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

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1. Introduction

When one considers the issue of exotic species and the risk they pose to ecosystem in terms of disease transmission, what generally springs to mind are the pathogens that these non-native species carry with them which could be introduced into the invaded habitats. Yet, despite numerous documented cases of co-introduced exotic parasites infecting resident species (Taraschewski, 2006; Lymbery et al., 2014), a growing number of studies suggest that the reverse situation - the invader being colonized by native parasites - is just as likely, if not more so, to occur over time (Torchin and Mitchell, 2004; Telfer et al., 2005; Gendron et al., 2012; Paterson et al., 2012; Sheath et al., 2015). As they integrate into the recipient food web, exotic species, especially those that spread and become invasive, may exert a significant influence on the transmission and population dynamics of native parasites (Telfer et al., 2005; Poulin et al., 2011; Flory et al., 2013; Young et al., 2017).

Depending on its competence as a host that permits the transmission of a given parasite, an exotic species could either act as a reservoir and amplify the abundance of that parasite among its
natural hosts, referred to as spillback (Kelly et al., 2009a) or, as a decay, leading to an overall loss of infection, also called the dilution effect (Kopp and Jokela, 2007). Both of these response patterns - parasite amplification and dilution - have been documented following the introduction of invasive species in taxonomically and geographically diverse systems (Kelly et al., 2009b; Hershberger et al., 2010; Mastitsky and Veres, 2010; Litvintseva et al., 2011; Paterson et al., 2011; Nelson et al., 2015; Ondrackova et al., 2015). However, host competence alone is not always a good predictor of the net effect of a non-native species in these systems, being edged out by demographic factors such as host density (Paterson et al., 2013b; Searle et al., 2016). When considering parasites with complex life cycles, the actual impact of an exotic host on infection dynamics is also likely to be driven by how different interspecific interactions (including competition and predation) develop over time between the exotic species and members of the recipient community. For instance, an invader could end up diluting a parasite, be it a competent host or not, if it downsizes the populations of its main first intermediate host by predation, or if it does not become a significant prey item in the diet of the main definitive host.

Most field evidence of parasite dilution or amplification induced by exotic species has been obtained by contrasting the abundance of parasites in natural hosts between invaded and non-invaded localities (Telfer et al., 2005; Kelly et al., 2009b; Lettoof et al., 2013), with limited knowledge of the pre-invasion levels of infection. In this study, a before-after approach was used to assess the influence of the Eurasian round goby (Neogobius melanostomus) on the transmission of parasites of the genus Diplostomum to native fish in the St. Lawrence River. These eye-dwelling digeneans, which commonly infected native fish in the St. Lawrence River before the introduction of the round goby (Marcogliese and Compagna, 1999; Marcogliese et al., 2001, 2006, 2010; Locke et al., 2013), were recently reported to have declined at localities where historic infection levels were previously very high (Désilets et al., 2013). To further document this apparent reduction in eyefluke abundance and clarify the role that the round goby may have played in it, infection levels in three native fish hosts, the spottail shiner (Notropis hudsonius), the golden shiner (Notemigonus crysoleucas) and the yellow perch (Perca flavescens), were compared before and after the goby’s establishment at sites where historical data on the parasite fauna of these fish species were available. The potential influence of the non-native goby was assessed while considering other key biotic and abiotic factors likely to modulate the transmission of eyeflukes from one host to the other. These include: (1) the hydrological conditions during the known periods of release of Diplostomum spp. cercariae and (2) the size of the populations of ring-billed gulls, which are the predominant definitive hosts for Diplostomum spp. in the St. Lawrence River.

Parasites of the genus Diplostomum have a three-host life cycle (Chappell, 1995). These digeneans use lymnaeid snails as first intermediate hosts, fish as second intermediate hosts, and gulls and terns as definitive hosts. Birds acquire the infection when they feed on infected fish. Worms mature and reproduce in their intestines and eggs are shed in feces. Miracidia hatch from eggs and infect the snails, which in turn release asexually-produced cercariae into the water. These free-living larvae penetrate the skin of fish and migrate to the eye, where they become metacercariae which are infective to the avian definitive hosts.

The genus Diplostomum is composed of numerous cryptic species (Locke et al., 2010, 2013), with at least fifteen occurring in the St. Lawrence River (Locke et al., 2015). Those establishing in the retina and vitreous humor of fish are more host-specific than lens forms (Locke et al., 2010; Blasco-Costa et al., 2014). Eyeflukes found to date in the round goby belong to a few generalist lens-dwelling species also common in the lens of the three native fishes considered herein (Désilets et al., 2013; Locke et al., 2015). The vast majority of non-lens forms in perch in the St. Lawrence River are D. baeri but other putative Diplostomum species also were found (Locke et al., 2010).

2. Material and methods

2.1. Sampling design

One-year old round gobies and spottail shiners were captured in June at two localities of the St. Lawrence River: Iles de la Paix (IPA: 4520.5740’N; 7350.9630’W) and Ilet Vert (IVT: 4542.4300’N; 7326.9600’W). The parasite load of one-year old fish collected in June reflects the infection acquired since the preceding fall and can thus be used unambiguously to follow year to year variations in parasite recruitment. At each sites, samples of both species were collected two years after the goby’s first sighting, in 2007 at IVT and in 2009 at IPA, and thereafter in 2011, 2013 and 2016 at both sites. Pre-invasion samples of 1-year-old spottail shiners obtained in June 1998, 1999, 2000 and 2004 (Marcogliese et al., 2006; Thilakarante et al., 2007) completed the chronological sequence. Additional data on Diplostomum spp. infection levels in native species along the St. Lawrence River were mined from the database on fish parasites maintained by the Department of Environment and Climate Change, which were collected for other research studies and have been published in part (Marcogliese et al., 2010; Désilets et al., 2013; Locke et al., 2013; Giraudo et al., 2016). Only samples comprising at least 15 fish of a given age class were considered for analyses. These include samples of 1 and 2-year-old perch and 1 year-old golden shiners collected between 1998 and 2016 from 6 sites situated within the three fluvial lakes of the St. Lawrence River: LSF-1 (45°09.7700’N; 74°25.514’W) and LSF-2 (45°07.646’N; 74°24.273’W) in Lake St. Francis, LSL-1 (45°24.415’N; 73°53.989’W) and IPA in Lake St. Louis and LSP-1 (46°12.000’N; 72°59.000’W) and LSP2 (46°8.895’N; 72°47.950’W) in Lake St. Pierre.

2.2. Fish capture and examination

Most fish were collected with a beach seine (22.6 m × 1.15 m; 3 mm mesh) towed on foot or partially deployed from a boat, with the exception of 2-year-old perch from Lake St. Pierre which were captured with fyke nets (in 2004 and 2005) or hoop nets (in 2013). Upon capture, fish were euthanized by immersion in a 400 mg/L Eugenol (clove oil) solution (American Veterinary Medical Association, 2013), placed in bags and frozen for subsequent analyses. Morphometric measurements (total, fork or standard lengths, to the nearest mm) and total weight (to the nearest 0.1 g) were obtained on thawed specimens. Minnows and gobies were divided into age classes using length frequency distributions. Perch were aged by counting the number of year marks (annuli) on their opercular bones or scales [see detailed methodology in (Marcogliese et al., 2005)]. Individuals of P. flavescens for which age was not available were a posteriori assigned to a given age class based on age-length curves generated for the species in each fluvial lake.

Parasitological examination followed standard procedures for macroparasites (Gendron et al., 2012). Parasite search was comprehensive and extended to the whole body with the exception of perch in 2011, 2013, 2014 and 2016 and golden shiners in 2011 for which only specific target organs/tissues were examined. In all instances, the left and right eyes of each fish were dissected out and their contents inspected under a stereomicroscope. The lens was detached from the vitreous humor and the eyeflukes were
identified to genus and counted. In spottail shiners, golden shiners and round gobies, eyeflukes were exclusively found in the lens whereas in perch they were primarily associated with the vitreous humor/retina, (less than 5% were lens dwelling forms). Subsamples of the parasites found were preserved in 95% ethanol for molecular analyses or fixed in ethanol 70% prior to being stained and mounted on slides. The taxonomic status of many species of eyeflukes being still unresolved (Locke et al., 2010, 2015), and in the absence of actual molecular data to ascertain the identification of each specimen, we refer to them collectively herein as Diplostomum spp.

2.3. Alternative explanatory variables

The effect of demographic changes in the predominant definitive hosts of Diplostomum spp. in the St. Lawrence River (Levy, 1997; Marcogliese et al., 2001) was evaluated using ring-billed gull counts in colonies along the St. Lawrence River from 1998 to 2012 that were accessed through the St. Lawrence Global Observatory-SLGO 2016 portal (www.ogsl.ca). More recent observations for Quebec populations (2016) and data relative to colonies in Ontario (west to Cornwall) were provided by the Canadian Wildlife Service.

To assess potential hydrological effects, mean daily water levels in the St. Lawrence River for the period ranging from January 1998 to September 2016 were extracted from the Department of Fisheries and Ocean archived hydrometric database (www.isdm-gdsi.gc.ca/isdm-gdsi/twl-mne/maps-cartes/inventory-inventaire-eng.asp). Data from Summerstown station were considered representative of Lake St. Francis where water levels are maintained stable throughout the year, whereas Montreal Jetty no 1 station was selected to represent the hydrological conditions of the river downstream from Lake St. Francis where water levels fluctuate.

2.4. Calculations and statistical analyses

To evaluate fish infection, the following parasitological descriptors were calculated (Bush et al., 1997): mean abundance, which is the mean number of parasites of a given taxon per fish in a sample, including uninfected fish; mean intensity, which is the mean number of individuals of a given parasite taxon in a sample, considering infected fish only; and prevalence, which is the proportion of parasitized fish in a given sample, expressed as a percent.

To account for overdispersion of data (variance being greater than the mean), a negative binomial model was fitted to the untransformed eyeflukes counts (using the GENMOD procedure of SAS). This model was used to test the effect of year and fish host for each sampling site. When more than two years were compared, differences between pairs were computed and a Tukey–Kramer adjustment of the p value was applied. For a number of historic samples or studies, an adjustment of the p value was applied. For a number of historic years and sites, a sharp decrease occurred two years after round gobies first established at that site (which has not been visited since 2006). A similar collapse in the parasite levels was observed across the three fluvial lakes in two-year old perch from 2011 to 2016 (Fig. 3), with the exception of one site in northern Lake St. Louis (LSL-1) where the round goby did not establish detectable populations (St. Lawrence Global Observatory-SLGO, 2016 portal, www.ogsl.ca). Mean abundance at LSL-1 in 2013 was elevated and not different from that in 2000 (P = 0.5800). The among-year change in abundance levels was significant but less marked at LSP-1, whereas the abundance of Diplostomum spp. after the goby’s detection (2013) was not statistically different (P = 0.1546) from the abundance at one previous year (2004). On the north shore of Lake St. Pierre, gobies, although present, remained in very low numbers compared to the south shore of the same fluvial lake (St. Lawrence Global Observatory-SLGO, 2016 portal, www.ogsl.ca).

Other than Diplostomum spp., metacercariae of ten trematode species transmitted by snails and using fish as second intermediate hosts were found in the tissues/organs of spottail shiners collected at IVT and IPA during the study period. When pooled, the abundance of these metacercariae was lowest in 1998 and 1999 and highest in 2013 at both sites (P < 0.0001) with considerable fluctuations between these two years. Fig. 4 illustrates the variation of abundance for the two most prevalent of these trematodes, Ornithodiplostomum spp. and Posthodiplostomum spp. Neither these species, nor the other metacercariae infecting spottail shiners, were found in any round gobies examined herein.

Metacercariae of Apophallus brevis commonly infect the flesh of perch in the St. Lawrence River. As shown in Table 1, the mean abundance of A. brevis in one-year old perch remained elevated at IPA across years (P = 0.1865) and significantly increased at IVT (P = 0.001) following the goby’s establishment, while perch infection by Diplostomum spp. decreased dramatically.

From 1997 to 2016, populations of ring-billed gulls downstream of Cornwall in the St. Lawrence River declined by approximately 36% (Fig. 5A). More than half of this reduction occurred after 2006. A number of nesting sites were completely abandoned over time. The largest known colony, located on lle Deslauriers close to IVT

3. Results

The abundance of Diplostomum spp. in one-year old spottail shiners at IVT and IPA varied significantly with year (P < 0.0001). At both sites, a sharp decrease occurred two years after round gobies were first observed in collections, that is in 2007 at IVT and in 2009 at IPA, and these low levels of infection were maintained up to 2016 (Fig. 1). Before the establishment of the round goby locally, eyeflukes were, by far, the most common helminths in juveniles of this native fish species. In some samples virtually all specimens examined were infected, the lowest prevalence being 97% at IPA and 80% at IVT during the pre-invasion period. During the post-invasion period, prevalence decreased to as low as 17% at IVT and 3% at IPA. From 2009 to 2016, the mean abundances of Diplostomum spp. were found significantly lower than in previous years at both localities as revealed by the post-hoc multiple comparisons (Fig. 1).

Diplostomum spp. were also the most frequent helminths found in one-year old round goby two years after that species’ first sighting. The parasite was however less abundant than in the native spottail shiner (Fig. 1), the difference between the two hosts being significant at IVT in 2007 (P < 0.0001). Parasite examinations, which were mostly performed on thawed fish, did not allow parasite viability to be determined, but only very rarely did we notice a metacercaria of Diplostomum spp. showing signs of degradation. However, a few gobies were examined shortly after being euthanized, and in all cases their eyeflukes were alive. From highs of 50% at IVT in 2007 and 30% at IPA in 2009, the prevalence of infection in gobies drastically dropped to less than 5% at both sites from 2011 to 2016.

As in spottail shiners, levels of infections reached record lows in one-year old perch and golden shiners in 2011 at LSP-1 (Fig. 2). Although considerable variations occurred prior to that year, Diplostomum spp. abundance in 2011 was significantly lower than in previous samples of both perch and golden shiners (P < 0.0001). The 2011 sampling coincided with the first record of gobies at that site (which has not been visited since 2006). A similar collapse in the parasite levels was observed across the three fluvial lakes in two-year old perch from 2011 to 2016 (Fig. 3), with the exception of one site in northern Lake St. Louis (LSL-1) where the round goby did not establish detectable populations (St. Lawrence Global Observatory-SLGO, 2016 portal, www.ogsl.ca).

Other than Diplostomum spp., metacercariae of ten trematode species transmitted by snails and using fish as second intermediate hosts were found in the tissues/organs of spottail shiners collected at IVT and IPA during the study period. When pooled, the abundance of these metacercariae was lowest in 1998 and 1999 and highest in 2013 at both sites (P < 0.0001) with considerable fluctuations between these two years. Fig. 4 illustrates the variation of abundance for the two most prevalent of these trematodes, Ornithodiplostomum spp. and Posthodiplostomum spp. Neither these species, nor the other metacercariae infecting spottail shiners, were found in any round gobies examined herein.

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From 1997 to 2016, populations of ring-billed gulls downstream of Cornwall in the St. Lawrence River declined by approximately 36% (Fig. 5A). More than half of this reduction occurred after 2006. A number of nesting sites were completely abandoned over time. The largest known colony, located on lle Deslauriers close to IVT
4. Discussion

From year to year throughout the entire study period.

Two gull colonies of the St. Lawrence River declined. Two (these populations were monitored on a decadal basis). Nevertheless, not all gull colonies of the St. Lawrence River declined. Two important populations, one on the south shore of Lake St. Louis (colony 2) and the other on the north shore of Lake St. Pierre (colony 3) have seen their numbers increased by respectively 30% and 20% from 2006 to 2016.

In Cornwall colonies, upstream of Lake St. Francis (colony 1), gull counts have fallen by 20% between 1999 and 2008 (these populations were monitored on a decadal basis). Nevertheless, not all gull colonies of the St. Lawrence River declined. Two important populations, one on the south shore of Lake St. Louis (colony 2) and the other on Île Lefèvre upstream of Lake St. Pierre (colony 4) have seen their numbers increased by respectively 30% and 20% from 2006 to 2016.

Fig. 5B illustrates the variation in water levels in early spring and late summer from 1998 to 2016 at two stations along the St. Lawrence River. Water levels in Lake St. Francis have been stable and did not show seasonal or yearly variations, whereas at the Montreal Jetty no. 1 station, spring highs and late summer lows fluctuated from year to year throughout the entire study period.

4. Discussion

Based on data collected over nearly two decades, this field study demonstrates that infection of native fish by eyeflukes (Diplostomum spp.) declined sharply throughout the St. Lawrence River in the years following the introduction of the invasive round goby. Analyses of the most likely explanatory factors (Fig. 6) strongly suggests that these two events which coincide in time are also causally related, and that the round goby clearly contributed to the collapse of this once widespread and abundant pathogen.

The alternate hypotheses attributing the eyefluke decline to a reduction in definitive host numbers or changes in hydrological conditions were not well supported. Between 2006 and 2016, while Diplostomum spp. abundance in fish dropped precipitously to very low levels, ring-billed gull numbers in the St. Lawrence River also experienced a marked decrease due in part to the control measures put in place to halt the expansion of this bird then considered a nuisance (Cotter et al., 2012). Given the implied potential reduction in the parasite’s reproductive capacity, the decline of the eyefluke’s main definitive host may seem a likely explanation for the reduction in fish infections. However, censuses show that the decrease in gull numbers had begun well before 2006 and that the species remained highly abundant across the St. Lawrence River up to 2016 (estimated as 73 000 individuals). Moreover, not all gull populations showed a declining trend; some sites where eyefluke abundance declined were located close to colonies which actually increased their numbers over the study period. Consequently, while the decrease in ring-billed gull populations has probably contributed to reducing the levels of fish parasitism by Diplostomum spp. in some areas, it cannot stand as the only and most satisfactory explanation for the observed phenomenon.

Hydrological conditions affect the ability of the free-living stages (cercariae) to reach and penetrate host fish (Moody and Gaten, 1982; Stables and Chappell, 1986; Marcogliese, 2001). However, there is no indication that the reduced infection levels reported herein can be attributed to unfavorable high water levels during critical time windows for cercarial recruitment. In fact, water levels in early spring and late summer fluctuated considerably from one year to the next with no apparent shift in seasonal pattern after 2006 nor any correlation with the abundance of Diplostomum spp. in fish. Furthermore, huge reductions in eyefluke counts were observed after 2006 in Lake St. Francis, where water levels have been stabilized since early 1960s by water control infrastructures, with annual fluctuations being limited to less than...
physid snails. Therefore, the decreased recruitment of *Diplostomum* spp. in fish could simply be symptomatic of the reduced abundance of the parasites’ first intermediate host (lymnaeids). In line with this hypothesis, we found that infection by *Apophallus brevis* which develops in hydrobiids (Miller, 1946; Sinclair, 1971), a gastropod family not affected by goby’s establishment (Kipp et al., 2012), remained stable in yellow perch or increased after the goby’s invasion. On the other hand, two common digeneans phylogenetically close to eyeflukes, *Ornithodiplostomum* spp. and *Posthodiplostomum* spp., did not decline in spottail shiners as would have been expected considering the goby-mediated depletion of physid snails (Kipp et al., 2012), their first intermediate hosts (Palmieri, 1975; Hendrickson, 1986). On the contrary, these two parasites have seen their abundance fluctuate over time, showing an increasing rather than a decreasing trend. Although temporal change is difficult to detect statistically in rare parasites, the same increasing trend was observed when all trematodes using snails and fish as intermediate hosts (except eyeflukes) were pooled together. Overall, *Diplostomum* spp. were both the only metacercariae declining after 2006 and the only ones acquired by the round goby.

To date, the round goby has been colonized by a limited number of generalist parasites in the St. Lawrence River, the most prevalent after *Diplostomum* spp. being *Neoechinorhynchus tenellus*, an acanthocephalan using gobies as paratenic hosts (Gendron et al., 2012). A large proportion of the cystacanths of *N. tenellus* died and degenerated rapidly in the tissues of the round goby suggesting that the exotic fish could act as a dead-end for that parasite (Gendron and Marcogliese, 2016). As of now, we have no evidence thereof for eyeflukes residing in the lens of gobies, all of which appeared intact. While it is true that eyeflukes are part of the parasite fauna of the round goby in Eurasia (Rolbiecki, 2006; Francová et al., 2011; Kvach and Winkler, 2011), the importance or the competence of the species as a host for *Diplostomum* spp. in its ancestral range is unknown. However, infection trials in which captive ring-billed gulls were fed each day for a 2-month-period with gobies collected in the St. Lawrence River resulted in only two adult worms established in the bird’s intestine, a particularly low yield compared to the tens of worms recovered in routine gull infection experiments using cercarial-exposed fish hosts (rainbow trout), especially considering that in the latter trials, gulls were exposed only once (A.R. Lapierre and J. Forest, Concordia University, Montreal, Canada, pers. com.). Such poor infection success could be attributed to either the low prevalence of *Diplostomum* spp. in the gobies used for these experiments or a reduced infectivity of the metacercariae they carry, or both.

Following the above, the dilution of *Diplostomum* spp. observed in the St. Lawrence River could have resulted, at least in part, from the integration of the round goby into the diet of the main definitive host. Ring-billed gulls are opportunistic omnivorous birds that feed on prey according to their availability and abundance (Cotter et al., 2012). A recent analysis of stomach contents of ring-billed gulls in Lake Michigan revealed that the round goby was the most frequent prey item (76%), replacing other native forage fish, notably during the gull’s post-breeding season (Essian et al., 2016). Assuming a comparable shift in the diet of gulls in the St. Lawrence River, it is reasonable to expect that they would have ingested fewer metacercariae of *Diplostomum* spp. than if they had fed exclusively on native fish, which were typically more heavily infected by eyeflukes than were gobies. This would lead in turn to a lowering of the abundance of adult *Diplostomum* spp., a decrease in the amount of eggs released in the aquatic environment, and therefore a general reduction in the recruitment of eyeflukes in all host fishes including the round goby itself. And the potential reduced infectivity of metacercariae from gobies could have enhanced this recruitment.

![Fig. 2](image-url). Variation in *Diplostomum* spp. mean abundance over years in Lake St. Francis (LSF-1) in (A) one-year old yellow perch (*Perca flavescens*) and (B) one-year old golden shiner. Data are expressed as mean number of metacercariae of the genus *Diplostomum* per fish including uninfected ones ± SEM. Significant differences among years within each locality are indicated by different letters above histograms. Black fish silhouettes within graph panels illustrate the occurrence of the invasive round goby among the fish captured at that site. See Fig. 3 for site location.
failure. Interestingly, a survey of the gastrointestinal parasite fauna of 25 ring-billed gulls in the region of Montreal in 2011 (Aponte et al., 2014) reported intensity of infection by *Diplostomum* spp. lower than that found before goby’s invasion in 1994 (Levy, 1997), although the prevalence of infection remained elevated (85%). Nonetheless, considering that ring-billed gulls can travel distances of up to 25 km (Belant et al., 1998), a shift in the composition of their diet can hardly explain why high eye flukes levels were maintained at the goby-free site in Lake St. Louis (LSL-1). Indeed, gulls foraging at that site would have visited surrounding areas as
Fig. 4. Temporal variation in parasitism of spottail shiners (Notropis hudsonius) by two trematodes (Diplostomidae) using snails as first intermediate hosts at Iles de la Paix (IPA) (A) and Ilet Vert (IVT) (B). Data are expressed as mean numbers of metacercariae (±SEM) of Ornithodiplostomum spp. (white circles) or Posthodiplostomum spp. (green circles) in the tissues of the native fish host. Samples with different lower- and upper-case letters have significantly different infection levels for Ornithodiplostomum spp. and Posthodiplostomum spp. respectively. For each site, the shaded area denotes the post-invasion period. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

| Parasite          | Site   | Year | Round goby | Abundance | Year effect |
|-------------------|--------|------|------------|-----------|-------------|
|                   |        |      |            | Mean      | SEM         | P value     |
| Apophallus brevis | IVT    | 2004 | no         | 11.63     | 1.98        | 0.185       |
|                   |        | 2016 | yes        | 14.67     | 3.01        |             |
|                   | IPA    | 2007 | no         | 3.33      | 0.57        | 0.001       |
|                   |        | 2016 | yes        | 7.78      | 0.92        |             |
| Diplostomum spp.  | IVT    | 2004 | no         | 6.52      | 1.58        | 0.0001      |
|                   |        | 2016 | yes        | 0.38      | 0.31        |             |
|                   | IPA    | 2007 | no         | NA        | NA          |             |
|                   |        | 2016 | yes        | 0.53      | 0.28        |             |

Fig. 5. Trends in ring-billed gull (Larus delawarensis) populations (A) and in water levels in the St. Lawrence River (B) during the study period (1998–2016). A: The solid black curve shows the variation over time of the total number of ring-billed gulls recorded along the St. Lawrence River from Cornwall to Trois-Rivières whereas dotted curves depict the change in gull counts in each of the main colonies within this area. The numbered bird icons match those shown in Figs. 1 and 3: Bird 1 – Cornwall; bird 2 – Beauharnois; bird 3 – Île Deslauriers; bird 4 – Île Lefebvre. B: Monthly mean water levels in April (green) and September (gray) at the Montreal Jetty no 1 station (solid lines) and at the Summerstown station (dashed lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
well, notably IPA, where gobies have become established and *Diplostomum* spp. have declined. Besides, the replacement of native fish by gobies in the diet of gulls should not only have induced a reduction in the abundance of *Diplostomum* spp. but also of *A. brevis*, which mature in the gastrointestinal tract of gulls (Levy, 1997; Aponte et al., 2014). However, *A. brevis* is known to have a much broader range of definitive hosts than *Diplostomum* spp. (Olsen, 1974; Hoffman, 1999; Kinsella and Forrester, 1999) and as such, would not have been expected to decrease to the same extent.

Alternatively, parasite dilution could have occurred if round gobies, which can reach high densities in shoreline habitats (Kipp et al., 2012), acted as physical sink and reduced encounters between competent native hosts and cercariae of *Diplostomum* spp. Free-living stages of eyeflukes are short-lived (Lyholt and Buchmann, 1996) and frequent encounters with non-competent fish can deplete their availability to competent hosts or damage them, reducing their success when attempting to penetrate these hosts. Cercariae could also have been eliminated prematurely, due to host-parasite incompatibility, in the tissues of gobies as they migrated to the eye, before they reach the low-immunity environment of the lens (Locke et al., 2010) leading to a dilution effect.

Similarly, native lungworms were suspected of succumbing to the immune response of invasive cane toads in Australia (Lettoof et al., 2013).

Encounter reduction by non-compatible or poor hosts has been documented in several studies involving trematodes transmitted through free-living stages (Johnson and Thieltges, 2010). Studying the transmission of the trematode *Ribeiroia ondatrae*, Johnson and Hartson (2009) found that when competent larval toad hosts were raised together with treefrog tadpoles (less suitable hosts), the total success of transmission of cercariae was greatly reduced, leaving few metacercariae available overall for transmission to the definitive bird hosts. Similarly, Thieltges et al. (2009) experimentally demonstrated that two introduced molluscs (*Crassostrea gigas* and *Crepidula fornicata*) filtered cercariae from the water column, leading to encounter reduction between the trematode *Himasthla elongata* and its native mussel hosts (*Mytilus edulis*) which, again, acquired much fewer infections than in the absence of these invasive species.

The mechanisms discussed above are not mutually exclusive and could be acting in concert totally or in part. However, in light of the discussion above, the infection loss through encounter reduction appears to more thoroughly explain our findings and could be a core driver of the collapse of *Diplostomum* spp. in the St. Lawrence River. Laboratory infection experiments evaluating the relative competence of the invasive round goby as a host for *Diplostomum* spp. are needed to further explore this hypothesis.

The presence of invasive species has sometimes led to a reduction in parasite loads of native species, as with lungworms in native frogs sympatric with invasive cane toads in recently invaded habitats.

**Fig. 6.** Potential mechanisms to explain the observed sharp decline of *Diplostomum* spp. infection in fish in the St. Lawrence River. Those involving a dilution effect induced by the exotic round goby (*Neogobius melanostomus*) appears in pale red rectangles with rounded corners. Other biotic or abiotic factors are displayed in blue rectangles. Gastropod illustration, representing lymnaeid snails, is a graphic art by Tracey Saxby, provided by the Integration and Application Network (IAN), University of Maryland Center for Environmental Science (www.ian.umces.edu/imagelibrary). The bird image, used to illustrate a ring-billed gull (*Larus delawarensis*), is a public domain clipart downloaded from www.openclipart.org. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
areas (Lettoof et al., 2013). However, despite accumulating evidence of exotic fish being poor hosts for native parasites (Paterson et al., 2011, 2013a; Gendron and Marcogliese, 2016), demonstrated cases of parasite dilution induced by introduced fish are few (Kelly et al., 2009b) and, to our knowledge, our study is the first to document such an effect potentially induced by the round goby. Studying the impact of the invasive brown trout in New Zealand streams, Kelly et al. (2009b) notably showed that abundances of three trematode species in two native species of fish were negatively correlated with the abundance of brown trout.

The dilution concept was first introduced to describe the protective role that species richness may have on animal community health and in turn, how biodiversity loss can mediate an increase in disease risk (Keesing et al., 2006). Hence, many view parasite dilution as one of the rare beneficial effects that exotic species may have on the communities they invade (Kopp and Jokela, 2007; Kelly et al., 2009b). For instance, in New Zealand streams the upland bully (Gobionymus breviceps) demonstrated an inverse relationship between hepato-somatic index and abundance of one species of trematode, which experienced a decline in the presence of invasive brown trout. Based on these results, the authors suggested dilution could have improved the general condition of native fish (Kelly et al., 2009b). Eyeflukes (Diplostomum spp.) are considered important parasitic pathogens in wild and farmed fish (Chappell, 1995). As metacercariae accumulate in the lens, visual acuity is impaired resulting in reduced feeding efficiency, emaciation and poor host growth (Chappell, 1995). In the St. Lawrence River, eyefluke-induced cataracts and blindness especially in benthic fish (such as catostomids and icthyids) frequently have been reported since the mid-1990s (Marcogliese and Compagna, 1999). If the low levels of eyefluke infection are maintained over the long term, we should see a reduction in these pathogenic effects.

Alternatively, given that parasites have direct effects on their hosts and indirect effects on species with which the host interacts (Prenter et al., 2004; Dunn et al., 2012), the collapse of a common pathogen may affect fish communities, their prey and predators in unpredictable ways. Furthermore, the reduction in Diplostomum spp. may impact the helminth communities of fish and other hosts in its life cycle through parasite-parasite direct competitive and indirect immune-mediated interactions (Dunn et al., 2012). Such interactions have been documented across parasite taxa (Lello et al., 2004; Telfer et al., 2010) and are not restricted to species inhabiting the same tissues or organ. In European perch (Perca fluviatilis), for example, infections with a larval cestode (Trienoporus nodulus) in liver have been associated with reductions in the gut-dwelling helminths Acanthocephalus lucii and Camallanus lacustris, presumably mediated via pathological or physiological processes (Morley and Lewis, 2017). In another study, two larval trematodes (R. ondatrae, Echinostoma trivolvis) infecting Pacific chorus frog (Pseudacris regilla) tadpoles were found to display apparent immune-mediated competition, each one reducing the intensity of the other (Johnson and Buller, 2011). Similarly here, the reduction in Diplostomum spp. infection in fish could have benefitted some other snail-transmitted trematodes for which abundance coincidently increased. Further research is needed to assess the net effect of the goby-mediated Diplostomum spp. decline in terms of disease risk and its effect on the St. Lawrence River ecosystem (Young et al., 2017).

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