al., 2014), including that of definitive hosts and trematode communities within snails (e.g., Hechinger and Lafferty, 2005), this was not necessarily seen for snail intermediate host diversity (e.g., Thieltges et al., 2011). However, spatial scale and level of taxonomic resolution may be important contributors to the variation in the host-parasite diversity association reported among studies (Wood and Johnson, 2016). For these reasons, we particularly wanted to gain a fine-scale understanding of how host-trematode interactions (via snail host specificity) could affect the timing of parasite peak abundance, the persistence of trematode species in the community, and their categorization as either dominant, common, or rare species.

When considering how and why trematode component communities within snail hosts may show spatiotemporal variation in temperate lakes, many biotic and abiotic factors can affect trematode presence, abundance, and successful life cycle completion in temperate lakes (Thieltges et al., 2008). At northern latitudes, lake ecosystems are highly dynamic, and strongly affected by seasonality (Goldammer and Furyaev, 1996; Elmqvist et al., 2004). Spring and summer reflect periods of peak productivity for many lake inhabitants, and variation in their growth and reproductive rates at these times can affect fluctuations in population sizes and generational turnover (Conover, 1992). When considering how such host population dynamics could ultimately affect trematode community assembly, there are both bottom-up and top-down processes at play, such as nutrient availability and food web dynamics, respectively (Lafferty et al., 2008). Notably, birds often serve as definitive hosts for trematodes (Schell, 1985), and many are migratory species that travel to northern latitudes for breeding during the spring and summer months. As such, the behaviour of migratory species, including timing of arrival, nesting location, and movement patterns could highly influence trematode community assembly dynamics through the variable input of parasite eggs into the ecosystem.

In addition to natural processes, various anthropogenic factors can affect the abundance and presence of both hosts and parasites through space and time. As mentioned above, nutrient availability can affect trematode communities, and cultural eutrophication (i.e. increased algal biomass via the runoff of fertilizers into lakes) can cause snail community shifts that favour certain trematode species (Johnson and Chase, 2004). In addition, other aquatic contaminants have been shown to affect trematode diversity within first and second intermediate hosts, as have landscape-level influences such as habitat change or recreational use that can affect definitive host habitat preferences (King et al., 2007; Koprivnikar et al., 2007; Sures et al., 2017). Although this is not an exhaustive list of possible drivers, there are many ecological and environmental factors that can possibly affect spatiotemporal patterns in trematode species richness and abundance, as well as the distribution of freshwater snails, within temperate lakes.

Environmental influences can directly affect parasite presence, or indirectly do so by affecting host assemblages, and thereby parasite communities (e.g., Berkhout et al., 2019); however, local site characteristics can be more influential in structuring trematode communities within intermediate hosts than regional ones (Richgels et al., 2013). As such, we not only investigated whether snail trematode community structure was influenced by select environmental factors that differed in spatial scale, but also in their seasonal variation. For example, dissolved oxygen levels and temperature can have strong seasonal variation in temperate, shallow lakes in Alberta (Mitchell and Prepas, 1990) and therefore potentially contribute to temporal differences in trematode community composition through effects on snails and free-swimming larval trematode stages. In contrast, relatively static variables related to landscape features could be better predictors of spatial variation in trematode community composition if they function as determinants of definitive host presence.

To examine biotic and abiotic influences on trematode community variation within their intermediate snail hosts, we built upon a recent survey that aimed to identify larval trematodes within snails from six lakes in central Alberta (Gordy et al., 2016; Gordy and Hanington, 2019). From a sample of nearly 18,000 snails over three summers (2013–2015), 79 trematode species were found based on molecular phylogenetic identifications from five snail host species (Gordy and Hanington, 2019), representing a fine level of taxonomic resolution over a relatively small spatial scale that allowed for an opportunity to focus on regional dynamics here. Previous studies examining this many trematode species did so over much larger spatial scales (Thieltges et al., 2011), or employed smaller spatial scales with fewer species (Richgels et al., 2013), and none utilized molecular approaches to characterize species.

Using this 2013–2015 survey data, we aimed to answer the question of whether broad and fairly static landscape variables (e.g., river basin, ecoregion) were stronger determinants of snail trematode community composition relative to more local and temporally variable abiotic factors (e.g., lake temperature, dissolved oxygen), hypothesizing that the latter would have greater impact. In addition, we hypothesized that snail community composition would play a role in determining trematode component communities given the specificity of host-parasite relationships.

2. Methods

2.1. Trematode and snail host diversity and associations

The collection of trematode cercariae from their snail first intermediate hosts in central Alberta has been previously described (Gordy et al., 2016), as were the methods used for species identifications through molecular and morphological assessments (Gordy et al., 2017; Gordy and Hanington, 2019), thus we only summarize the key findings here. The final dataset from this survey resulted in 2452 trematode samples that ultimately represented 79 trematode species identified from infections within five freshwater snail species: *Stagnicola elodes, Lymnaea stagnalis, Helisoma trivolvis, Physa gyrina,* and an unidentified planorbid. The collections took place from June to September 2013–2015 on a biweekly basis across six lakes in central Alberta: Buffalo Lake, Gull Lake, Isle Lake, Lac La Nonne, Pigeon Lake, and Wabamun Lake (for specific site information, see Gordy et al., 2016; Gordy and Hanington, 2019).

2.2. Environmental data

During each field collection, measurements were taken using a YSI Professional Plus multiparameter meter (Xylem, Inc.) for the following water-based parameters: temperature (°C), salinity (ppt), pH, dissolved oxygen (ppm), and barometric pressure (pKa). The meter was placed in the water at no less a depth of 1 m directly in the collection area. Microhabitat-specific measurements included sediment type and anthropogenic use (site type). Sediment was haphazardly sampled from each collection location with a grab sample, and sediment type was broadly characterized based on the relative size of the most dominant feature in the sample area (silt, sand, or pebble). Site type was categorized as either a boat launch or a beach for swimming. Each lake was also characterized by its river basin and ecoregion (macrohabitat defined by physiography, vegetation, and soils and influenced by climate -also referred to as Natural Regions) as determined by using the most recent delineations from the Government of Alberta (Alberta Government, 2016; https://rivers.alberta.ca/).

2.3. Statistical analyses

Trematode component communities are defined here as all the trematode species found among all snails of a given species within a

defined geographical space, i.e. collection sites (see Bush et al., 1997 for standard definitions). To best align with the definitions of Bush et al. (1997), we use the term cumulative component community when referring to analyses that focus on assessment of multiple component communities in snail hosts defined by lake. Trematode component and cumulative communities were derived from the subset of snail samples that contained patent trematode infections, while snail community data was calculated from all snails in each collection, including uninfected snails. Because very few samples came from Pigeon Lake, these were not included in diversity analyses beyond richness calculations.

2.4. Community diversity

The following packages were utilized in R version 3.4.3 (R Core Team, 2017) to calculate diversity metrics and plot them: *vegan* (Oksanen et al., 2018), *BiodiversityR* (Kindt and Coe, 2005), *dplyr* (Wickham et al., 2017), *ggpubr* (Kassambara, 2017), and *ggplot2* (Wickham, 2009). Effective species (exp(*H*)) were calculated from normalized species abundance data for each sample, using the Shannon index (*H*). For each lake, a Spearman rank correlation was used to test the relationship between snail and trematode richness, as well as between snail and trematode effective species (*ggpubr*). This correlation test was repeated for the three sites within Buffalo Lake (Pelican Point, Rochon Sands, and The Narrows) for effective species and pooled richness for all sites because it was the only lake sampled at multiple sites.

To examine how the species diversity of trematode component communities changed over time, each unique combination of collection site and date was considered as a separate sample unit and assigned a unique number. Both alpha diversity (α) (*vegan*:diversity) and effective species (exp(*H*)) were then calculated from normalized species abundance data for each sample unit using the Shannon index (*H*). Community evenness was calculated as effective species/species richness. These metrics were derived for all sites.

2.5. Community comparisons and species dominance

When comparing communities to each other, a shortened dataset was used that only included lakes which were sampled all three years: Buffalo, Gull, Isle, and Wabamun. Because Buffalo Lake had three sites, these were all included separately rather than pooled so as not to inflate the results. For these six sites, α diversity was based on Shannon entropy (exp(*H*)) and calculated as described above. Gamma diversity (γ) was calculated as the pooled Shannon entropy for each site by using the diversity comp (*vegan*) command, of which the exponent was taken for the calculation of effective species. Beta diversity (β) was then calculated based on effective species as exp(γ)/exp(α). These indices were also calculated in the same manner for different groupings of these sites based on micro- or macrohabitat attributes such as site type (beach or boat launch) or landscape (ecoregion and river basin), respectively.

Multivariate homogeneity of group dispersion was used to explore β diversity among the four different groupings of the sample units (site, site type, ecoregion, and river basin). The vegdist (*vegan*) command was used to generate a Bray-Curtis dissimilarity matrix based on normalized (*vegan*:decostand) abundance data. This dissimilarity matrix was then used in a multivariate dispersion model (*vegan*:betadisper), the results of which were reduced into principal coordinates to assess the differences in two-dimensional space. A permutation test was then run using the dispersion model result with the command permutest (*vegan*) and run for 999 permutations. Finally, an ANOVA was used on the results of the model to look for any significant differences in group dispersion, followed by Tukey's Honest Significant Different (HSD) posthoc tests to further examine pairwise differences.

In contrast to group dispersion, we also calculated community overlap in trematode species composition among the four different groupings. Overlap was analyzed in terms of effective species using a formula [(α/γ - 1/N)/(1 - 1/N)], where N represents the number of communities, or separations, within each grouping. An analysis of similarity was also conducted by comparing the dissimilarities within and between groups using a permutation test via anosim (*vegan*) that was run for 999 permutations.

Rank-abundance dominance (RAD) models were used to analyze species' dominance in each community. The radfit (*vegan*) command was used to find the best model based on Akaike Information Criterion (AIC) values. The best fit model was then applied to each individual site. Rényi entropy was used (*BiodiversityR*:renyiresult) to analyze the role of rare species in communities.

2.6. Environmental influences

To test whether our environmental variables were associated with trematode species composition (relative abundance), we first utilized an ordination method via canonical correspondence analysis (CCA) (*vegan*: cca) so as to examine whether species composition was affected by specific combinations of environmental factors. Within the CCA, we used model-building methods to find the best fit model with particular variables included or not based on AIC values via a permutation test (*vegan*: add 1, test = "permutation"). The variables tested were: water temperature, month (a proxy for seasonality as it can be associated with either relative average air temperature or life cycle timing), salinity, dissolved oxygen, pH, barometric pressure, sediment type, latitude, longitude, primary productivity status of the lake (eutrophic or hypereutrophic), river basin (North Saskatchewan or Red Deer), and ecoregion (Aspen Parkland, Boreal Forest, or Mixed).

Because CCA is not considered a traditional hypothesis testing method, we then tested the same environmental variables as fixed effects in a Generalized Linear Mixed Model with a multinomial distribution and generalized logit link function in IBM® SPSS® Statistics Premium version 24. The response variable used in all models was trematode species absence or presence in each sample after reducing the dataset to the top five most abundant species (*Australapatemon burti* LIN1, *Diplostomum* sp. 4, *Echinoparyphium* sp. Lineage 2, *Notocotylus* sp. A, and *Plagiorchis* sp.). Categorical random effects included in the model were year and collection site. Owing to the number of environmental predictors, and the decreased power to detect significant effects with high degrees of freedom, we first tested each predictor separately for significance to ensure that only potentially influential variables were ultimately considered. Those that were significant fixed effects on their own were then combined into a full model, with subsequent iterations removing insignificant terms in sequential fashion until only significant predictors remained in the final model. The final model was run with the response variable in both ascending and descending order to retrieve specific effects for the intercept.

3. Results

3.1. Community diversity

There was a significant positive relationship between pooled snail host and trematode richness for all sites ($r = 0.67$, $P = 0.046$) (Fig. 1A). Sample-based analyses (i.e. separate for each lake, excluding Pigeon Lake) revealed a positive correlation between snail and trematode richness for Buffalo Lake ($r = 0.32$, $P < 0.001$, Gull ($r = 0.22$, $P < 0.001$), and Lac La Nonne ($r = 0.37$, $P < 0.001$), whereas a negative correlation was found for Isle ($r = -0.53$, $p < 0.001$) and Wabamun ($r = -0.22$, $P < 0.001$) lakes (Fig. 1B). Effective species (a measurement of diversity that incorporates relative abundance) was related to snail species richness in all lakes except Wabamun (Buffalo: $r = 0.084$, $P = 0.033$; Lac La Nonne: $r = 0.17$, $P < 0.001$; Isle: $r = -0.27$, $P < 0.001$; Wabamun: $r = -0.046$, $P = 0.48$; Gull: $r = -0.16$, $P < 0.001$) (Fig. 1C). In terms of effective species, the strongest correlation between snail and trematode diversity was found at The Narrows site at Buffalo Lake ($r = 0.71$, $P < 0.001$) (Fig. 1D).

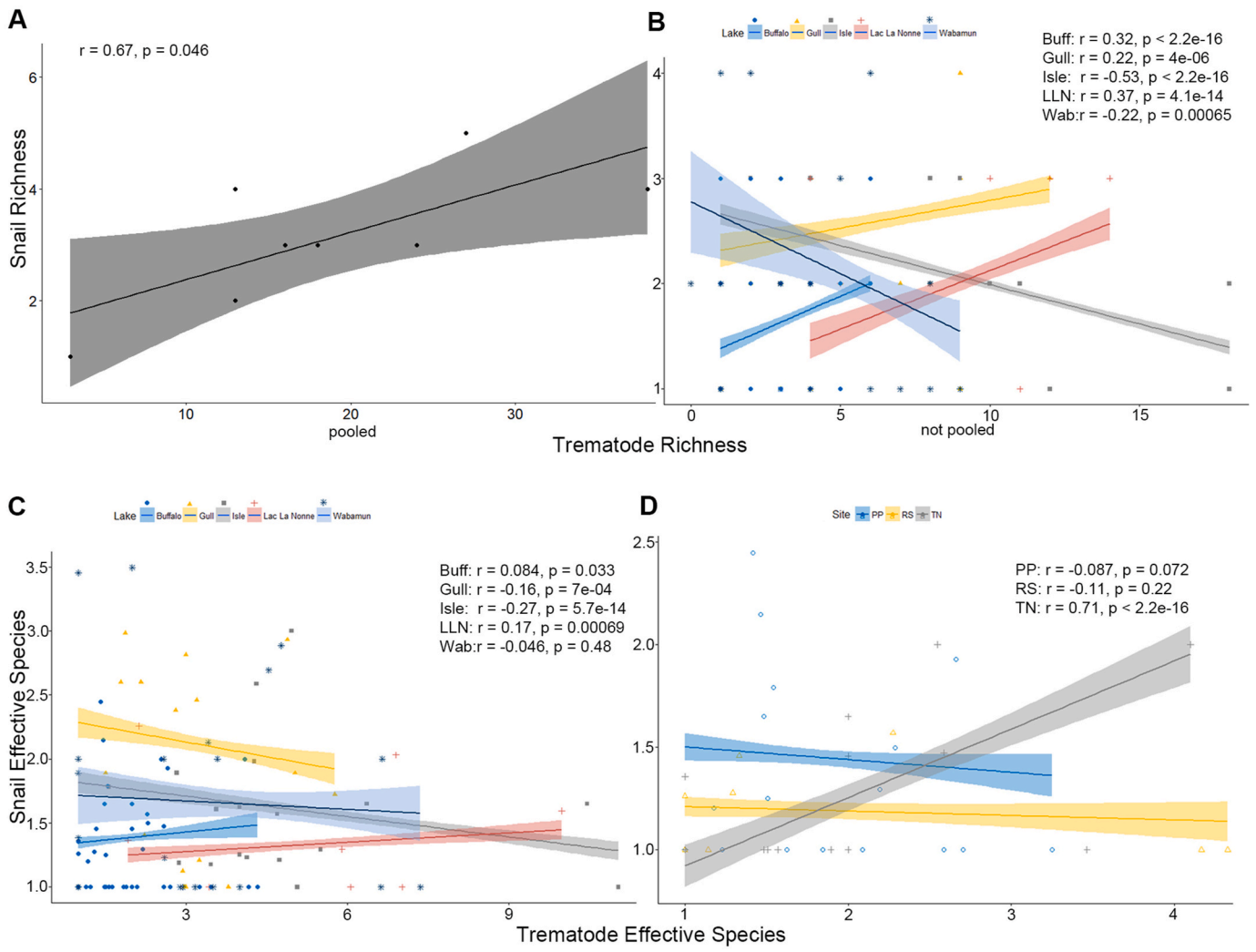

Fig. 1. Host-Parasite diversity correlations. Spearman rank correlations of A) snail and trematode richness, pooled by site, B) non-pooled, sample-based, snail and trematode richness, C) snail and trematode effective species based on Shannon index (exp(*H*)) for all lakes, and D) effective species by each site at Buffalo Lake. PP = Pelican Point, RS = Rochon Sands, TN = The Narrows.

3.2. Community comparisons and species dominance

Community evenness (a measure of homogeneity in species abundance) was highest at Lac La Nonne Site #2 (e = 0.402), followed by Wabamun Lake (e = 0.355), Buffalo Lake – The Narrows (e = 0.323), Lac La Nonne Site #1 (e = 0.306), Isle Lake (e = 0.286), Buffalo Lake – Rochon Sands (e = 0.263), Gull Lake (e = 0.208), and finally Buffalo Lake – Pelican Point (e = 0.139). However, all of the communities generally exhibited low evenness. This is likely due to high quantity of rare species found in all communities (although more so in the southern lakes, Gull and Buffalo), and the presence of a few highly dominant species that stayed in the community for long periods of time (Supplementary Figures 1–7 Panels B–C & Supplementary Figure 8).

Trematode diversity fluctuated over time such that there was no strong or consistent pattern from year to year. However, there were peaks in trematode richness (α diversity) in each community, primarily in July and August. Snail richness fluctuated seasonally but was less extreme in comparison to trematode richness. Trematode community evenness, or lack thereof, therefore fluctuated with changes in α diversity, as expected given the formula used for calculations, but otherwise showed little fluctuation overall (Supplemental Figures 1–7 Panel A).

Analyses of homogeneity of variance using Bray-Curtis dissimilarities showed that the effect of site on β diversity was significant ($F_{5,93} = 5.631$, $P < 0.001$). This was specifically driven by the difference between the Pelican Point at Buffalo Lake site with all others (Tukey HSD: PP-Gull: $P = 0.050$, PP-Isle: $P = 0.0008$, PP-Wab: $P = 0.0002$, PP-RS: $P = 0.0036$, PP-TN: $P = 0.015$) (Supplementary Figure 1B). Grouped by our geographic and anthropogenic activity categories for sites, there was a significant effect of ecoregion on β diversity ($F_{2,96} = 4.589$, $P = 0.0124$). Specifically, there was a difference between Aspen Parkland and Boreal Forest (effect size = 0.165, $P = 0.011$, CI: [0.0316, 0.298]), the river basin from which the lakes were a part, either the North Saskatchewan River or the Red Deer River basin ($F_{1,97} = 8.3205$, $P = 0.0048$; effect size = −0.15, CI: [-0.2533, −0.0468]), but no significant effect of site type (beach or boat launch) on β diversity ($F_{1,97} = 2.9557$, $P = 0.0887$) (Table 1 & Fig. 2).

The analysis of similarity by comparing within and between group dissimilarity ranks also confirmed that trematode species composition was significantly dissimilar among sites (R = 0.2581, $P = 0.001$). Species overlap among sites was 52.7% (in terms of Shannon diversity), and 54.7% by ecoregion. By river basin, the overlap was 72.2%, and the highest overlap was found among site types (beach or boat launch) at 84.8%.

Table 1
Location-based trematode diversity summary.

Lake	Site	Richness	α (mean ES)	γ (pooled ES)	β (gamma/ alpha)
	Pelican Point (PP)	16	1.77	2.43	1.37
Buffalo	Rochon Sands (RS)	13	1.49	4.49	3.01
	The Narrows (TN)	13	1.93	5.80	3.00
Gull	Gull	24	2.75	6.56	2.38
Isle	Isle	38	3.82	10.20	2.67
Wabamun	Wabamun (Wab)	27	3.02	10.45	3.46
		River basin			
	PP	Red Deer	1.97	6.42	3.25
	RS	Red Deer	1.97	6.42	3.25
	TN	Red Deer	1.97	6.42	3.25
	Gull	Red Deer	1.97	6.42	3.25
	Isle	North Saskatchewan	3.41	12.67	3.71
	Wab	North Saskatchewan	3.41	12.67	3.71
		Ecoregion			
	PP	Aspen Parkland	1.74	5.41	3.11
	RS	Aspen Parkland	1.74	5.41	3.11
	TN	Aspen Parkland	1.74	5.41	3.11
	Gull	Mixed	2.75	6.56	2.38
	Isle	Boreal Forest	3.42	12.67	3.71
	Wab	Boreal Forest	3.42	12.67	3.71
		Site Type			
	PP	Beach	2.24	7.76	3.47
	RS	Beach	2.24	7.76	3.47
	TN	Boat Launch	2.88	10.90	3.78
	Gull	Beach	2.24	7.76	3.47
	Isle	Boat Launch	2.88	10.90	3.78
	Wab	Beach	2.24	7.76	3.47

3.3. Environmental influences

Of all the environmental variables tested, only the trophic status of the lake, and the landscape variables of ecoregion and latitude, were significant within the CCA model. Together, these explained 22.9% of the variance for the sample-based trematode community composition (Overall: $F(4) = 7.139$, $P = 0.001$; trophic: $F(1) = 13.209$, $P = 0.001$; ecoregion: $F(2) = 6.299$, $P = 0.001$; latitude: $F(1) = 2.748$, $P = 0.007$, permutation test of 999 permutations) (Fig. 3).

However, when examining environmental factors as predictors for the occurrence of the five most abundant trematode species, as opposed to overall community composition, we found that lake trophic status was no longer significant (GLMM: $F_{4,969} = 1.742$, $P = 0.138$). In the final significant model (Trem_species ~ Month + Ecoregion + Latitude + Dissolved Oxygen + (1|Year) + (1|Site)), dissolved oxygen and month were additional significant predictors to those also found in the CCA (GLMM (full model): $F_{28,\ 945} = 8.006$, $P < 0.001$).

Ecoregion was significantly associated with each of the most abundant species (*A. burti* LIN1: Boreal Forest, *coeff.* = −48.264, $P < 0.001$; *Diplostomum* sp. 4: Boreal Forest, *coeff.* = 13.720, $P = 0.005$; *Echinoparyphium* sp. LIN2: Aspen Parkland, *coeff.* = −2.646, $P < 0.001$; *Notocotylus* sp. A: Aspen Parkland, *coeff.* = −1.773, $P = 0.020$; *Plagiorchis* sp.: Boreal Forest, *coeff.* = 48.121, $P < 0.001$). For seasonality, the month of August was a positive predictor for the presence of *A. burti* LIN1 (*coeff.* = 2.800, $P = 0.009$), but negative for that of *Plagiorchis* sp. (*coeff.* = −2.895, $P = 0.007$) and *Diplostomum* sp. 4 (*coeff.* = −2.767, $P = 0.030$). Month had no significant association with the other species. Lastly, *A. burti* LIN1 was found at lower dissolved oxygen environments (*coeff.* = −0.149, $P < 0.001$), in comparison to the positive association seen for *Plagiorchis* sp. (*coeff.* = 0.152, $P < 0.001$), *Notocotylus* sp. A (*coeff.* = 0.129, $P = 0.003$), *Echinoparyphium* sp. LIN2 (*coeff.* = 0.198, $P = 0.010$), and *Diplostomum* sp. 4 (*coeff.* = 0.164, $P < 0.001$).

4. Discussion

We found trematode communities within Alberta lake ecosystems to be highly dynamic in space and time. While there were many occurrences of rare trematode species among the snail samples, making it difficult to assess how host- and environment-related factors affected their presence/absence, we gained insight into variables that affected trematode species which were highly abundant and consistently present within these lakes. Notably, a significant positive relationship between trematode species richness and snail host species richness was found at all sites. This being said, trematode diversity showed variation from year to year, with peaks in species richness observed in the late summer months. Environmental factors found to be influential on trematode and snail communities in these Alberta lakes included the trophic status of the lake, month of sample collection, dissolved oxygen concentrations, and landscape variables such as ecoregion and latitude.

When pooling the species richness data for all sites, we saw a positive relationship between host and parasite richness, although the strength of the relationship was weaker than that reported in the first study of these lakes (Gordy et al., 2016). This is likely due to the increasing number of trematode species found in subsequent years, and with greater taxonomic resolution, while the number of snail species remained the same. However, when considering each site separately, some continued to exhibit a positive (although weaker) correlation for snail and trematode richness, while other sites showed a negative correlation. When considering diversity by accounting for effective species rather than just species richness, host and parasite diversity were still significantly associated, although the correlations became weaker for most sites relative to those for richness. Our overall findings are thus in line with various studies that have reported a positive relationship between host and parasite diversity irrespective of spatial scale (e.g., Thieltges et al., 2011; Kamiya et al., 2014; Wood and Johnson, 2016), supporting that this holds even when finely delineating among related parasites (79 trematode species here). Other measures of diversity (i.e. beyond species richness) used to test this relationship for trematodes and their hosts also came to similar conclusions, including that for β diversity, which describes changes in species turnover between single sites (α) and all sites (γ) (Johnson et al., 2016), as well as the fundamental biodiversity number – a constant central to the neutral theory of biodiversity (Hechinger and Lafferty, 2005).

Our findings suggest that the presence of more snail host species can create greater colonization opportunities for different parasites (demonstrated by the overall strong positive relationship between snail and trematode richness), but the domination of a few trematode species also indicates that parasite diversity is strongly driven by the presence of certain susceptible host snail species. This would then explain the relatively weaker relationship of trematode diversity with snail richness after accounting for trematode community evenness. In line with the host encounter and susceptibility "filters" suggested by Combes (2001) to explain parasite absence or presence, snails in these Alberta lakes seem to be acting as biotic filters for regional trematode diversity by virtue of their compatibility (i.e. susceptibility to successful infection). For parasites such as trematodes, with complex, multi-host life cycles, their relatively motile definitive hosts thereby act as the first biotic filter (encounter) by bringing infectious propagules from the regional species pool to each site, followed by a second snail-mediated susceptibility filter.

Although we do not currently have definitive host diversity information for these lakes, even sites with a rich array of definitive hosts introducing the eggs of many trematode species may see limited colonization by these trematodes based on snail diversity considering the

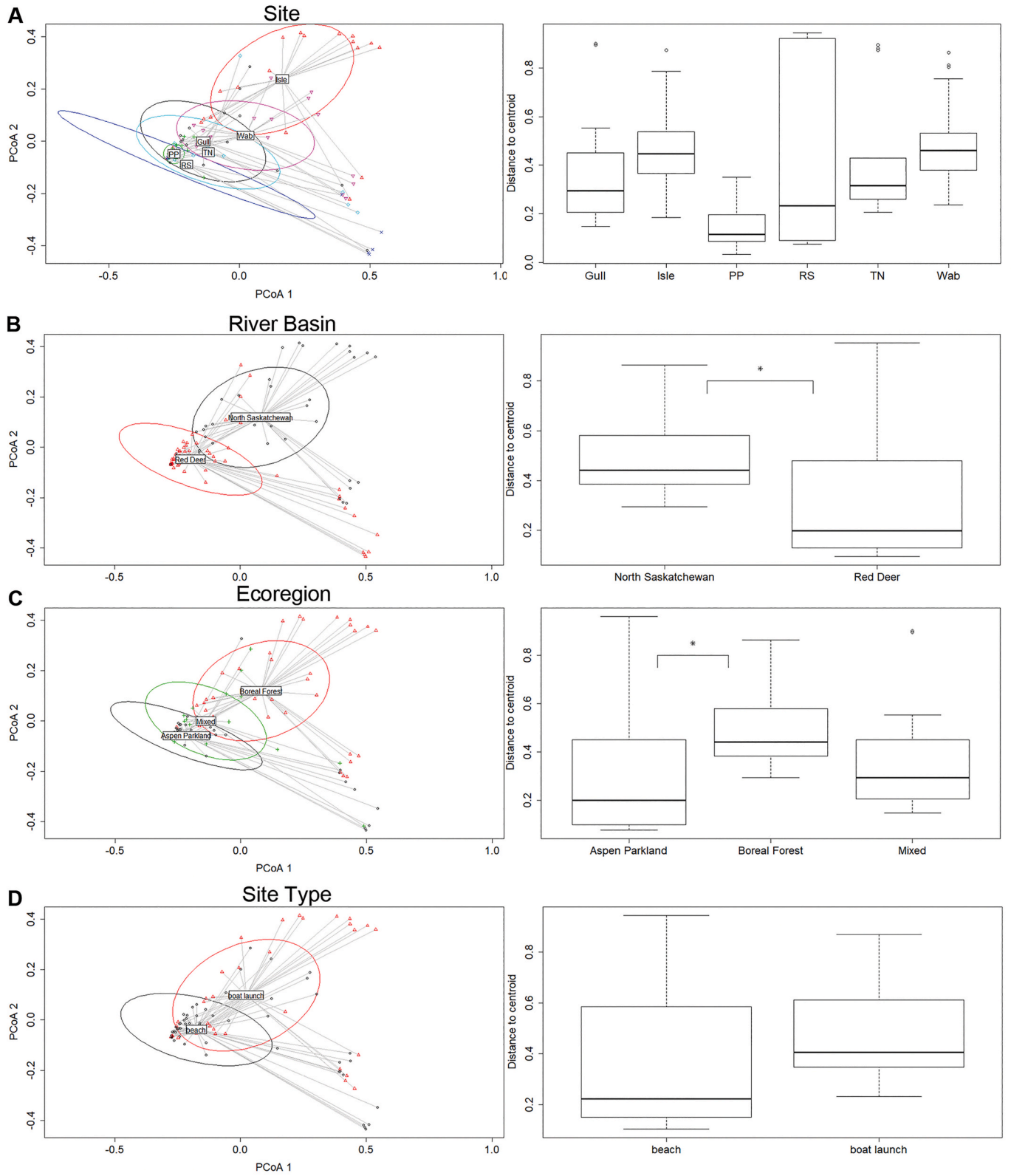

Fig. 2. Multivariate Homogeneity of Group Dispersion for Trematode Communities. Bray-Curtis dissimilarities were used to examine the homogeneity of variance among samples (trematode species counts) when grouped by different geographical or anthropogenic-use distinctions. The left panels show the two-dimensional visualizations of the data by Principal Coordinate Analysis (PCA) plots. Each grouping is labeled in the center, and ellipses represent 95% confidence intervals. The right panels provide a boxplot of the distance to centroid for each group in the multivariate analysis. A) samples grouped by site, B) grouped by river basin, C) grouped by ecoregion, D) group by site-type or anthropogenic use (beach or boat launch). Statistical significance for differences between groups is indicated by an asterisk.

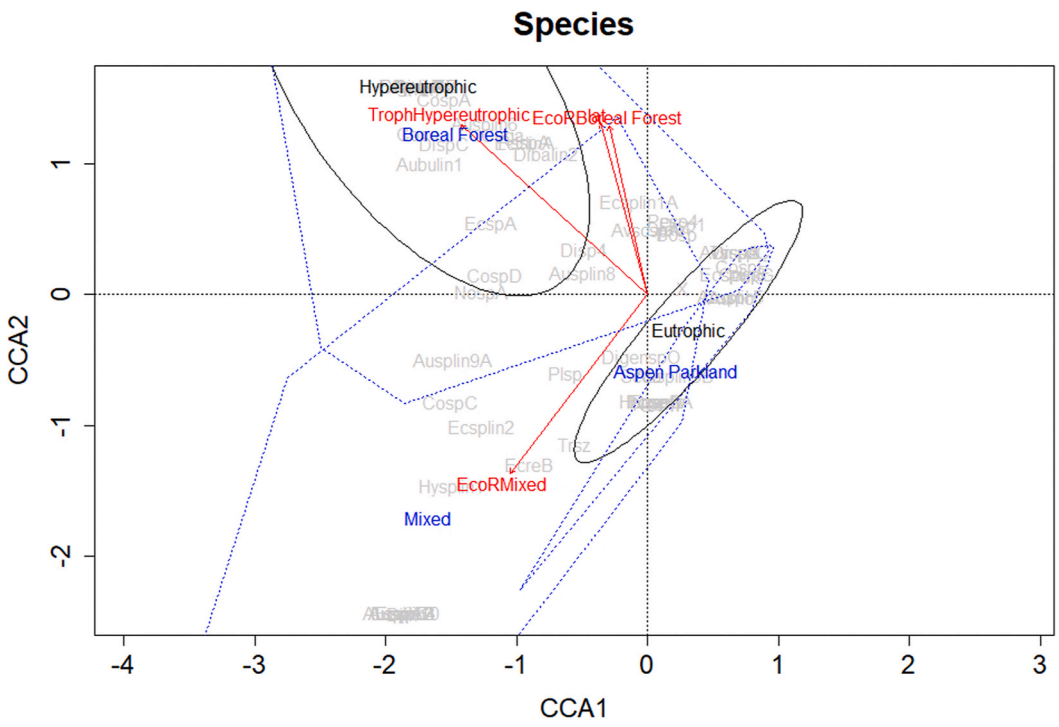

Fig. 3. Canonical correspondence analysis (CCA) of trematode component communities. Relative abundances of trematode species by sample are constrained by environmental variables from the best-fit model (community \lake trophic status \+ ecoregion \+ latitude). Trematode species abbreviations are shown in grey. CCA results are in red as eigenvectors. Ecoregions are identified with a blue dotted line. The trophic status of each lake is identified with an ellipse.

obligatory role that snails have in furthering the trematode life cycle. Snails and trematodes have been associated for 200 million years (Blair et al., 2001). Because of the close association, trematodes can exhibit relatively high specificity for their first intermediate hosts, typically only infecting snails that belong to the same family or genus (Ewers, 1964; Adema and Loker, 1997; Lockyer et al., 2004). Correspondingly, if the predominant snail species, acting as susceptibility filters at any given site, have narrow "membrane pores" (i.e. susceptible to infection by few trematode species), there should be less trematode diversity than if their "pores" are wide (i.e. permissive hosts susceptible to many trematodes). We found that two of our collected snail species (an unidentified planorbid and *Valvata tricarinata*) never had patent (active) trematode infections, suggesting they may be strong filters for trematode establishment.

However, because it is not clear if diverse snail communities harbor relatively greater or fewer permissive host species, this warrants investigation in future studies given its potential importance. Notably, host communities often assemble or disassemble in non-random ways, with implications for the relationship between host richness and susceptibility to parasitism, as demonstrated for trematodes and second intermediate hosts (Johnson et al., 2019). For the present study, if there is a positive relationship between snail host diversity and susceptibility to infection, then this should result in higher trematode diversity at sites with diverse snail assemblages. In contrast, trematode community composition may be minimally affected by any reductions in snail host species richness if the hosts that remain are relatively permissive. It is therefore vital to determine if diverse freshwater snail communities consist of many species that are relatively susceptible to infection, or composed of a combination of host species that have either wide or narrow "membrane pores" as filters.

Understanding the extent to which diverse snail communities contain susceptible host species, and what may drive this, is important given our finding that each trematode cumulative component community was typically composed of a few highly abundant species that were also common and consistent among sites. While trematode community evenness fluctuated very little over time, the observed richness of both trematode larvae and snails showed significant temporal variation, although less so for the latter. Across all sites, peaks in trematode diversity were typically observed in July and August. Although not specifically tested here, these peaks are likely driven by intermediate host population dynamics (birth and growth rate, as well as timing of infection assuming an age-based immunity profile), as well as the length of time for trematode development within the snail host, all of which may be influenced by environmental variables.

The most common and abundant trematode species in all the sites sampled was *Plagiorchis* sp. Four other trematode species were also highly abundant: *Australapatemon burti* LIN1, *Diplostomum* sp. 4, *Echinoparyphium* sp. LIN2, and *Notocotylus* sp. A. For these five species, we found that ecoregion, month, and dissolved oxygen level were strong overall predictors for their presence, but not necessarily in the same manner. For instance, we found that *A. burti* LIN1 was more common at lower dissolved oxygen levels compared to the other highly abundant trematode species which showed a positive association. In addition, *A. burti* LIN1 was more commonly found in August, whereas *Plagiorchis* sp. and *Notocotylus* sp. A were less frequent during that month. Considering that *A. burti* LIN1 was mostly found in nutrient-rich, hypereutrophic lakes in the Boreal Forest ecoregion of Alberta, there could be an interaction occurring between high temperature (likely in August) and algal communities that potentially impacts dissolved oxygen (DO) levels. Increased nutrient levels in lakes can increase algal biomass, which can initially increase both food and DO levels for aquatic invertebrates like snails, but this is then followed by a dramatic decrease in DO as bacterial decomposition spikes (Hem, 1985). Depending on the tolerance of a particular organism to DO availability, this could have significant effects on mortality rates (Misra and Chaturvedi, 2016).

Anoxic conditions have been reported as having differential effects on snail mortality in infected snails in salt marsh ecosystems (Sousa and Gleason, 1989). Related to this, a given snail host species can react very differently to stress depending on the trematode that it is infected with (Bates et al., 2011; Koprivnikar and Walker, 2011). In freshwater

ecosystems, it has been noted that most gastropods are unable to withstand anaerobic conditions for more than 48 h (Pennak, 1978). However, pulmonate gastropods will surface for air, perhaps eliminating the negative effects of low dissolved oxygen. From the perspective of trematodes, it appears as if there is a correlation between the presence of *A. burti* LIN1 and its snail hosts at sites with low dissolved oxygen. We do not see this association with the other four abundant trematode species. This could be because their second intermediate hosts or definitive hosts cannot tolerate these conditions, or because either the eggs, miracidia, or both stages of *A. burti* LIN1 are more tolerant to lower dissolved oxygen conditions. As such, dissolved oxygen concentrations are an interesting environmental variable that should be considered in future studies.

When considering how spatiotemporal heterogeneity in environmental factors may affect trematode cumulative component community composition, contrary to our hypothesis, we found that the environmental variables which fluctuate the most across seasons and sites had no significant impact on trematode species relative abundances at local scales. Rather, the relatively static landscape divisions by ecoregion and lake trophic status (e.g., hypertrophic) were the strongest predictors of trematode species presence. On the other hand, significant spatiotemporal differences in trematode β diversity were found based on divisions by site, river basin, and ecoregion, but there was no significant effect of anthropogenic activity (beach or boat launch). As an example, the Pelican Point site at Buffalo Lake experienced the lowest rate of trematode species turnover compared to all the other sites. One possible explanation for this difference could be that snails from at Pelican Point were almost always found in small, dug-out pools that had collected on the shore of the lake where contact with the primary lake water was infrequent, except during storms or disturbances of the lake shore. This reduced exposure to the main lake could have multiple effects on the overall community assembly process, such as improved trematode success in infecting snail hosts because of the limited space. However, it could have also limited the infection success of certain trematode species by reducing exposure to the necessary snail host species. In addition, if the life cycles of particular trematode species are dependent on hosts such as fish, motile infectious stages (cercariae) may not reach them because of the physical barrier to the main lake. All of these factors could work to reduce the turnover of trematode species in the community if some are simply not able to establish under the constraints posed by certain site conditions.

Taken together, we found considerable variation within and among trematode component communities in these Alberta lakes, whether examining the presence of individual species, or their community composition through space and time, as well as the variation in the influence of environmental factors. When considering 79 different trematode species at a regional scale, our results support those of previous studies with fewer species at similar spatial scales, or many species at larger scales, by demonstrating a strong overall positive relationship between snail and trematode species richness. We also found a weaker but still significant association between host richness and trematode community composition when accounting for evenness given the dominance of five trematode species at many sites. Considering the relatively high degree of host specificity in this system, this suggests that the presence of a few key host species is central to understanding trematode community assembly in snails. As such, reductions in snail species richness may cause reductions in trematode species richness, but may not necessarily alter trematode community dynamics (i.e. dominance by particular species). Identifying the forces structuring trematode communities in snails is important for many reasons, including understanding the risk of infection to hosts "downstream" in the life cycle, as well as public health impacts, such as the possibility of contracting cercarial dermatitis, a. k.a. "swimmer's itch" from avian schistosome trematodes (Cort, 1928a, 1928b; Kolářová et al., 1999; Verbrugge et al., 2004; Brant and Loker, 2009; Gordy et al., 2018). Future studies to elucidate host-parasite associations and parasite life cycles will be needed to incorporate more host and parasite species when examining the associations among these two communities in order to predict the possible repercussions of biodiversity changes in this context, as well as to explore the potential use of parasites as bioindicators.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijppaw.2020.11.008.

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