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Infection of *Diplostomum* spp. in invasive round gobies in the St Lawrence River, Canada

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Abstract

The round goby (*Neogobius melanostomus*) is a successful invader of the Great Lakes–St Lawrence River basin that harbours a number of local parasites. The most common are metacercariae of the genus *Diplostomum*. Species of *Diplostomum* are morphologically difficult to distinguish but can be separated using molecular techniques. While a few species have been sequenced from invasive round gobies in this study system, their relative abundance has not been documented. The purpose of this study was to determine the species composition of *Diplostomum* spp. and their relative abundance in round gobies in the St Lawrence River by sequencing the barcode region of cytochrome *c* oxidase I. In 2007–2011, *Diplostomum huronense* (=*Diplostomum* sp. 1) was the most common, followed in order by *Diplostomum indistinctum* (=*Diplostomum* sp. 4) and *Diplostomum indistinctum sensu* Galazzo, Dayanandan, Marcogliese & McLaughlin (2002). In 2012, the most common species infecting the round goby in the St Lawrence River was *D. huronense*, followed by *D. indistinctum* and *Diplostomum gavium* (=*Diplostomum* sp. 3). The invasion of the round goby in the St Lawrence River was followed by a decline of *Diplostomum* spp. in native fishes to low levels, leading to the previously published hypothesis that the presence of the round goby has led to a dilution effect. Herein, it is suggested that despite the low infection levels in the round goby, infections still may lead to spillback, helping to maintain *Diplostomum* spp. in native fishes, albeit at low levels.

Introduction

Invasive species often acquire native parasites in their adopted habitats. Indeed, native species compose the majority of parasites in invasive hosts (Kelly *et al*., 2009; Poulin *et al*., 2011; Paterson *et al*., 2012). These parasites tend to be generalists (Kelly *et al*., 2009), and are frequently helminths with complex life cycles that are transmitted through the food chain (Poulin *et al*., 2011). If the invasive host is a competent one, it may act as a reservoir for native parasites, which can then be transmitted back to native hosts – a process termed ‘spillback’ (Kelly *et al*., 2009; Poulin *et al*., 2011; Hatcher *et al*., 2012). Such a process may amplify infections and lead to an increased risk of disease for native hosts (Poulin *et al*., 2011; Dunn & Hatcher, 2015).

The round goby (*Neogobius melanostomus*) successfully invaded the Great Lakes in 1990 and the St Lawrence River in 1997 (Gendron *et al*., 2012; Kornis *et al*., 2012). Since its first appearance, gobies have acquired >20 species of helminths in Lake St Clair alone (Muzzall *et al*., 1995; Pronin *et al*., 1997; Kvach & Stepien, 2008; Gendron *et al*., 2012). Although a more recent introduction in the St Lawrence River, by 2009 gobies were infected with eight species of helminths (Gendron *et al*., 2012). In both the Great Lakes and the St Lawrence River, the most prevalent and abundant parasite was the digenean *Diplostomum* spp. (Muzzall *et al*., 1995; Pronin *et al*., 1997; Camp *et al*., 1999; Kvach & Stepien, 2008; Gendron *et al*., 2012).

Diplostomum spp. have a three-host life cycle (Chappell *et al*., 1994). Definitive hosts include birds of the families Laridae, Anatidae, Gaviidae and Ardeidae, from which parasite eggs pass in the feces. Free-swimming miracidia hatch from eggs and infect the first intermediate host, a lymnaeid snail. The parasites reproduce asexually inside the snail, which then releases free-swimming cercariae that infect the fish second intermediate host. In the St Lawrence River, there are at least 21 known fish hosts including cyprinids, centrarchids, percids, catostomids, ictalurids, percopsids, fundulids, esocids and atherinopsids (Marcogliese & Compagna, 1999; Marcogliese *et al*., 2001; Locke *et al*., 2010a). The life cycle is completed when the definitive host consumes an infected fish. In the fish, the parasite resides in either the eye or brain. Larvae are morphologically difficult to distinguish to species, and, using molecular methods, Locke *et al*. (2010a, 2015) found 12 different species in fishes from the

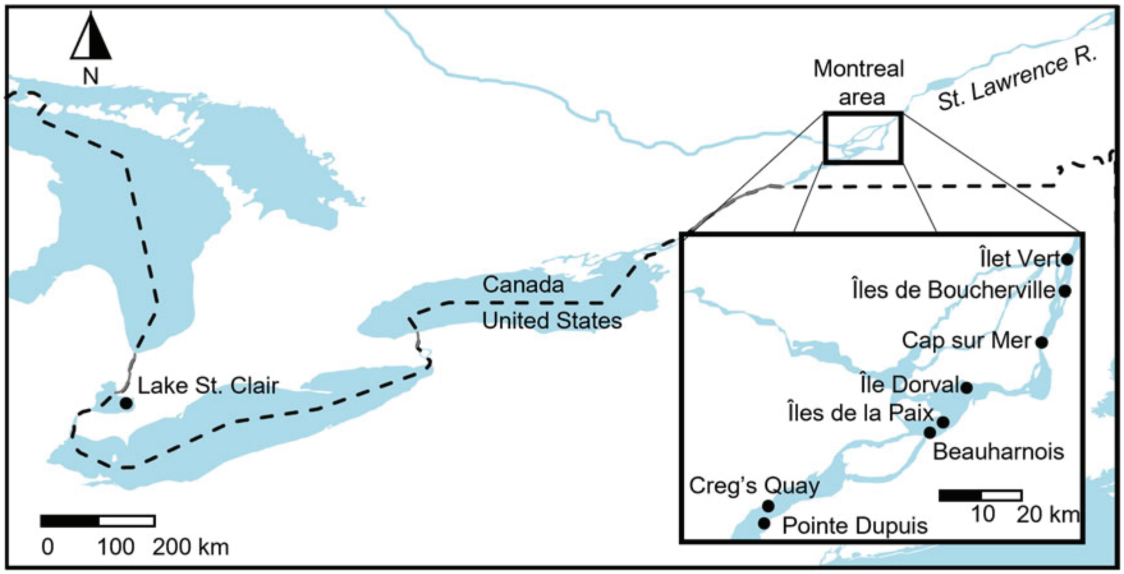

Fig. 1. Collection localities of invasive round gobies (*Neogobius melanostomus*) in the St Lawrence River and Great Lakes region from which metacercariae of *Diplostomum* spp. were sequenced in the present and prior studies (see tables 1 and 2).

Great Lakes–St Lawrence River basin. Those infecting the brain and non-lens parts of the eye were host specialists, while those infecting the lens were host generalists (Locke *et al*., 2010a, b, 2015). Given that *Diplostomum* spp. cause cataracts in their hosts, leading to reduced growth, emaciation and other detrimental effects (Chappell *et al*., 1994), it is important to identify the species of *Diplostomum* infecting the round goby, which have become widespread and integrated into local food webs in both the Great Lakes and the St Lawrence River (Johnson *et al*., 2005; Reyjol *et al*., 2010). An extensive study of round gobies in the St Lawrence River showed that *Diplostomum* spp. only occurred in the lens (Gendron & Marcogliese, 2017). However, to date, five species have been found in the lenses of native fishes in that river, all of which can be considered generalists (Locke *et al*., 2010a, 2015). Previous molecular work on a limited number of specimens of *Diplostomum* spp. from gobies in the Great Lakes and St Lawrence River has shown them to be infected with three species – *Diplostomum indistinctum*, *Diplostomum huronense* and *Diplostomum indistinctum sensu* Galazzo, Dayanandan, Marcogliese & McLaughlin (2002) (nomenclature as in Achatz *et al*., 2021; and see Galazzo *et al*., 2002; Locke *et al*., 2010a, 2015; Désilets *et al*., 2013) – but their relative abundance was not quantified. In this study, we expand upon those results in greater detail and provide further data from more specimens infecting round gobies in the St Lawrence River to determine the relative abundance of the different species of *Diplostomum*. Our hypothesis was that round gobies should be infected with all species of generalists occurring in the St Lawrence River. We use these results in addition to the results of previous studies to pose the suggestion that spillback may be occurring in this system, and that spillback and dilution need not be mutually exclusive.

Materials and methods

Given that previous studies on molecular sequences of *Diplostomum* spp. in round gobies only published summary information, data were extracted and compiled from all prior publication records (Locke *et al*., 2010a, 2015; Désilets *et al*., 2013).

To obtain specimens for the current study, round gobies were collected by beach seine (22.6 m × 1.15 m; 3 mm mesh) towed by hand or partially deployed from a boat from Îles de la Paix (45° 20.574′N, 73°50.963′W), Île Dorval (45°26.018′N, 73°44.257′W) and Pointe Dupuis (45°7.683′N, 74°24.233′W) in June, August and September 2012 (fig. 1). Given that infection rates were extremely low at the time of sampling (Gendron & Marcogliese, 2017), many hundreds of round gobies had to be examined to obtain an adequate number of specimens. However, the total number of fish was not tabulated in this aspect of the study, and, hence, infection parameters could not be determined. Fish were euthanized by immersion in a 400 mg/l eugenol (clove oil) solution, placed in bags and frozen for subsequent analyses. Upon thawing, eyes were removed, and dissected and examined using a stereomicroscope. All 128 specimens of *Diplostomum* spp. were removed and fixed in 95% ethanol for subsequent molecular analyses.

To determine infection levels of *Diplostomum* spp. in round gobies, fish were collected, stored and processed using the same techniques from four localities in June 2012 and June 2013. The localities included Îles de la Paix, Île Dorval, Pointe Dupuis and Creg's Quay (45°9.678′N, 74°25.582′W; fig. 1). Twenty fish were collected from each locality on each occasion.

Extraction, amplification and sequencing of the barcode region of cytochrome *c* oxidase 1 from metacercariae was conducted at the Canadian Centre for DNA Barcoding (Canada) using the protocols and Mplat or plat-diplo primers of Moszczynska *et al*. (2009).

Results

A total of 41 metacercariae were sequenced from 15 invasive round gobies in previous studies (Locke *et al*., 2010a, 2015; Désilets *et al*., 2013). These include 37 specimens of *D. huronense* (=*Diplostomum* sp. 1), three specimens of *D. indistinctum* (=*Diplostomum* sp. 4) and a single specimen of *D. indistinctum*

Table 1. *Diplostomum* species determined by molecular sequencing of 41 specimens recovered from invasive round gobies (*Neogobius melanostomus*) in the St Lawrence River (SLR) and Great Lakes (GL) derived from original data summarized in Locke *et al.* (2010a, 2015) and Désilets *et al.* (2013).

Date	Species	Specimens (*N*)	Site
September 2007	*Diplostomum huronense* (=*Diplostomum* sp. 1)	3	Beauharnois, SLR
June 2008	*Diplostomum indistinctum* (=*Diplostomum* sp. 4)	1	Îlet Vert, SLR
	Diplostomum indistinctum sensu Galazzo, Dayanandan, Marcogliese & McLaughlin (2002)	1	
June 2009	*Diplostomum huronense*	10	Îles de Boucherville, SLR
	Diplostomum huronense	3	Cap-sur-Mer, SLR
September 2009	*Diplostomum huronense*	4	Lake St Clair, GL
	Diplostomum indistinctum	1	
	Diplostomum huronense	5	Beauharnois, SLR
	Diplostomum indistinctum	1	
September 2011	*Diplostomum huronense*	12	Creg's Quay, SLR

Table 2. *Diplostomum* species determined by molecular sequencing of 69 specimens recovered from invasive round gobies (*Neogobius melanostomus*) in the St Lawrence river in 2012.

Month	Species	Specimens (*N*)	Site
June	*Diplostomum huronense* (=*Diplostomum* sp. 1)	1	Îles de la Paix
	Diplostomum gavium (=*Diplostomum* sp. 3)	2	
	Diplostomum indistinctum (=*Diplostomum* sp. 4)	2	
	Diplostomum huronense	1	Île Dorval
	Diplostomum huronense	7	Point Dupuis
August	*Diplostomum huronense*	2	Îles de la Paix
	Diplostomum gavium	3	
	Diplostomum indistinctum	13	
September	*Diplostomum huronense*	1	Îles de la Paix
	Diplostomum gavium	3	
	Diplostomum huronense	34	Île Dorval

sensu Galazzo, Dayanandan, Marcogliese & McLaughlin (2002) (table 1). Multiple metacercariae were sequenced from nine fish, of which three contained mixed infections. One fish collected in June 2008 from the St Lawrence River was infected with one *D. indistinctum* and one *D. indistinctum sensu* Galazzo, Dayanandan, Marcogliese & McLaughlin (2002). Six specimens from a single fish collected from the St Lawrence River in September 2009 consisted of five *D. huronense* and a single *D. indistinctum*. Lastly, of three metacercariae sequenced from a single fish collected in the Great Lakes in September 2009, two were *D. huronense* and one was *D. indistinctum*.

From the 2012 sampling for molecular species discrimination, 69 parasites were sequenced successfully, yielding 40 distinct haplotypes. Of these, 46 corresponded to *D. huronense*, 15 were *D. indistinctum* and eight were *Diplostomum gavium* (=*Diplostomum* sp. 3) (table 2). All metacercariae were lens forms. Another 59 metacercariae could not be sequenced successfully. Sequenced specimens originated in 15 different individual fish, and multiple specimens of *Diplostomum* were sequenced in ten fish. Mixed infections were observed in four individual hosts, all from Îles de la Paix. They comprised a co-infection with three *D. gavium* and one *D. huronense*; with two *D. gavium* and one *D. indistinctum*; with eight *D. indistinctum* and one *D. huronense*; and, lastly, with one *D. huronense*, one *D. gavium* and one *D. indistinctum*. A notably homogeneous infection was observed in one fish from Île Dorval, in which sequences obtained from 32 of 46 specimens all corresponded to *D. huronense*. All the sequences newly obtained from 2012 material were either identical to or differed by at most two nucleotides (>0.5%) from haplotypes of *D. huronense*, *D. gavium* and *D. indistinctum* previously published by Locke *et al.* (2010a, b, 2015) under the names *Diplostomum* sp. 1, 3 and 4, respectively. Newly generated sequences are deposited in GenBank under accession numbers MZ563479–MZ563546.

Mean total length (±standard deviation) of round gobies collected to measure overall infection rates pooled from the four localities was 62.2 (±8.16) mm in 2012 and 61.5 (±10.92) in 2013. Prevalence of infection of *Diplostomum* spp. in round gobies pooled from the four localities was 3.75% and 6.25% in 2012 and 2013, respectively. Mean abundance (±standard error) from the pooled localities was 0.04 (±0.02) in 2012 and 0.11 (±0.05) in 2013. Intensity was 1.0 in 2012 and ranged from one to three in 2013.

Discussion

Results confirm previous studies showing that round gobies in the St Lawrence River become infected with lens forms of *Diplostomum*, all of which are putative generalists. *Diplostomum gavium* was found for the first time in the round goby. In 2012, *D. huronense* was clearly the most common, followed by *D. indistinctum* and *D. gavium*. Similarly, *D. huronense* was the most common form in round gobies collected from 2007 to 2011, followed by *D. indistinctum* (Locke *et al*., 2010a, 2015; Désilets *et al*., 2013). In contrast to previous studies (Locke *et al*., 2010a; Désilets *et al*., 2013), *D. indistinctum sensu* Galazzo, Dayanandan, Marcogliese & McLaughlin (2002) was not found. However, these parasites were collected in 2007–2009, and this parasite has only been observed once since that time (2011) in a golden shiner (*Notemigonus crysoleucas*), out of a total of 703 specimens sequenced from various fish hosts (Désilets *et al*., 2013). Mixed infections of *Diplostomum* spp. do occur in round gobies, although they are not common, likely due to the overall low infection levels, especially of the less abundant species. Among native host species, any one *Diplostomum* species may be the most common, with *D. huronense* predominant in five fish species, *D. indistinctum* predominant in five species and *D. gavium* predominant in two species, with *D. gavium* and *D. indistinctum* co-dominant in another three species (Désilets *et al*., 2013; Locke *et al*., 2013). However, the relative distribution of *Diplostomum* spp. among these hosts is not completely consistent between these two studies, suggesting temporal variation in species composition. Given that there is no phylogenetic pattern to the distribution of these species among fish hosts, the reason for the relatively high abundance of *D. huronense* in the round goby in this or previous studies is not known. All of the above species are considered host generalists (Locke *et al*., 2010a, b, 2015), but both *D. huronense* and *D. indistinctum* were the most common in native fishes (Désilets *et al*., 2013; Locke *et al*., 2013). Curiously, another generalist found in the St Lawrence River, *D. huronense sensu* Galazzo, Dayanandan, Marcogliese & McLaughlin (2002) has never been found in the round goby. This species was not common in 2011 (Désilets *et al*., 2013), while being relatively abundant in 2006 (Locke *et al*., 2013). Its limited occurrence may explain its absence from the round goby.

The round goby is among the most successful invasive species in the St Lawrence River, becoming one of the most abundant fish species in the littoral zone (Morissette *et al*., 2018). Gobies have inserted themselves into local food chains and are now prey to avian and piscine piscivores in the Great Lakes–St Lawrence River basin (Johnson *et al*., 2005; Reyjol *et al*., 2010; Essian *et al*., 2016). Indeed, the round goby is now one of the most important prey items for ring-billed gulls, an important definitive host for *Diplostomum* spp., in the Great Lakes (Essian *et al*., 2016). Furthermore, the round goby was shown to be a potentially competent host for species of *Diplostomum*. In a preliminary experiment, ring-billed gulls (*Larus delarewarensis*) fed wild-caught gobies from the St Lawrence River became infected with adult *Diplostomum* spp., suggesting some degree of competency (see Gendron & Marcogliese, 2017). Host competence, the capacity of a host to transmit infection (Stewart Merrill & Johnson, 2020), is a required characteristic of an invasive species for spillback to occur (Kelly *et al*., 2009; Poulin *et al*., 2011).

Spillback also depends on the relative abundance of native and invasive hosts (Paterson *et al*., 2013). In the areas sampled in the St Lawrence River, the round goby comprises 11.6–35.4% of fish captured in the littoral zone (Morissette *et al*., 2018). Of the native hosts of *Diplostomum* spp., some increased in abundance, some decreased, and others remained stable since the invasion of the round goby (Morissette *et al*., 2018). Thus, other than the huge numerical increase in the round goby population, there is no clear trend one way or another across native host fish species that could influence transmission of *Diplostomum* spp. Indeed, the fact that the round goby is numerically so abundant lends support to the idea that spillback could occur (Paterson *et al*., 2013). Confirmation would require determination of the actual relative transmission rates of *Diplostomum* spp. to the definitive hosts from round gobies compared to native fishes.

Spillback of native parasites from invasive species can result in amplification, an increase in prevalence and abundance in native hosts (Kelly *et al*., 2009; Poulin *et al*., 2011). Amplification depends on the invading host's abundance, its susceptibility and the transmission potential (Rohr *et al*., 2020). However, abundance and prevalence of *Diplostomum* spp. in round gobies in the St Lawrence River is quite low (Gendron & Marcogliese, 2017; this study). Yet, abundance and prevalence of the parasites in numerous native host species has also declined to low levels following the round goby introduction (Gendron & Marcogliese, 2017). These authors suggested that the presence of the round goby has led to a dilution effect, reducing parasite loads in native fishes, and also explored the potential mechanism(s) in detail as well as alternate explanations (Gendron & Marcogliese, 2017). These authors found that possible declines in first intermediate hosts or definitive hosts, or changes in hydrological conditions, could not explain the declines in *Diplostomum* spp. We suggest the infections in the round goby, one of the most abundant hosts in the river, may assist in maintaining infections of *Diplostomum* spp. in native fishes, albeit at low levels, via spillback, despite the low levels of infection in that host. The abundance of the round goby, its importance as prey for piscivorous birds and its potential competence as a host for the parasite lend support for this idea. This raises the intriguing possibility that dilution and spillback may not be mutually exclusive.

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Conflict of interest. None.

Ethical standards. Collection and treatment of animals follows the guidelines of the Canadian Council for Animal Care and was approved by the

Environment Canada Animal Care Committee. Fish were collected under a permit from the Quebec Ministère des Ressources Naturelles et de la Faune.

References

Achatz TJ, Martensa JR, Kostadinova A, *et al*. (2021) Molecular phylogeny of *Diplostomum*, *Tylodelphys*, *Austrodiplostomum* and *Paralaria* (Digenea: Diplostomidae) necessitates systematic changes and reveals a history of evolutionary host switching events. *International Journal for Parasitology* (in press). doi: 10.1016/j.ijpara.2021.06.002.

Camp JW, Blaney LM and Barnes DK (1999) Helminths of the round goby, *Neogobius melanostomus* (Perciformes: Gobiidae), from southern Lake Michigan, Indiana. *Journal of the Helminthological Society of Washington* **66**, 70–72.

Chappell LH, Hardie LJ and Secombes CJ (1994) Diplostomiasis: the disease and host-parasite interactions. pp. 59–86 *in* Pike AW and Lewis JW (*Eds*) *Parasitic diseases of fish*. Dyfed, Samara Publishing Limited.

Désilets HD, Locke SA, McLaughlin JD and Marcogliese DJ (2013) Community structure of *Diplostomum* spp. (Digenea: Diplostomidae) in eyes of fish: main determinants and potential interspecific interactions. *International Journal for Parasitology* **43**, 929–939.

Dunn AM and Hatcher MJ (2015) Parasites and biological invasions: parallels, interactions, and control. *Trends in Parasitology* **31**, 189–199.

Essian DA, Chipault JG, Lafrancois BM and Leonarda JBK (2016) Gut content analysis of Lake Michigan waterbirds in years with avian botulism type E mortality, 2010–2012. *Journal of Great Lakes Research* **42**, 1118–1128.

Galazzo DE, Dayanandan S, Marcogliese DJ and McLaughlin JD (2002) Molecular systematics of some North American species of *Diplostomum* (Digenea) based on rDNA sequence data and comparisons with European congeners. *Canadian Journal of Zoology* **80**, 2207–2217.

Gendron AD and Marcogliese DJ (2017) Enigmatic decline of a common fish parasite (*Diplostomum* spp.) in the St. Lawrence River: evidence for a dilution effect induced by the invasive round goby. *International Journal for Parasitology-Parasites and Wildlife* **6**, 402–411.

Gendron AD, Marcogliese DJ and Thomas M (2012) Invasive species are less parasitized than native competitors, but for how long? The case of the round goby in the Great Lakes-St. Lawrence Basin. *Biological Invasions* **14**, 367–384.

Hatcher MJ, Dick JTA and Dunn AM (2012) Disease emergence and invasions. *Functional Ecology* **26**, 1275–1287.

Johnson TB, Bunnell DB and Knight CT (2005) A potential new energy pathway in Central Lake Erie: the round goby connection. *Journal of Great Lakes Research* **31**(Suppl. 2), 238–251.

Kelly DW, Paterson RA, Townsend CR, Poulin R and Tompkins DM (2009) Parasite spillback: a neglected concept in invasion ecology? *Ecology* **90**, 2047–2056.

Kornis MS, Mercado-Silva N and Vander Zanden MJ (2012) Twenty years of invasion: a review of round goby *Neogobius melanostomus* biology, spread and ecological implications. *Journal of Fish Biology* **80**, 235–285.

Kvach Y and Stepien CA (2008) Metazoan parasites of introduced round and tubenose gobies in the Great Lakes: support of the "enemy release hypothesis". *Journal of Great Lakes Research* **34**, 23–35.

Locke SA, McLaughlin JD, Dayanandan S and Marcogliese DJ (2010a) Diversity and specificity in *Diplostomum* spp. metacercariae in freshwater fishes revealed by cytochrome *c* oxidase I and internal transcribed spacer sequences. *International Journal for Parasitology* **40**, 333–343.

Locke SA, McLaughlin JD and Marcogliese DJ (2010b) DNA barcodes show cryptic diversity and a potential physiological basis for host specificity among Diplostomoidea (Platyhelminthes: Digenea) parasitizing freshwater fishes in the St. Lawrence River, Canada. *Molecular Ecology* **19**, 2813–2827.

Locke SA, McLaughlin JD and Marcogliese DJ (2013) Predicting the similarity of parasite communities in freshwater fishes using the phylogeny, ecology and proximity of hosts. *Oikos* **122**, 73–83.

Locke SA, Al-Nasiri FS, Caffara M, *et al*. (2015) Diversity, specificity and speciation in larval Diplostomidae (Platyhelminthes: Digenea) in the eyes of freshwater fish, as revealed by DNA barcodes. *International Journal for Parasitology* **45**, 841–855.

Marcogliese DJ and Compagna S (1999) Diplostomatid eyeflukes in young-of-the-year and forage fishes in the St. Lawrence River, Quebec. *Journal of Aquatic Animal Health* **11**, 275–282.

Marcogliese DJ, Dumont P, Gendron AD, Mailhot Y, Bergeron E and McLaughlin JD (2001) Spatial and temporal variations in abundance of *Diplostomum* sp. in walleye (*Stizostedion vitreum*) and white sucker (*Catostomus commersoni*) from the St. Lawrence River. *Canadian Journal of Zoology* **79**, 355–369.

Morissette O, Paradis Y, Pouliot R and Lecomte F (2018) Spatio-temporal changes in littoral fish community structure along the St. Lawrence River (Québec, Canada) following round goby (*Neogobius melanostomus*) invasion. *Aquatic Invasions* **13**, 501–512.

Moszczynska A, Locke SA, McLaughlin JD, Marcogliese DJ and Crease TJ (2009) Development of primers for the mitochondrial cytochrome *c* oxidase I gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminths. *Molecular Ecology Resources* **9**, 75–82.

Muzzall PM, Peebles R and Thomas MV (1995) Parasites of the round goby, *Neogobius melanostomus*, and tubenose goby, *Proterohinus marmoratus* (Perciformes: Gobiidae), from the St. Clair River and Lake St. Clair, Michigan. *Journal of the Helminthological. Society of Washington* **62**, 226–228.

Paterson RA, Townsend CR, Tompkins DM and Poulin R (2012) Ecological determinants of parasite acquisition by exotic fish species. *Oikos* **121**, 1889–1895.

Paterson RA, Rauque CA, Fernandez MV, Townsend CR, Poulin R and Tompkins DM (2013) Native fish avoid parasite spillback from multiple exotic hosts: consequences of host density and parasite competency. *Biological Invasions* **15**, 2205–2218.

Poulin R, Paterson RA, Townsend CR, Tompkins DM and Kelly DW (2011) Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. *Freshwater Biology* **56**, 676–688.

Pronin NM, Fleischer GW, Baldahova DR and Pronina SV (1997) Parasites of the round goby (*Neogobius melanostomus*) and tubenose goby (*Proterohinus marmoratus*) (Cottidae) from the St. Clair River and Lake St. Clair, Michigan, USA. *Folia Parasitologica* **44**, 1–6.

Reyjol Y, Brodeur P, Mailhot Y, Mingelbier M and Dumont P (2010) Do native predators feed on non-native prey? The case of round goby in a fluvial piscivorous fish assemblage. *Journal of Great Lakes Research* **36**, 618–624.

Rohr JR, Civitello DJ, Halliday FW, Hudson PJ, Lafferty KD, Wood CL and Mordecai EA (2020) Towards common ground in the biodiversity-disease debate. *Nature Ecology & Evolution* **4**, 24–33.

Stewart Merrill TE and Johnson PTJ (2020) Towards a mechanistic understanding of competence: a missing link in diversity-disease research. *Parasitology* **147**, 1159–1170.