

Variation in parasite communities and health indices of juvenile *Lepomis gibbosus* across a gradient of watershed land-use and habitat quality

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ABSTRACT

Parasites of fishes vary in community structure and species abundance in response to environmental conditions and pollutants. As a result, the use of parasites as bioindicators of habitat degradation has been proposed and successfully applied in recent years. Here, helminth parasites of juvenile pumpkinseed *Lepomis gibbosus* from three streams representing a gradient of watershed development and habitat quality were examined to assess variation in parasite communities. Health assessment indices were also generated for each host to quantify the influence of habitat on the observed health of individuals. A total of 22 parasite taxa were recovered from examined fish, comprising 11 digeneans, 3 cestodes, 2 acanthocephalans, 5 monogeneans and 1 crustacean. In the most disturbed stream, parasite species richness was lowest and total abundance was highest, while parasite abundance was lowest and diversity highest in the least disturbed stream. There was no significant difference in health indices among streams. Analysis of similarities (ANOSIM) and Bray–Curtis dissimilarity in species composition (SIMPER) identified *Posthodiplostomum* spp. and *Actinocleidus* sp. as the species driving parasite community dissimilarity. These taxa are relatively easy to identify to genus level and thus could be appropriate for use as indicators of environmental health, where increased abundance would suggest negative changes in habitat quality. However, larger scale study including more streams would be necessary to establish baseline community data before such implementation would be feasible.

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1. Introduction

The impact of anthropogenic development on stream ecosystems is of great concern for environmental managers. These systems are currently among the most threatened environments in the world (Richter et al., 1997; Malmqvist and Rundle, 2002), and recent analyses have shown that freshwater ecosystems are degrading more rapidly than terrestrial ecosystems (Ricciardi and Rasmussen, 1999). Unfortunately, restoration efforts are often limited or ineffective (Palmer et al., 2010). Consequently, the importance of management to monitor and mitigate degradation is increasingly evident as intensification of agriculture and urban

development associated with increased crop production and population growth continues (Lambin and Meyfroidt, 2011).

To monitor the effects of development, environmental managers use a variety of aquatic sampling techniques to quantify the health of aquatic ecosystems. For example, aquatic organism community surveys are often used as a surrogate for watercourse health. In particular, numerous indices have been created to quantify fish and invertebrate communities, including the fish index of biologic integrity (IBI; Karr, 1981) and Hilsenhoff index for invertebrates (Hilsenhoff, 1988). While these indices are effective for comparative purposes, they are only able to identify changes to populations after they have occurred, and are consequently slow to indicate environmental change (Adams et al., 1993). Moreover, the level of sampling required to document changes in community structure can be extensive, and IBIs may need to be locally calibrated (Frausch et al., 1984). As a result, there has been interest

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in identifying other potential endpoints beyond fish and invertebrate community structure that have the potential to be indicative of aquatic ecosystem health.

The interest in using parasites as bioindicators of aquatic ecosystem status in the face of anthropogenic degradation has grown over recent years (see Lafferty, 1997; Marcogliese, 2005; Blana et al., 2009; Marcogliese and Pietrock, 2011 for extensive reviews); certain parasite taxa are more sensitive to environmental perturbation than their host species, and are thus a more sensitive indicator of various contaminants and trophic changes (Marcogliese, 2005). Two groups identified as potential bioindicators are monogeneans, monoxenous gill infesting ectoparasites, and digeneans, heteroxenous trematodes that typically include at least one invertebrate in their life cycle and various free-living stages during their transmission between hosts. Monogeneans are considered to be good indicators of water quality because they typically infest gill tissue and are exposed to the environment throughout their life cycle; for example, monogenean species sensitive to water quality may decrease in abundance (Bagge and Valtonen, 1996; Zargar et al., 2012). Because digeneans have direct and indirect transmission pathways, digenean species recovered in a host population may indicate multiple ecosystem and environmental characteristics, such as changes in food-web dynamics, invertebrate populations and densities, and water quality (Marcogliese and Cone, 1997; Soucek and Noblet, 1998; Marcogliese, 2005). In addition to the complex relationships that may occur as a result of parasite interactions with environmental and intermediate host dynamics, fish themselves that experience environmental stress can experience immunosuppression, increasing their susceptibility to parasite infection and disease (Lafferty and Kuris, 1999; Marcogliese, 2004).

Assessing changes in parasite species community in resident fish hosts can thus indicate potential changes in aquatic ecosystems on a variety of levels. For example, eutrophication has been identified as a factor that influences overall parasite species composition in fish residing in affected waters (Valtonen et al., 1997; Zagar et al., 2012), and can either increase or decrease infection properties depending on the parasite taxon's life history traits, i.e. the presence, absence and densities of intermediate or definitive hosts, and sensitivity of hosts and parasites themselves to environmental change (Sures, 2004). Overall parasite taxa diversity has also been used to measure restoration success. For example, Huspeni and Lafferty (2004) monitored increasing trematode diversity over six years of post-restoration in a coastal salt marsh, where the increase in snail-borne trematodes indicated the return of various bird species to the area. Further, Marcogliese and Cone (1997) demonstrated low pH affected trematode diversity in North American eels (*Anguilla rostrata*), a pattern that was also reflected in free-living diversity, by influencing the abundance and distribution of intermediate hosts. This suggests parasite diversity could be used to estimate the ecological impact of acid rain.

As freshwater fishes are hosts to a taxonomically diverse suite of parasites, with estimates running upwards of 30,000 parasitic helminth species alone (Williams and Jones, 1994), the importance of parasites in aquatic ecosystems is under-appreciated where monitoring is concerned (Marcogliese and Cone, 1997). As a result of the high parasite diversity of intermediate trophic fishes – i.e., those that consume invertebrates and are prey for both predatory fishes and piscivorous birds – and the ability to estimate age and thus parasite recruitment time, juvenile fish are excellent models to investigate the potential efficiency of parasites as bioindicators for use in environmental monitoring; however, due to the time and knowledge required to sample and identify all parasites present within a host species, previous authors advocate monitoring only individual parasite taxa using prior knowledge of their biology and distribution within a system (Blana et al., 2011). Additionally, selection of model species for habitat specific data requires that

Table 1

Creek watershed land-use, water quality and benthic scores for three creeks in eastern Ontario, Canada. Watershed data was determined using Quantum GIS and land use data from the Southern Ontario Land Resource Information System, total phosphorous levels and ranges were extracted from a Ontario the Environment Report (AECOM, 2009), and benthic scores from the Raisin Region Conservation Authority Watershed Report Cards (2006). Benthic scores are presented as a Hilsenhoff index, where lower values indicate greater benthic diversity.

	Gunn Creek	Gray's Creek	Hoople Creek
Watershed area (ha)	1037.5	4450.8	9534.8
% Forest	5	8.7	13.9
% Water & Wetland	2.9	16.9	26.7
% Disturbed	91.8	68.3	58.5
Mean annual TP ($\mu\text{g/L}$)	82	66	54
Annual TP range ($\mu\text{g/L}$)	140	80	30
Benthic Score	7.08	6.73	5.4
Bacteria Score	83	56	39

the species have high site fidelity and be common across habitats in question, requiring preliminary survey and species biology.

The aim of the present study is to define relationships between habitat quality (approximated using proportional watershed land-use, phosphorous levels and benthic and bacteria scores) and parasite community structure, and to determine whether helminth parasites of juvenile sunfish, pumpkinseed *Lepomis gibbosus*, a common species across eastern North America, could be used as a tool for environmental monitoring in stream ecosystems. In addition, we sought to describe the relationship between parasite species richness, parasite abundance, and fish health.

2. Methods

2.1. Watershed summarization and fish collection

This study took place in a portion of the St. Lawrence River near Cornwall, ON (45°01'17.5" N, 74°43'50.42" W). Using previous reports of water quality, benthic invertebrate scores, and habitat variables from the Raisin Region Conservation Authority, three streams that represented a range in habitat quality were selected to test whether or not changes in habitat quality were reflected in helminth community (Table 1). Land use data were obtained from the Southern Ontario Land Resource Information System (SOLRIS) and were combined with a GIS layer defining watershed boundaries developed by the local Raisin Region Conservation Authority using Quantum GIS Lisboa (1.8.0). Watershed land cover variables are presented as proportions of the total watershed size, in hectares (ha). Disturbed land was calculated by combining the proportions of urban, agricultural and recreational lands (Table 1).

Total phosphorous levels and ranges were extracted from the Ontario Ministry of the Environment Report (AECOM, 2009), and benthic and bacteria scores from the Raisin Region Conservation Authority Watershed Report Cards (available at www.rrca.on.ca). These values combined indicate the level of organic nutrient loading experienced by each stream. Annual total and range in phosphorous indicate the severity and fluctuation of nutrient loading from fertilizers and runoff, and bacteria score monitor the *Escherichia coli* entering the streams from fecal contamination from farmland and septic fields. Benthic invertebrate scores assign a weighting to each taxon depending on its tolerance to organic pollution. Scores higher than 5.00 are above local provincial guidelines (AECOM, 2009). Because no major changes to land cover or habitat quality occurred in sampled streams between the collection of phosphorous and benthic data and the commencement of this study (RRCA, personal communication), these data are used to represent current watershed characteristics.

To ensure fish were representative of stream conditions, fish were sampled from areas approximately 1.5 km upstream of the

confluence with Lake St. Francis, in the first Wadeable portions of the streams. Fish were captured between June 4th and 13th, 2012, using a beach seine (22.6 m × 1.15 m; 5 mm mesh) and all *L. gibbosus* at each site were measured to construct length frequency histograms to determine the size class of age 0+ y fish, which were recruitment from the previous summer (i.e. young-of-the-year; YOY). Fish collected were from the smallest age class and thus assumed to have had ~1 year for parasite recruitment (Busacker et al., 1990). Because of the intimate relationship between watershed landscape characteristics and a stream's biotic and abiotic characteristics (Hynes, 1975; Jeffries et al., 2015), and the high site fidelity of juvenile *L. gibbosus* (McCairns and Fox, 2004), individuals captured were likely exposed to water quality conditions reflective of watershed characteristics throughout their entire lives.

2.2. Heath assessment and parasitological examination

Fish were collected and immediately euthanized by the dropwise addition of clove oil and 10% EtOH, put in individual bags and placed on ice for transport back to the lab. Within 2 h of euthanasia, dissection based health assessment indices (HAI) were conducted (as per Adams et al., 1993, with modification as described below) to quantify the general health status of individual fish resident in each stream, and the influence that overall parasite infection may have on this measurement. After external examination for abnormalities of the fins, eyes, scales, and gills, a single incision was made along the ventral portion of the fish to assess the color, size, and condition of major organs (alimentary tract, heart, liver, and spleen). Scores for each organ category ranged from 0 to 30 and were combined to produce a single HAI value for each fish. An HAI score of zero indicates a fish that did not have any appreciable abnormalities, whereas a high score reflects an individual perceived to be unhealthy. Throughout this process care was taken not to disturb tissues to avoid damaging parasite fauna within the host. After the HAI was completed, fish were placed back in their original bag and stored at -20 °C until examined.

Prior to dissection fish were weighed to the nearest 0.01 g and fork length was recorded. Fish and bag contents were first rinsed and checked for external parasites using a dissecting microscope following routine parasitological techniques. Parasitological examination was conducted in accordance with Environment Canada's Freshwater Monitoring Protocols (Marcogliese, 1998). All organs and tissues were examined for parasites, which were excysted if necessary, enumerated, and fixed in 70% ethanol for later staining and identification when species were unknown. Cysts were either opened manually using fine forceps or insect pins, or chemically dissolved in a dilute hypochlorite solution. This is a useful technique that has previously been used for excysting multi-layered gelatinous cysts of digeneans such as *Apatemon* spp. Nematodes were also excysted and removed though not identified for this analysis, as nematodes have not demonstrated strong patterns of response to eutrophication (Blonar et al., 2009). Additionally, larval nematodes recovered in this study require expertise in identification beyond what we consider appropriate for potential bioindicators. Parasites were identified using the keys in Gibson (1996), Beverly-Burton (1984) and Hoffman (1999).

2.3. Calculations and statistical analysis

Quantitative descriptions of parasite infection properties used throughout are as in Bush et al. (1997) and defined as follows: prevalence is the proportion of examined hosts infected with a given species, mean intensity is the mean number of individuals of a given species found per infected host, and mean abundance is the mean number of individual parasites per host including all hosts examined. Also included in this analysis are descriptors of

parasite community, including component community richness – the assemblage of parasites found in the entire sample of hosts examined – and infracommunity – the assemblage of parasites found within an individual host. Differences in fish weight, length, total abundance of all parasites, and HAI values among sites were tested using one-way Analysis of Variance (ANOVA) and post hoc Tukey HSD. Simple linear regression was used to test the relationship between total parasite abundance and HAI score. In typical parasite communities, many individual hosts in a population have few parasites and few hosts have many, resulting in non-normal distribution. Kruskal–Wallis non-parametric analysis of variance was thus used to test differences in mean abundance of parasite species among streams. Assumptions of normality and equal variance were tested using Shapiro–Wilk and Levene's test, respectively. Above analyses were conducted in JMP® V 9.0.1.

Non-parametric species accumulation curves for sample-based data were constructed using EstimateS V 9.1.0 and were extrapolated by 25 samples to estimate required host sample size to account for all species (Colwell et al., 2012). As we were interested in identifying potential bioindicators, examinations of hosts concluded when species accumulation curves plateaued, or increased at a gradient such that multiple hosts would need to be examined to add a single species to the model.

To investigate parasite component communities of each stream, several methods were used to compare species composition. Firstly, Shannon–Wiener diversity index (H') was calculated for each stream to quantify species diversity and evenness (Shannon and Weaver, 1949). To further quantify observed differences, non-parametric statistics based on the Bray–Curtis dissimilarity index were used following procedures outlined in Oksanen et al. (2013). This analytical technique accounts for both abundance and species identity in a single measurement of dissimilarity. An analysis of similarities (ANOSIM) on Bray–Curtis dissimilarity matrices was conducted to test if there was indeed a significant difference in parasite species composition among streams. To quantify the contribution of each parasite species to the observed dissimilarity, Bray–Curtis dissimilarity in species composition (SIMPER) was calculated with paired-comparisons between streams. This method indicates the parasite species that drive the overall dissimilarity between paired streams. Finally, a 2D non-metric multidimensional scaling ordination (NMDS) with 95% confidence limits around the weighted averages for each contributing site was created to visually interpret community dissimilarity. All analyses were conducted in R for Statistical Computing (V 2.14.1; R Development Core Team 2013) using the “vegan package” (Oksanen et al., 2013).

3. Results

Land cover within the sampled watersheds followed several patterns associated with habitat quality (Table 3). The proportion of forest and undisturbed lands was highest in Hoople Creek and lowest in Gunn Creek, and total phosphorous (TP), annual TP range, benthic and bacteria scores increased with increasing proportion of disturbed watershed. The proportion of agriculture and urban land use did not follow similar patterns, as Gray's Creek was found to have the lowest proportion of agriculture and the highest proportion of urban land use. These combined however created an intermediate level of disturbance consistent with water quality and benthic score data. Considering watershed land cover and habitat variables, Hoople Creek is the least degraded stream, Gunn Creek the most degraded stream, and Gray's Creek is intermediate between the two.

Forty-nine fish (total length range of 42–56 mm) were examined to evaluate parasite community structure. Both mean total

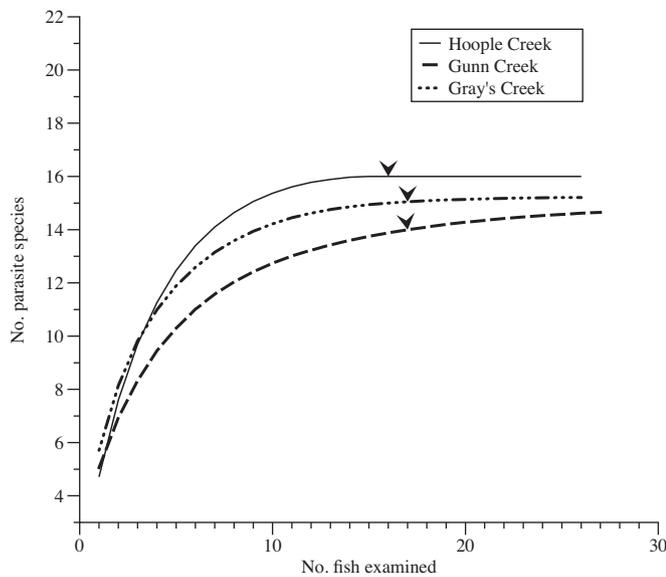


Fig. 1. Species accumulation curves with extrapolation calculated for the parasite fauna of overwinter juvenile pumpkinseed *Lepomis gibbosus* collected from three streams in Eastern Ontario. Arrows indicate the number of hosts examined from each stream.

length (one way ANOVA, $F_{(2,46)} = 1.58$, $P = 0.22$) and weight (one way ANOVA, $F_{(2,46)} = 1.47$, $P = 0.24$) of sampled *L. gibbosus* did not differ significantly among streams. Total parasite abundance per fish was significantly different among streams (one way ANOVA, $F_{(2,46)} = 12.75$, $P \leq 0.0001$), while mean number of taxa per fish was not (one way ANOVA, $F_{(2,46)} = 1.14$, $P = 0.33$) (Table 3). Post hoc tests revealed total parasite abundance in Hoople Creek (mean = 14.69) to be significantly different from Gunn (mean = 37.06) and Gray's Creek (mean = 28.63) (Fig. 1). HAI scores decreased with increasing habitat quality (Table 3), though differences among streams were not statistically different (one way ANOVA, $F_{(2,46)} = 2.405$, $P = 0.10$).

Twenty-two parasite taxa were identified from examined fish, comprising 11 digeneans, 3 cestodes, 2 acanthocephalans, 5 monogeneans and 1 crustacean (Table 2). Groups such as *Apatemon* spp., *Posthodiplostomum* spp. and *Diplostomum* spp. have recently been demonstrated to contain cryptic species in the St. Lawrence River through DNA analysis (Locke et al., 2010, 2013); as such, no attempt was made to identify individuals past genus to avoid improper identification. Species accumulation curves indicated that the majority of parasite taxa were likely accounted for by the analysis of ~13 hosts (Fig. 1). Given that between 16 and 17 hosts were examined, common parasite species that make good candidates as bioindicators in YOY *L. gibbosus* were likely encountered. The majority of parasites found were monogeneans and larval trematodes (metacercaria), while larval cestodes (plerocercoids) and glochidia were comparatively rare (Table 2). All individual hosts examined contained at least two taxa, and the highest parasite species diversity within a single host (collected from Gray's Creek) was 10 species. The most common parasites found across streams were *Posthodiplostomum* spp. metacercariae and the monogenean *Actinocleidus* sp. The acanthocephalan *Neoechinorhynchus tenellus* was moderately prevalent in two of the three streams. Of the 22 taxa found, eight were shared among streams. All cestodes were present in Gunn Creek, and *Proteocephalus* sp. was the only cestode recovered from all three streams. The average number of taxa found per fish was highest in Gray's Creek and lowest in Hoople Creek (Table 3). Total parasite abundance was significantly correlated with HAI score in Gunn Creek ($r^2 = 0.25$, $P = 0.043$) and Gray's Creek ($r^2 = 0.32$, $P = 0.023$), though not in Hoople Creek ($P = 0.67$).

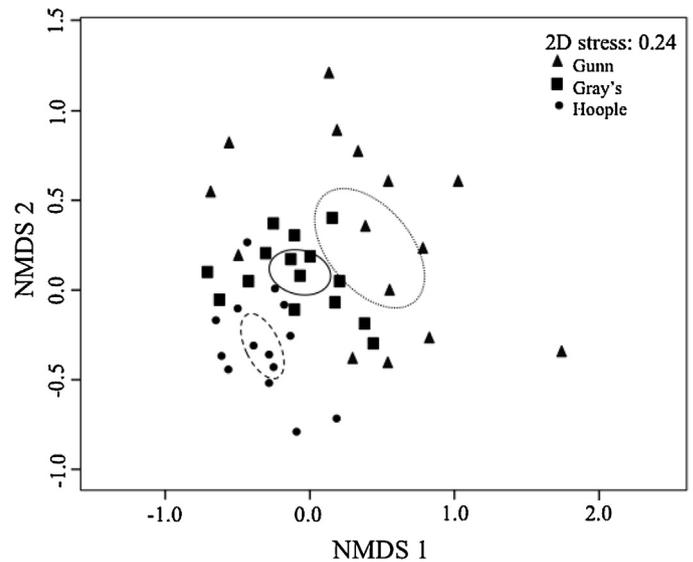


Fig. 2. Non-metric multidimensional scaling (NMDS) output with 95% confidence limits around the centroid (weighted average) of each stream of parasite species assemblage from overwintered YOY pumpkinseed *Lepomis gibbosus* collected in streams in Eastern Ontario.

Shannon–Weiner diversity was highest in fish collected from Hoople Creek and lowest in fish collected from Gunn Creek, indicating an increase in parasite diversity and species evenness with increasing condition in these streams. Gray's Creek fell consistently intermediate in terms of total parasite abundance, H' and HAI values. In addition, Hoople Creek had lower overall prevalence of *Posthodiplostomum* spp. and *Actinocleidus* sp., the highest prevalence species shared among streams (Table 2).

Global ANOSIM comparison indicates the overall parasite species composition among streams was significantly different (R statistic = 0.43, $P < 0.001$). Observed differences are qualitatively displayed in the NMDS plot, where relative similarities among localities are presented by clusters of site-specific data points (Fig. 2). A stress value (0.24) indicates that the ordination should be interpreted with caution, likely a result of high similarity and relatively low diversity in parasite species composition in the streams; of eight species shared among streams, only two are significantly different (Table 2). Further investigation using SIMPER analysis revealed the species driving the differences between good and poor habitat quality were the most abundant species collected: the trematode *Posthodiplostomum* spp., and the gill monogenean *Actinocleidus* sp. (Table 4). *Posthodiplostomum* spp. contributed the greatest proportional dissimilarity to comparisons between the most degraded and least degraded streams, while *Actinocleidus* sp. was the top contributor to dissimilarity between the less degraded streams. In total, these two species contributed the greatest amount to the observed dissimilarity between the streams, followed by the digenean *Apatemon* sp. and acanthocephalan *Neoechinorhynchus tenellus*; all other species contributed <5% to the total dissimilarity. Total dissimilarity values presented through SIMPER analysis confirmed trends observed in the H' indexes, ANOSIM analysis, and strengthen interpretation of the NMDS ordination.

4. Discussion

This is the first study to examine the parasite communities of fish across a gradient of watershed land cover and habitat variables. In this study we are assuming that overall habitat structure and *L. gibbosus* parasite populations would be the same if not for anthropogenic influences associated with land use, and thus trends

Table 2
Prevalence (P), mean intensity (MI), and mean abundance (MA) (\pm SE) of macroparasites collected from overwintered YOY pumpkinseed in three streams of different habitat quality in Cornwall, Ontario.

Class	Species	Gunn Creek			Gray's Creek			Hoople Creek			Sig ^a
		P	MI	MA	P	MI	MA	P	MI	MA	
Monogenea	<i>Cleidodiscus robustus</i>	0.118	1.50 \pm 0.50	0.18 \pm 0.13	0.188	2.00 \pm 0.58	0.38 \pm 0.22	0.312	1.40 \pm 0.24	0.44 \pm 0.18	0.43
	<i>Cleidodiscus venardi</i>	0.294	1.60 \pm 0.18	0.47 \pm 0.21	–	–	–	–	–	–	0.006*
	<i>Onchocleidus ferox</i>	0.235	2.25 \pm 0.67	0.53 \pm 0.26	0.500	2.38 \pm 0.60	1.19 \pm 0.42	0.312	2.60 \pm 0.69	0.81 \pm 0.37	0.35
	<i>Actinocleidus sp.</i>	1.000	13.50 \pm 2.30	13.50 \pm 2.30	1.000	14.00 \pm 2.31	14.00 \pm 2.31	0.938	6.73 \pm 1.17	6.31 \pm 1.17	0.0085*
	<i>Lyrodiscus sp.</i>	–	–	–	0.375	1.17 \pm 0.17	0.44 \pm 0.16	0.312	1.20 \pm 0.20	0.38 \pm 0.16	0.024*
Trematoda	<i>Phyllodistomum sp.</i>	0.118	2.00 \pm 1.00	0.24 \pm 0.18	–	–	–	0.375	1.50 \pm 0.20	0.56 \pm 0.20	0.017*
	<i>Plagioporus sp.</i>	–	–	–	–	–	–	0.125	4.00 \pm 1.00	0.50 \pm 0.35	0.12
	<i>Posthodiplostomum spp. m</i>	1.000	15.30 \pm 2.20	15.30 \pm 2.23	1.000	7.62 \pm 3.90	7.63 \pm 0.98	0.250	1.25 \pm 0.25	0.31 \pm 0.15	<0.0001*
	<i>Azygia angusticauda</i>	0.176	1.00	0.18 \pm 0.10	–	–	–	0.125	1.00	0.13 \pm 0.09	0.24
	<i>Apatemon ssp. m</i>	0.294	1.20 \pm 0.20	0.35 \pm 0.15	0.625	3.00 \pm 1.17	1.86 \pm 0.81	0.500	4.25 \pm 1.15	2.13 \pm 0.78	0.085
	<i>Uvulifer sp. m</i>	0.294	2.20 \pm 0.97	0.65 \pm 0.36	0.375	1.17 \pm 0.34	0.56 \pm 0.22	0.250	1.25 \pm 0.25	0.31 \pm 0.15	0.73
	<i>Clinostomum marginatum m</i>	–	–	–	0.250	1.25 \pm 0.25	0.31 \pm 0.15	–	–	–	0.012*
	<i>Crepidostomum cooperi</i>	–	–	–	–	–	–	0.125	1.00	0.13 \pm 0.09	0.12
	<i>Diplostomum ssp. m</i>	0.353	2.83 \pm 1.01	1.00 \pm 0.48	0.188	3.00 \pm 1.15	0.56 \pm 0.39	0.375	3.38 \pm 0.83	1.44 \pm 0.56	0.42
	<i>Apophallus sp. m</i>	–	–	–	0.188	1.33 \pm 0.33	0.25 \pm 0.14	0.188	2.67 \pm 0.67	0.50 \pm 0.29	0.17
	<i>Crassiphiala bulboglossa</i>	–	–	–	0.125	1.00	0.13 \pm 0.09	–	–	–	0.12
	<i>Proterometra macrostoma</i>	0.059	2.00	0.12 \pm 0.12	–	–	–	–	–	–	0.12
Cestoda	<i>Haplobothrium globuliforme pl</i>	0.176	1.00	0.18 \pm 0.10	–	–	–	–	–	–	0.053
	<i>Proteocephalus sp. pl</i>	0.059	11.00	0.65 \pm 0.65	0.125	2.00 \pm 1.41	0.25 \pm 0.19	0.188	1.00	0.19 \pm 0.10	0.61
Acanthocephala	<i>Neoechinorhynchus tenellus c</i>	0.882	4.27 \pm 0.67	3.76 \pm 0.66	0.500	1.50 \pm 1.07	0.75 \pm 0.27	–	–	–	<0.0001*
	<i>Leptorhynchoides thecatus c</i>	–	–	–	0.062	1.00	0.06 \pm 0.09	0.125	1.00	0.13 \pm 0.09	0.33
Mollusca	Unionidae Glochidia	–	–	–	0.188	3.00 \pm 0.33	0.25 \pm 0.06	0.188	2.33 \pm 0.33	0.44 \pm 0.24	0.17

m, metacercaria; pl, plerocercoid; c, cystacanth.

^a Kruskal–Wallis nonparametric analysis of variance

Table 3

Abundance and diversity data for infracommunity and component community of parasites and health assessment index scores of the hosts *Lepomis gibbosus* examined. Hosts were collected from three streams in Eastern Ontario in June 2012.

	Gunn Creek	Gray's Creek	Hoople Creek
Number of fish hosts examined	17	16	16
No. of parasite taxa	14	15	16
Mean taxa/fish	5.06 ± 0.5	5.69 ± 0.5	4.69 ± 0.4
Total abundance	37.0 ± 3.68	28.63 ± 3.54	14.69 ± 1.91
Common taxa (prevalence >50)	3	3	1
Intermediate taxa (prev < 10–50%)	9	11	15
Rare taxa (prev < 10%)	2	1	0
Shannon diversity (<i>H'</i>)	1.49	1.59	2.03
Health assessment score	35.9 ± 5.8	28.8 ± 6.0	17.7 ± 6.0

observed are a result of landscape alteration(s). Further, we are assuming that individuals are not moving between systems. These assumptions are based on local knowledge of historical fish populations, the proximity of sample sites (and thus historical similarities in land cover, climate and water chemistry), and knowledge of host species home range and distribution within the area. This is also the first study to compare modified health assessment indices of a YOY stream fish to parasite abundance and diversity in degraded areas.

Differences in fish condition among streams was marginally non-significant, likely due to high variation in HAI scores for each stream, however the significant correlation between total parasites/host and HAI score in Gunn and Gray's Creek leads to the question of whether host health is influencing parasite infection dynamics, if parasites themselves are causing decreased host health, and whether these properties are influenced by the habitat quality observed in the two systems.

Cases where total parasite abundance was high were generally a result of high infection levels of *Posthodiplostomum* spp. and *Actinocleidus* sp. which were found in highest intensity in Gunn and Gray's Creek (Table 2). Pathology associated *Posthodiplostomum* spp. has been observed only at very high experimental infection levels (Hoffman, 1958); even in these cases, hepatocytes in livers heavily infected with *Posthodiplostomum* spp. metacercaria maintain normal appearance and function (Mitchell et al., 1983), and mortality only occurred during experimental infections involving thousands of metacercaria in a single host similarly sized to fish examined here (adult fathead minnow *Pimephales promelas*; Hoffman, 1958), well beyond what was observed here. Intensity of infections of *Actinocleidus* sp. observed is well below levels that would be considered pathogenic for monogeneans (Rohlenová et al., 2011), and pathology of this species has not been demonstrated to date. Consequently, parasite community dynamics observed here are not likely to result in pathology or alter infection dynamics among hosts.

However, attempting to interpret causal mechanisms regarding observed health of fish at time of collection and how this may relate to the host's parasite community requires great caution, particularly when fish are collected from the wild. Recent analyses by Blanchet et al. (2009) have suggested a reciprocal relationship between host phenotype prior to infection and pathology of parasites, i.e. that direct effects of parasites may in part influenced by the phenotype of the host prior to infection, and host phenotype variation during infection was not purely a result of parasites. Further, genetic variation within a population has been shown to influence both resistance and tolerance to parasite species, and environmental factors such as temperature can influence host tolerance to parasites (Blanchet et al., 2010). As both poor habitat quality (Jeffries et al., 2015) and parasite burden (Sitjà-Bobadilla, 2008) have been related to decreased fish health and survival, disentangling the relationship between HAI scores, parasite abundance, and habitat is beyond the scope of field surveys such as this. Indeed an array of potential stressors, anthropogenic or natural, can influence the health of an organism. In this case, parasites have been demonstrated to be significantly associated with the health of fish, and these relationships are present in areas with poor habitat quality. Cumulative effects of multiple stressors on organism health, including parasites, are an important consideration for ecosystem management (Marcogliese and Pietrock, 2011).

Parasite diversity appears to decrease as watershed size becomes smaller and habitat quality (TP, benthic, and bacteria scores) decreases. In contrast, total parasite abundance, largely driven by changes in prevalence and intensity of *Posthodiplostomum* spp., follows an opposite pattern, increasing as watershed size becomes smaller and habitat quality decreases. Decreasing habitat availability may increase densities of host population, increasing the likelihood of transmission from host to host for direct lifecycles or exposing a greater number of fish to intermediate hosts where habitat requirements overlap (Marcogliese, 2005). Literature on species-area relationships indicates larger riverine systems

Table 4

Results of SIMPER analysis of parasite species extracted from overwintered YOY pumpkinseed *L. gibbosus* in three streams in Eastern Ontario. Only species contributing >3% of dissimilarity are listed.

Site comparisons	Total dissimilarity	Species	Avg. A	Avg. B	Contribution
Gunn (A) Hoople (B)	117.0	<i>Posthodiplostomum</i> spp.	15.29	0.3125	28.5
		<i>Actinocleidus</i> sp.	13.47	6.3	17.2
		<i>Neoechinorhynchus tenellus</i>	3.76	0	7.6
		<i>Apatemon</i> sp.	0.35	0	4.6
		<i>Diplostomum</i> spp.	1	1.44	3.7
Gunn (A) Gray's (B)	66.2	<i>Posthodiplostomum</i> spp.	15.29	7.63	14.2
		<i>Actinocleidus</i> sp.	13.47	14	14
		<i>Neoechinorhynchus tenellus</i>	3.76	0.75	5.5
Hoople (A) Gray's (B)	48.8	<i>Actinocleidus</i> sp.	6.31	14	20.3
		<i>Posthodiplostomum</i> spp.	0.31	7.63	16.9
		<i>Apatemon</i> spp.	2.13	1.88	7
		<i>Onchocleidus ferox</i>	0.8125	1.1875	3.5
		<i>Diplostomum</i> spp.	1.44	0.56	3.5

often support higher diversity (Hugueny, 1989); consequently, the observed changes in parasite diversity may be positively influenced by increased habitat complexity and connectivity found in larger systems. However, previous research has demonstrated similar changes in parasite communities in relation to habitat quality as observed here (Hartmann and Nümann, 1977; MacKenzie, 1999; Zargar et al., 2012), and nutrient loading may dampen the species-area effect (Brönmark et al., 1984), providing further support for parasites as effective indicators of environmental characteristics. Truly disentangling the causal mechanisms regarding changes in parasite diversity such as what is observed here would require large-scale, systematic research program incorporating laboratory and field trials.

Streams sampled in the present study are notably impacted by urban and agricultural development (e.g. stream channelization, culvert insertions) and so do not include a true reference habitat. It is therefore possible the least degraded stream has already passed a threshold for some parasite species that would otherwise be present, thus excluding sensitive parasites from this study; however, as the goal of this study was to identify potential bioindicators, the findings address the overall research objectives to identify relative changes in parasite community and species indicative of habitat quality.

Species recovered were similar to those found in previous research on adult *L. gibbosus* in the St. Lawrence River (Locke et al., 2013), except that fewer adult trematodes were recovered. This is likely a result of using YOY hosts, as a juvenile's diet is not as diverse as an adult and so excludes transmission pathways present in older populations of *L. gibbosus*, and recruitment and maturation time is less (i.e. one growing season). Further, fish examined in Locke et al. (2013) were from larger, more complex habitats on the St. Lawrence River compared to the wadeable streams sampled here. Fish species diversity and more elaborate trophic dynamics (e.g. presence of piscivorous fishes) likely contributed to the increased adult parasite diversity found in the previous study. Consequently, certain species and forms of parasites present within the entire *L. gibbosus* population in each sampling location may be under-represented in this study. The majority of parasites encountered were larval stages, most notably trematode metacercariae. This is a trend consistent with other studies on fish parasite communities in the St. Lawrence River itself (Marcogliese et al., 2006; Thilakaratne et al., 2007; Krause et al., 2010; Locke et al., 2013), and is likely a result of the intermediate trophic status of hosts sampled (Marcogliese, 2004). Fish positioned in the middle of local food webs such as juvenile *L. gibbosus* consume mainly invertebrates (Scott and Crossman, 1973) and provide both nutrients and transmission pathways for developing helminth parasites because these intermediate fish hosts are common prey for the parasite's respective definitive host (Marcogliese, 2004; Marcogliese and Cone, 1997; Valtonen et al., 2010). Esch (1971) proposed that larval forms of parasites that mature in piscivorous birds and mammals would dominate eutrophic systems. As all streams included in this study are eutrophic at some level (AECOM, 2009), these data support this idea.

Differences in parasite communities and infection dynamics recovered from watersheds in this study may indicate that parasite burden increases in more impacted systems, a finding that is consistent with previously reported trends. A positive relationship between nutrient loading found in eutrophic fresh waters and fish parasitism has previously been reported in Eurasian perch *Perca fluviatilis* and the Himalayan cyprinid *Schizothorax niger* inhabiting eutrophic lakes (Hartmann and Nümann, 1977; Zargar et al., 2012), and spottail shiner *Notropis hudsonius* exposed to nutrient rich urban effluent (Marcogliese and Cone, 2001; Marcogliese et al., 2006). In this study mean total abundance of parasites was significantly higher, while species diversity was lower, in more eutrophic

streams. MacKenzie (1999) summarized effects of eutrophication as being overall positive for monogeneans, digeneans, cestodes and acanthocephalans; however a detailed meta-analysis conducted by Blanar et al. (2009) reported an overall significant negative effect of eutrophication on digenetic trematodes, and no effect on other groups. The disparity in summarized effects compared to individual study effects suggests that changes in water quality associated with eutrophication may only benefit a minority of species, in particular generalist parasite taxa such as *Posthodiplostomum* spp., beyond a certain level of disturbance. In addition, the cause for presence or absence of a given species at a site may be a result of multiple factors, as fish immunity, parasite survival and intermediate host populations are not independent from one another, making determination of cause and effect problematic (Poulin, 1992; Marcogliese, 2005).

The species found in all streams that significantly increased in abundance and prevalence in eutrophic habitats were also identified as species driving dissimilarity between localities, and thus identified as potential bioindicators for these streams (Tables 3 and 4). However, *Actinocleidus* sp. did not follow the gradient of habitat quality as closely as *Posthodiplostomum* spp., and was found to be the species driving differences in community composition between the two less impacted creeks. The significant decrease in this species' abundance in Hoople Creek may be a result of natural variation in *Actinocleidus* sp. distribution, or a change in biotic or abiotic characteristics shared between Gray's and Gunn that impacts transmission dynamics in some way. Consequently, *Posthodiplostomum* spp. appears to follow habitat characteristics more consistently in abundance and intensity, and thus be a more appropriate bioindicators compared to *Actinocleidus* sp. in this case.

These parasites are extremely different in their transmission pathways and physiology; parasites of the genus *Posthodiplostomum* are heteroxenous trematodes found in many freshwater fish species (Miller, 1954; Hoffman, 1956; Gibson, 1996), while *Actinocleidus* sp. are monoxenous ectoparasites specific to centrarchids and commonly found on *L. gibbosus* (Beverly-Burton, 1981, 1984; Locke et al., 2013). As a result, these taxa interact with their hosts and the environment in different ways, and thus may indicate unique characteristics of the ecosystem and health of host fish. In addition, *Posthodiplostomum* spp. in particular has extensive distributions and are relatively easy to identify with a standard stereomicroscope, two qualities that enhance their potential use as bioindicators.

The *Posthodiplostomum* spp. lifecycles involves snails of the genera *Physa* sp. and *Physella* sp. as the first intermediate host (Hoffman, 1956), so increased abundance of this parasite may indicate an increase in number or density of snails (Shah et al., 2013). In contrast, the lifecycle of *Actinocleidus* sp. is direct, i.e. is transferred from fish to fish, requiring no intermediate host to facilitate development or transmission (Beverly-Burton, 1981). These helminths are hermaphroditic and able to proliferate rapidly under ideal conditions including warm temperatures and high host density. Consequently, synergistic mechanisms may result in the increased *Posthodiplostomum* spp. and *Actinocleidus* sp. prevalence and intensity observed in Gunn and Gray's creek compared to Hoople. Habitat characteristics associated with eutrophic and developed streams include high and fluctuating water temperatures (Bly and Clem, 1992), pollutants (Duffy et al., 2002) and nutrient loading (Johnson et al., 2010), factors that can alter fish physiology, particularly immune function, perhaps increasing the likelihood of infection of certain parasites (Secombes and Chappell, 1996; Rohlenová et al., 2011; Jeffries et al., 2015). Consequently, fish living in sub-optimal habitat may demonstrate higher rates of infection of specific parasites when compared to those residing in optimal conditions. For example, water temperatures and poor water quality associated with effluents may cause immunosuppression, which could ease

penetration and migration through host tissue (Jokinen et al., 1995; Khan, 2004; Inendino et al., 2005).

In the watersheds examined both parasite and benthic invertebrate communities decreased in diversity with decreasing stream condition. This decrease in benthic diversity may be a factor that excludes certain parasites from more degraded streams by eliminating transmission pathways (Marcogliese and Cone, 1997). In this study, the greater species diversity of trematodes in Hoople Creek compared to Gunn Creek suggests there may be potential alteration of invertebrate transmission pathways in degraded streams (Marcogliese, 2004). For example, Hechinger et al. (2007) found a consistent positive correlation between trematodes and large benthic invertebrate species in coastal wetlands of California. Hoople Creek and Gray's Creek, with high component community and infracommunity diversity, also scored higher on benthic diversity indices (Table 3). Disturbed areas such as Gunn Creek, an area with intense eutrophication, may not support invertebrate species necessary for the transmission of certain parasites. Indeed, glochidia were absent in Gunn Creek, suggesting that freshwater clams may be infrequent or absent in this system; a similar trend was seen in pulp-effluent polluted lakes in Finland by Valtonen et al. (1997). Increased nutrient loading associated with high proportions of agriculture may also facilitate an increase in snail density (Shah et al., 2013). This could explain the increase in *Posthodiplostomum* spp. prevalence and intensity with increasing habitat degradation compared to other species, as snails are required for this parasite's life cycle. Thus, if more snails found within a system, there is a higher potential for transmission. Shah et al. (2013) noted increased densities of planorbid and lymnaeid snails in a eutrophic lake, and attributed elevated *Diplostomum* spp. levels in fish hosts from the area to increased densities and spatial distribution of snails.

5. Conclusions

This study demonstrated potential for certain parasite species to be used as indicators of habitat quality in eastern Ontario. While the mechanisms driving differences in parasite community dynamics are not well understood, species diversity indices were highest in the least degraded stream, perhaps indicating potential sensitivity to habitat quality changes similar to those seen in previous studies (e.g., Huspeni and Lafferty, 2004). HAI scores were variable and while trending a decrease in fish condition as habitat quality decreased, such an assessment technique may not be appropriate to use on small young fish. To fully investigate the relationship between parasite abundance and host health assessment, a more extensive survey or elaborate assessment protocol would be required. The changes in abundance of *Posthodiplostomum* spp. and *Actinocleidus* sp., two large and rather easily identifiable parasites, suggests that they may be appropriate bioindicators to monitor changes in habitat quality for this area. Again, validation in the form of a larger-scale study involving more watersheds, anthropogenic land-use types, and habitat characteristics would be necessary prior to the implementation of this monitoring technique. Additionally, the incorporation of multiple host species from a related group such as centrarchids (as conducted by Esch, 1971) would identify whether parasite taxa shared among host species such as *Posthodiplostomum* spp. follow similar response patterns, allowing for the use of multiple fish species in a single survey.

With the intensive effort required to extract and identify all parasites present within a host, small-scale parasite surveys such as this would be useful to establish baseline community compositions and focal species for use in monitoring. After such a survey, the incorporation of parasites into sampling regimes such as Before-After-Control-Impact designs may increase the breadth of information acquired while monitoring ecosystems

post-restoration. Managers could use parasites to assess changes in food web dynamics (e.g. benthic invertebrates in this case) such as the return of certain species known to be required for parasite transmission. Indeed, individual-level metrics such as fish condition and parasite infection/infestation dynamics have much to offer the evaluation of habitat quality, restoration activities, and overall ecosystem health (Cooke and Suski, 2008; Marcogliese and Pietrock, 2011).

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