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Host-switching among crabs: species introduction results in a new target host for native parasites

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ABSTRACT: Invasive species can introduce parasites to, and/or acquire new parasites from, novel regions, thereby greatly influencing community interactions, including symbiotic relationships involving parasites. Host-switching of native and non-native parasites could enhance or dilute parasite transmission and spread among hosts. We investigated the effect of host invasion on trematode parasitism in 2 Newfoundland (Canada) bays: one invaded by European green crabs *Carcinus maenas* and the other not yet invaded. To determine the influence of *C. maenas* on host–parasite relationships, we assessed trematode prevalence in 3 native hosts: 2 *Littorina* spp. snails and Atlantic rock crabs *Cancer irroratus* (first- and second-intermediate hosts for microphallid trematodes, respectively). We found no difference in trematode prevalence between the bays among the 4 host species. However, cyst abundance was significantly higher in *C. maenas* versus *C. irroratus* in the bay where the crab distributions overlap, while it was lower in *C. irroratus* in the invaded versus uninvaded bay, suggesting a dilution of infection in the native host. Sequencing data of microphallid trematodes detected 4 genetically divergent lineages: a cosmopolitan lineage found in all host species; 2 lineages dominant in *C. irroratus*, suggesting a native origin for the trematodes that now use *C. maenas* as an additional host; and 1 lineage represented just in *C. maenas* in Europe. This is the first study to demonstrate the magnitude of trematode infection in crab hosts in Newfoundland, including the commercially valuable native *C. irroratus*. Our results demonstrate the influence that species introductions can have on parasite life cycles in native systems under recent host invasion.

KEY WORDS: Invasion · Dilution · Trematode · *Carcinus maenas* · *Cancer irroratus* · *Littorina* · Newfoundland

1. INTRODUCTION

Marine communities involve a complex web of direct and indirect interactions among free-living species and those that are in intimate symbiotic relationships. Of the major symbioses, parasitism is particularly significant and can be a key driver of host ecology and evolution in systems where host health, survival, and reproduction are considerably impacted (Alvarez et al. 1995, Meißner & Bick 1999, Lafferty & Kuris 2009, Sures et al. 2017). Moreover, marine parasites can influence population and community dynamics by changing host densities, distributions, and interactions with competitors, predators, and prey (Marcogliese & Cone 1997, Mouritsen & Poulin 2002, Hudson et al. 2006, Ricklefs 2015). Marine parasites also form numerous links across multiple free-living species (especially parasites with complex life cycles; Rohde 2005), ranging from small benthic organisms to highly mobile vertebrate predators, making them some of the most influential organisms in marine communities. Yet investigations examining their strong ecological roles are often lacking in marine systems, likely because parasites

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are an inherently 'hidden' component of these systems. Even so, emerging research is beginning to recognize the importance of incorporating ecological principles into the study of both human and non-human parasites and diseases, given the inherent complexity that exists in many parasite life cycles, where multiple intermediate species may be involved (Buhnerkempe et al. 2015, Johnson et al. 2015).

Presently, community interactions among free-living and parasitic species are being modified, disrupted, or even augmented by the establishment of novel species into marine systems around the world (Ruiz et al. 1999, Prenter et al. 2004, Dunn & Hatcher 2015, Jackson 2015). These introductions are becoming increasingly frequent given progressively efficient and widespread global trade and shipping methods (Ruiz et al. 2000, Seebens et al. 2013) which have made species boundaries more fluid in recent decades (Byers & Pringle 2006, Zanolla & Andreakis 2016). Consequently, the community ecology and evolutionary biology of numerous marine biota are being influenced by novel and highly dynamic ecological forces and species interactions (Lavergne et al. 2010, Viard et al. 2016), including tightly linked symbiotic relationships (e.g. Jackson 2015, Allen et al. 2016). In fact, the presence or absence of symbiotic organisms such as parasites in an introduced range could have a strong impact on host abundance, distribution, and demographics (Torchin et al. 2001). Specifically, many introduced species see a significant reduction in parasite diversity in their introduced versus native ranges (Ross et al. 2010), even when similar-sized sample areas are compared (Blakeslee 2016). In some systems, such an escape from a host's native parasite burden could confer a fitness benefit (Torchin et al. 2001) or provide a competitive advantage (Aliabadi & Juliano 2002) to the introduced host, thereby contributing to its success in the novel region (e.g. Torchin et al. 2003, Prenter et al. 2004, Torchin & Mitchell 2004, Torchin & Lafferty 2009, Blakeslee et al. 2013). Yet this escape from parasites could diminish with time if introduction vectors remain active and/or if introduced species become competent hosts for native parasites (Torchin & Mitchell 2004, Torchin & Lafferty 2009, Kroft & Blakeslee 2016, Goedknegt et al. 2017). Recent work has shown that microparasites, like bacteria and protists, may be more readily acquired in, or spread to, introduced ranges (Bojko et al. 2018), while among macroparasites, certain taxa such as trematodes are more commonly detected in introduced regions (Blakeslee et al. 2013).

The establishment of introduced hosts and parasites may also impact native parasite life cycles. This is because native hosts could contract parasites carried with an introduced species, or a native host could contribute parasites that can infect a competent introduced host; i.e. 'host switching' (Hall et al. 2009, Kelly et al. 2009, Goedknegt et al. 2016, 2017). Here, we focus on 3 major ways in which host switching has been identified as an influential factor in marine host–parasite communities in an invaded region (Hall et al. 2009, Kelly et al. 2009, Goedknegt et al. 2016; Fig. 1). (1) Parasite spillover occurs when introduced parasites associated with introduced hosts infect (i.e. spill over to) native hosts, which then field greater infection intensities than the introduced hosts. (2) Parasite spillback occurs when an introduced species is a competent host for native parasites, and the presence of a new competent host increases parasite transmission among native hosts, enhancing overall infection prevalence and intensity in (i.e. spilling back to) the native hosts. (3) Parasite dilution occurs when native parasites begin to use competent introduced hosts, and the addition of novel hosts in the system decreases (i.e. dilutes) infection intensity in the native hosts.

Among marine macroparasites, digenean trematodes represent a key group to investigate host-switching in invaded communities. This is because trematodes commonly use organisms such as mollusks and crustaceans as intermediate hosts, and these are 2 of the most introduced taxa globally (Ruiz et al. 2000, Blakeslee et al. 2013). In turn, trematodes are the most represented metazoan parasite group in introduced regions (Blakeslee et al. 2013). Trematode life cycles typically include 2 or more hosts, in which the trematode cycles between asexual and sexual reproduction. Gastropods often serve as first-intermediate hosts, in which asexual reproduction results in the production of free-living larval stages (cercariae) that enter the water column to locate the next host. In a 3-host infection cycle, the cercariae encyst as metacercariae in a second-intermediate host, which may include an array of taxa (e.g. mollusks, crustaceans, polychaetes, fish). Trophic transmission via vertebrate definitive host predation (often shorebird, fish, reptile, or mammal) transfers the parasite to the definitive host gut, where the trematode matures and sexually reproduces. Trematode eggs are released into the environment through the definitive host's feces, where a snail may inadvertently ingest the eggs, or eggs may hatch in the environment and a ciliated miracidial

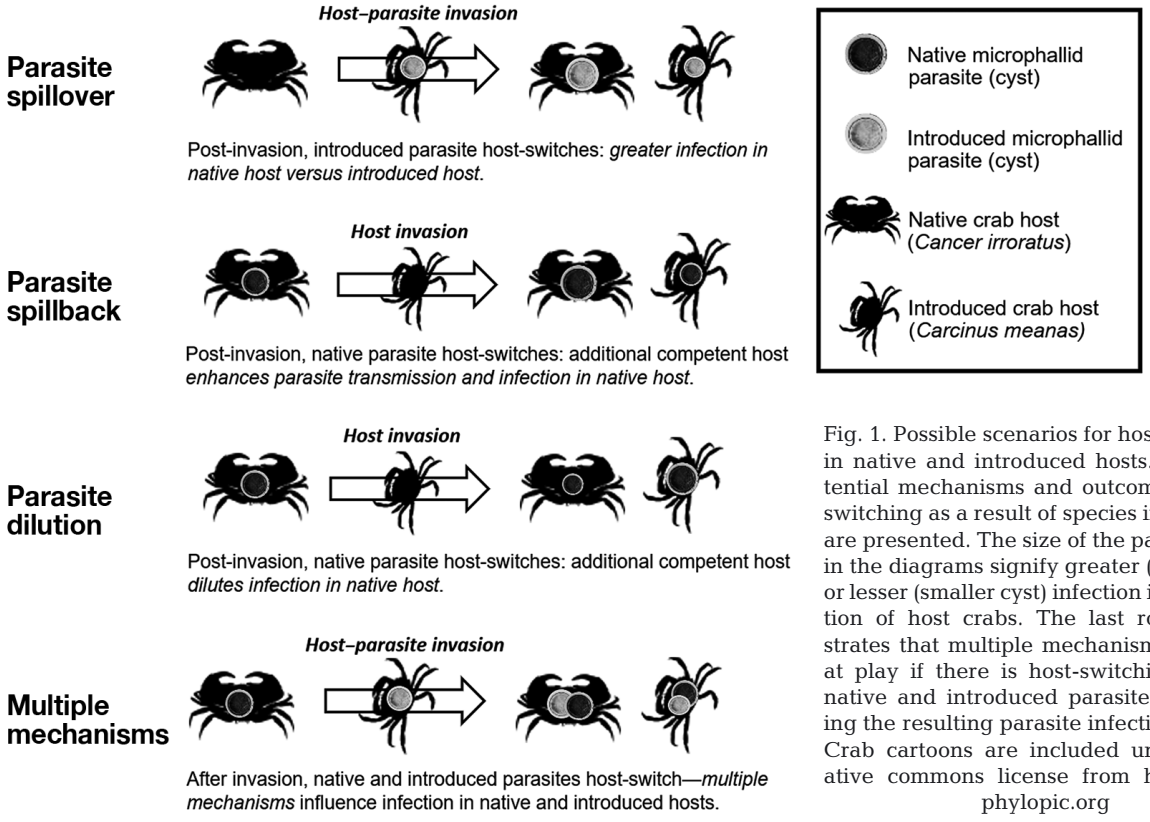

Fig. 1. Possible scenarios for host-switching in native and introduced hosts. Three potential mechanisms and outcomes of host-switching as a result of species introduction are presented. The size of the parasite cysts in the diagrams signify greater (larger cyst) or lesser (smaller cyst) infection in a population of host crabs. The last row demonstrates that multiple mechanisms could be at play if there is host-switching in both native and introduced parasites, influencing the resulting parasite infection in hosts. Crab cartoons are included under a creative commons license from http://www.phylopic.org

stage actively penetrates the snail host. The cycle then continues (Rohde 2005).

In this study, we focused on a coastal ecosystem (eastern Newfoundland, Canada) in which we could investigate the influence of a prominent introduced crab species (European green crab *Carcinus maenas*) on native trematode communities in populations where the invasive crab is present versus absent. We examined how the invader's presence or absence influenced trematode infection prevalence and abundance at 2 stages of the trematode life cycle: the first-intermediate *Littorina* spp. snail host stage and the second-intermediate crab host stage involving both the invasive crab and a native crab species (Atlantic rock crab *Cancer irroratus*). Collected parasite data were then used to evaluate patterns of parasite spillover, spillback, and/or dilution in the intermediate hosts (Fig. 1). In addition, because morphological identification of trematodes is challenging in second-intermediate hosts, we used molecular tools (18S rRNA barcoding marker) to resolve trematode identities among Atlantic populations of snails and crabs from Newfoundland, eastern USA, and the native range of *C. maenas* in Europe.

2. MATERIALS AND METHODS

2.1. Study system

Carcinus maenas is a globally invasive species (Carlton & Cohen 2003, Darling et al. 2008). Its oldest introduced range is eastern North America, where it is also an important host to trematode parasites (Torchin et al. 2001, Blakeslee et al. 2009). The eastern North American range of *C. maenas* first expanded into Placentia Bay, Newfoundland, in the early to mid-2000s, likely through intracoastal shipping from Nova Scotia (Blakeslee et al. 2010, McKenzie et al. 2010). Since then, the crab has continued to spread in coastal regions of the island, and it is now well established in an adjacent southeastern bay (Fortune Bay) as well as in a large swath of the western coast (DFO 2017, Lehnert et al. 2018). The crab is associated with numerous negative impacts in Newfoundland, including predation of local crabs and shellfish and competition with native crustacean species, as well as destruction and degradation of subtidal habitats (DFO 2009, Matheson & Gagnon 2012a,b, Matheson & McKenzie 2014, Matheson et al. 2016). Furthermore, as its range has expanded into ar-

eas crucial to the lobster fishery, there are major concerns of it filling lobster pots and competing with lobsters for food and shelter (Rossong et al. 2012, DFO 2017); in fact, dive surveys have confirmed spatial overlap between lobsters and green crabs in shallow subtidal ecosystems (K. Matheson unpubl. data). *C. maenas* also destroys large areas of eelgrass beds, an ecologically significant habitat for numerous species in the region (DFO 2009, Matheson et al. 2016). Of relevance to our study, the native rock crab *Cancer irroratus* is outcompeted by *C. maenas* for prey and shelter (Matheson & Gagnon 2012a,b), and local monitoring programs have observed decreased catches of *C. irroratus* in traps, likely due to predation by *C. maenas* or competitive displacement to deeper areas of the coastal zone (DFO 2011, K. Matheson & C. H. Matheson pers. obs.). Notably, following focused *C. maenas* mitigation harvests, native *C. irroratus* crabs have been observed to return to shallower nearshore waters (DFO 2011).

Prior research has found that 1 trematode species, *Microphallus similis*, infects *C. maenas* in eastern North America (Torchin et al. 2001, Blakeslee et al. 2009, 2015, Bojko et al. 2018). This microphallid species has also been identified in first-intermediate *Littorina* spp. snail hosts, particularly *L. saxatilis* and *L. obtusata* and rarely *L. littorea*, from Long Island, USA, to Newfoundland, Canada (Pohley 1976, Blakeslee & Byers 2008). In some populations, this trematode can be highly prevalent, particularly in crab hosts (e.g. as high as 100% of sampled crabs; Blakeslee et al. 2009). *M. similis* has also been documented in *C. maenas* in Europe, along with *M. primas*, which uses hydrobiid snails as first-intermediate hosts and *C. maenas* as a second-intermediate host (James 1968, Thieltges et al. 2009, Pina et al. 2011). Regarding *C. irroratus*, very little is known of its macroparasite infection. Only 1 study has investigated crab macroparasites in Nova Scotian waters and detected unidentified microphallid trematodes in both *C. maenas* and *C. irroratus* (Brattey et al. 1985).

2.2. Sampling

We selected 2 proximally close Newfoundland bays (Fig. 2; see Table 1) to examine microphallid prevalence and intensity in host snails and crabs. (1) Con-

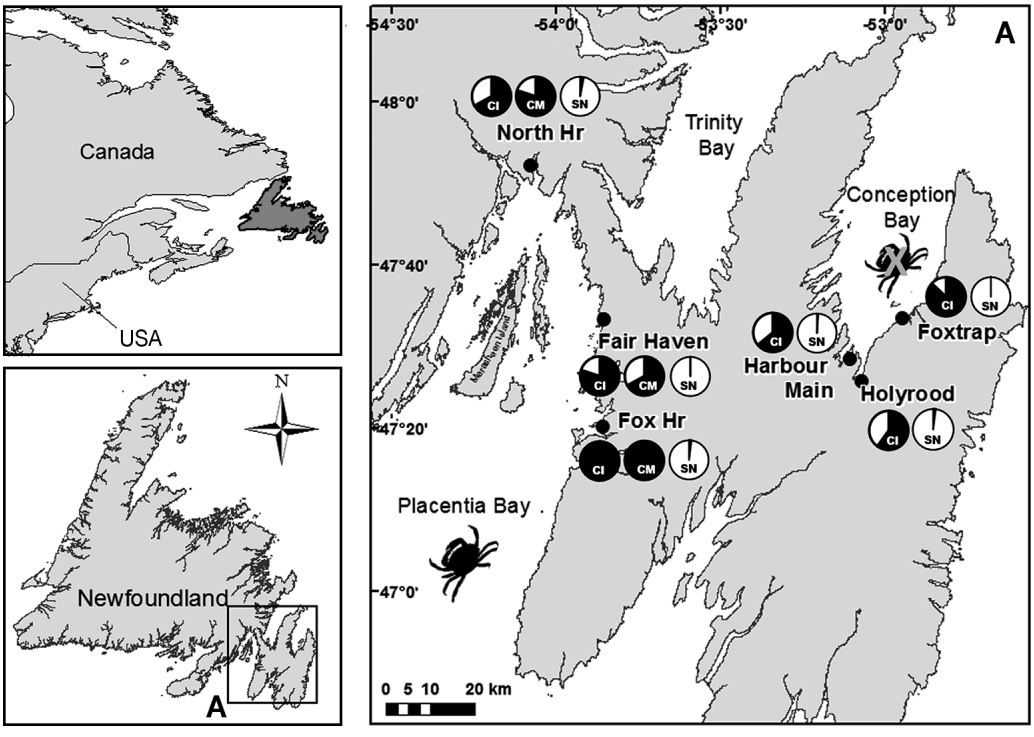

Fig. 2. Sampling site locations in Newfoundland, Canada, and prevalence of microphallid trematodes in *Cancer irroratus* (CI) and *Carcinus maenas* (CM) crabs and *Littorina* spp. snails (SN). In Placentia Bay, the crab image demonstrates the presence of *C. maenas* versus its absence in Conception Bay (crab crossed out). Black pieces in pie charts represent the proportion of infected crabs or snails; white represents uninfected. Prevalence values: North Hr = 67% (CI), 80% (CM), 4% (SN); Fair Haven = 80% (CI), 67% (CM), 0% (SN); Fox Hr = 100% (CI and CM), 2% (SN); Foxtrap = 87% (CI), 0% (SN); Harbour Main = 64% (CI), 1% (SN); Holyrood = 60% (CI), 2% (SN)

ception Bay, where *C. maenas* has not yet invaded, is located on the southeast corner of Newfoundland, approximately 20 km west of the capital city of St. John's. Three sites (Foxtrap, Holyrood, and Harbour Main) were sampled in this bay. (2) Placentia Bay is where *C. maenas* was first documented in 2007 (Klassen & Locke 2007, Blakeslee et al. 2010). This bay is also located in southeastern Newfoundland, approximately 90 km west of St. John's. Three sites were sampled (Fox Harbour, Fair Haven, and North Harbour, the latter being the oldest documented population; Klassen & Locke 2007). At each site, crabs were collected using baited Fukui traps attached to docks and left for approximately 24 h in the shallow subtidal (see Table 1). In Conception Bay, we collected 15 *C. irroratus* per site. In Placentia Bay, we attempted to collect 15 crabs per species per site. However, *C. irroratus* were less abundant in Placentia Bay, and at 1 of our sites (Fox Harbour), we were only able to collect 9 individuals. In contrast, *C. maenas* were extremely abundant in Placentia Bay, often completely filling traps (ca. 350 *C. maenas*) overnight (McKenzie et al. 2010, K. Matheson, C. H. McKenzie, & A. M. H. Blakeslee pers. obs.). In both bays, we also attempted to collect approximately 100 each of 2 periwinkle snail species (*L. saxatilis* and *L. obtusata*) serving as first-intermediate hosts. While we were unable to locate both species at all sites, we obtained at least 1 of the 2 species at every site (see Table 1).

2.3. Snail infection prevalence

Snails were measured in mm from the tip of the aperture to the tip of the spire using digital calipers. To identify characteristic swimming and movement behaviors of trematodes at the cercarial stage, live snails were dissected by cracking the shell with a hammer, extracting the snail from the shell, and placing it into a clear watch glass. The gonad and digestive regions were then torn open using sterilized forceps. The watch glass was scanned at 4× power under a stereomicroscope for parasites. Because trematodes at the redial/sporocyst stage go through asexual reproduction (Rohde 2005), mature infections are readily apparent. When snails were infected, trematode species were morphologically identified using published keys (e.g. James 1968, Werding 1969, Stunkard 1983). Prevalence of infection, species identities, and species richness were recorded for each site. It is not possible to record trematode abundance in snails because asexual reproduction at this stage produces clones that do not represent genetically distinct individuals. Occasionally, snails may harbor multiple infections of the same or differing trematode species, but the former is rare and the latter was not observed in our study. We examined patterns of infection prevalence in snails by assigning a (0,1) value for uninfected and infected snails, respectively. A total of 860 *Littorina* spp. snails were examined in this way over the 2 bays. We used a generalized linear mixed model (GLMM) (binomial probability distribution and logit link function) with bay, snail size, and the interaction of bay and snail size as fixed effects, with site nested in bay as a random effect. We combined the 2 *Littorina* species in statistical analyses because there were no significant differences in prevalence between them. In addition, the 2 species were of very similar average size and size range (*L. obtusata* and *L. saxatilis* mean ± SD = 7.46 ± 2.35 and 7.68 ± 2.55 mm; max size = 14.2 and 16.6 mm).

2.4. Crab infection prevalence and abundance

Crabs were euthanized by freezing at 0°C. This preservation technique does not adversely affect detection of trematode cysts in crab tissues and also allows for downstream genetic analyses. Prior to dissection, each crab was measured (maximum carapace width in mm) using digital calipers, and sex was determined. Crabs were then dissected by separating the upper from the lower carapace using a sterilized razor blade. A series of 8 tissue squashes (or 'snips') containing the amount of tissue needed to fill the area of a 22 × 22 mm glass cover slip were removed from each crab to capture a large subset of the crab's hepatopancreas (snips 1–6), gonad (1 snip), and ganglia (1 snip). We concentrated on the hepatopancreas because prior work indicated the greatest concentration of cysts in these tissues (Torchin et al. 2001, Blakeslee et al. 2009, 2015). Each slide was viewed under a compound microscope at 4× (10× oculars). The number of crabs infected out of the total number analyzed per sample site was then recorded (i.e. infection prevalence). In addition, the abundance of microphallid trematode cysts was enumerated per crab using a handheld tally counter. Parasite abundance was defined as cyst counts across all sampled individuals, including those individuals where no parasites were detected (i.e. abundance also includes zero values; Bush et al. 1997). We were interested in the effect the invasive crab may have on the presence or absence of metacercarial cysts in and across individuals (native and non-native), as well as how many cysts were detected

in infected crabs; thus the use of abundance as our measure of microphallid cyst counts allowed us to look at both of these aspects together. These cyst counts were collective across all microphallids, which are difficult to identify morphologically to species at the cyst stage. We therefore used genetic analyses in a subset of samples to confirm identities (see Section 2.5).

Cyst counts provided a raw measure of parasite abundance per crab based on 8 tissue snips. Thus, for comparative purposes, cyst abundance was analyzed as cysts per gram hepatopancreas for each of the 2 crab species, particularly given their differences in average size (carapace width mean ± SD = 87 ± 15 mm for *C. irroratus*; 61 ± 14 mm for *C. maenas*). The cysts per gram hepatopancreas value was estimated from an average number of cysts per snip (across the 6 hepatopancreas snips), which was then multiplied by the differential for a gram of hepatopancreas tissue using a standardized tissue weight per snip (0.116 grams) based on a number of prior data points (n = 75) (from Blakeslee et al. 2015 and crabs in this study).

Crab infection prevalence and abundance were analyzed in 2 main comparisons: (1) across the 2 bays for *C. irroratus* only (i.e. the native crab found in both bays), and (2) just within Placentia Bay, where both crabs are established. For (1), we used GLMMs, with sex and bay (and their interaction) as fixed effects, and site nested in bay as a random effect. For (2), we used GLMMs, with sex and species (and their interaction) as fixed effects, and site as a random effect. In both, prevalence GLMMs had a binomial probability distribution with a logit link function, and abundance GLMMs had a Poisson probability distribution with a log link function. Statistical analyses were performed using SPSS v26.

2.5. DNA sequencing, phylogenetic relationships, and haplotype networks

When microphallid cysts were detected in crabs during dissections, a subset was saved for DNA analysis (Table S1 in the Supplement at www.int-res.com/articles/suppl/m636p091_supp.pdf), including a total of 37 cysts from *C. irroratus* (14 from Conception Bay, 23 from Placentia Bay) and 24 cysts from *C. maenas* (all from Placentia Bay). We also included 2 microphallid samples found in *L. saxatilis* and *L. obtusata* snails from Placentia Bay. Additionally, for comparison to other North Atlantic regions harboring microphallid infections, we included samples detected in *C. maenas* and *C. irroratus* crabs and *L. saxatilis* and *L. obtusata* snails from 2 populations in the USA (Rye, New Hampshire, and Scituate, Massachusetts; R. B. Barnard unpubl.). We also sequenced microphallid samples from the native range of *C. maenas* in Europe, including metacercarial cysts detected in *C. maenas* from Texel (Netherlands), Cork (Ireland), and Esbjerg (Denmark), and from *L. saxatilis* and *L. obtusata* snails sampled from Portland (England), Tjarno (Sweden), and Moss (Norway; Table S1).

Trematode samples were placed into 1.5 ml tubes and frozen at −20°C until processing. DNA was extracted using a standard CTAB/chloroform/ethanol precipitation (France et al. 1996), and DNA concentration and purities were determined using a Nanodrop 8000 (Thermo Scientific). A 468 bp fragment of the 18S rRNA gene was amplified using primers that were designed from a *Microphallus turgidus* sequence in GenBank (accession no. EU825773.1): MTURG_18S_F (5′-ACG GAT ACG GGA CTC AAC AG-3′) and MTURG_18S_R (5′-TGG CAT CGT TTA TGG TCA GA-3′), under the following PCR profile: 95°C for 2 min; 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s; and 72°C for 5 min (Steinberg et al. 2008). PCR amplicons were purified using ExoSAP-IT™ (ThermoFisher Scientific), and purified amplicons were Sanger sequenced by Macrogen USA (Rockville, Maryland). Sequences were manually cleaned, inspected for ambiguities, and aligned without gaps to the *M. turgidus* reference sequence from GenBank using Geneious10.1.2 (Biomatters). To build our phylogenetic tree, we also included 4 additional microphallid sequences: (1) the *M. turgidus* sequence described above; (2) a sequence from *M. primas* (accession no. AJ287541.1), which infects *C. maenas* in Europe (James 1968); (3) a consensus sequence made from 10 individual sequences of cercariae/sporocysts sampled from *Gynaecotyla adunca* (A. M. H. Blakeslee unpubl.), which infects the mud snail *Tritia* (= *Ilyanassa*) *obsoleta* as its first-intermediate host (Hunter & Vernberg 1953, Blakeslee et al. 2012); and (4) a sequence from *Maritrema arenaria* (A. M. H. Blakeslee unpubl.), which we sampled from an *L. saxatilis* snail in Maine (USA) and was morphologically identified from its cercariae/sporocysts using published keys (Hadley & Castle 1940). This latter parasite infects *L. saxatilis* and *L. obtusata* as first-intermediate hosts and barnacles as second-intermediate hosts (Hadley & Castle 1940). We used *M. arenaria* as the outgroup to root our phylogenetic tree.

To determine trematode identities and to explore phylogenetic relationships among trematodes in crab and snail species in Newfoundland and also in com-

parison to Europe, Geneious10.1.2 (Biomatters) was used to first select the optimal nucleotide substitution model with JMODELTEST (Posada 2008), and then to create Bayesian phylogeny reconstructions (burn-in: 100 000; total chain length: 1 000 000) using MrBayes3.2.6 (Huelsenbeck & Ronquist 2001). PopArt (http://popart.otago.ac.nz/index.shtml) was used to graphically demonstrate haplotype networks (based on a TCS network) that were categorized by microphallids in crabs, Newfoundland bays, and those in USA and/or Europe. In addition, we used a Shannon diversity test to analyze haplotype richness and relative abundance to compare haplotype diversity among bays and crabs.

3. RESULTS

3.1. Snail infection prevalence

In *Littorina* spp. snails, prevalence of infection in Conception Bay was 0.00–0.04 for all trematode species (mean ± SE = 0.010 ± 0.006) and 0.00–0.02 for microphallid infection prevalence. In Placentia Bay, prevalence of infection was 0.00–0.08 for all trematode species and 0.00–0.04 for microphallid infection prevalence (0.020 ± 0.006; Table 1, Fig. 2). Bay was not identified as a significant factor affecting snail parasite prevalence (all trematodes: F = 0.919; p = 0.338; microphallid trematodes: F = 0.085; p = 0.771), but snail size significantly affected prevalence for all trematodes (F = 15.626; p < 0.001), with larger snails demonstrating greater prevalence of infection (Fig. S1). This same trend was not observed for microphallid trematodes (F = 0.003; p = 0.957), although this may have been a result of smaller sample size. There was no significant interaction between bay and size (all trematodes: F = 0.951; p = 0.330; microphallid trematodes: F = 0.176; p = 0.675).

3.2. Crab infection prevalence and metacercarial abundance

In crabs, prevalence of infection was 0.60–0.87 in *Cancer irroratus* at the 3 sites in Conception Bay (mean ± SE = 0.700 ± 0.083); 0.67–1.00 in *C. irroratus* in Placentia Bay (0.820 ± 0.097); and 0.67–1.00 in *Carcinus maenas* in Placentia Bay (0.820 ± 0.097; Table 1, Fig. 2).

Table 1. Site-level information on host sampling for each site within 2 bays in Newfoundland, Canada (Placentia Bay [PB] or Conception Bay [CB]). Columns include site name and ID; bay; number of samples; number of microphallids (micro.) detected during dissection and processing, either as metacercarial cysts in second-intermediate crab hosts or as sporocysts/cercariae in first-intermediate snail hosts; the proportion of microphallids out of all sampled individuals (Prev: micro.); average abundance of microphallid cysts in crab hosts; SD of microphallid metacercarial cysts in crab hosts; and the proportion of all trematodes infecting snail hosts, including microphallid and non-microphallid trematodes (Prev: all trem.). 'na' refers to the bay where *C. maenas* has not yet invaded; thus it was not possible to collect *C. maenas* crabs in that region; '–' refers to locations where we attempted to find both *Littorina* spp. snails but could only find 1 species at that location

Site name	Site ID	Bay	*Carcinus maenas*					*Cancer irroratus*				
			No. of samples	No. of micro.	Prev: micro.	Abundance Average	Abundance SD	No. of samples	No. of micro.	Prev: micro.	Abundance Average	Abundance SD
Fair Haven	FH	PB	15	10	0.67	2.53	3.89	15	12	0.80	10.20	19.11
Fox Harbour	FOX	PB	19	19	1.00	63.21	52.81	9	9	1.00	87.11	158.08
North Harbor	NH	PB	15	12	0.80	18.60	21.93	15	10	0.67	4.47	7.40
Harbour Main	HM	CB	na	na	na	na	na	14	9	0.64	72.89	183.73
Holyrood Marina	HLY	CB	na	na	na	na	na	15	9	0.60	68.89	78.06
Royal Newfoundland Yacht Club, Foxtrap	FT	CB	na	na	na	na	na	15	13	0.87	38.38	90.59

Site name	Site ID	Bay	*Littorina obtusata*				*Littorina saxatilis*			
			No. of samples	No. of micro.	Prev: micro.	Prev: all trem.	No. of samples	No. of micro.	Prev: micro.	Prev: all trem.
Fair Haven	FH	PB	47	0	0.00	0.00	100	0	0.00	0.01
Fox Harbour	FOX	PB	86	2	0.02	0.06	109	1	0.01	0.02
North Harbor	NH	PB	100	2	0.02	0.06	106	4	0.04	0.08
Harbour Main	HM	CB	72	1	0.01	0.01	83	0	0.00	0.00
Holyrood Marina	HLY	CB	–	–	–	–	85	2	0.02	0.02
Royal Newfoundland Yacht Club, Foxtrap	FT	CB	75	0	0.00	0.04	–	–	–	–

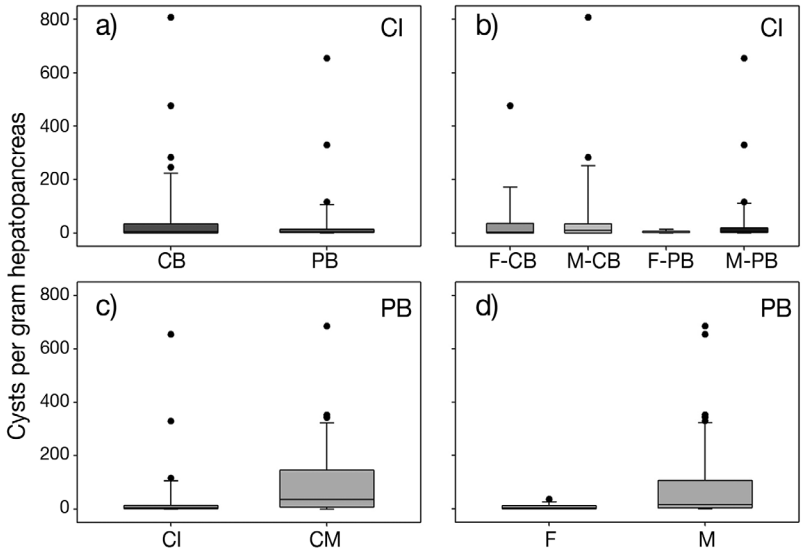

Fig. 3. Metacercarial cyst abundance of microphallid trematodes (cysts per gram hepatopancreas) in *Cancer irroratus* (CI) and *Carcinus maenas* (CM) in 2 Newfoundland bays: Conception Bay (CB) (where *C. maenas* is absent) and Placentia Bay (PB) (where *C. maenas* is introduced). (a) Comparison between the 2 bays for *C. irroratus* only; (b) comparisons of bay and sex for *C. irroratus* only; (c) comparisons between crab species in Placentia Bay; (d) comparisons of sex (both crab species combined) for Placentia Bay. F: female; M: male. Box plots display the 10th, 25th, 75th, and 90th percentiles as boxes and error bars, with the line representing the median. Individual dots represent outliers

Regarding microphallid infections in *C. irroratus*, prevalence did not differ between sex, host size, and bay, and there were no significant interaction terms (Table 1). In the metacercarial abundance GLMM, bay (F = 4.065, p = 0.047), sex (F = 139.173, p < 0.001), and the interaction between bay and sex (F = 58.041, p < 0.001) were all identified as significant factors, with microphallid abundance being higher in Conception Bay than Placentia Bay, and males in Conception Bay having higher abundance than females in Conception Bay (Fig. 3a,b).

When comparing microphallid prevalence between both crab species in Placentia Bay, we found no significant effect of any factor (sex, size, species) nor their interactions on microphallid trematode infection prevalence (all p > 0.50). However, in the abundance GLMM, both species (F = 15.141, p < 0.001) and sex (F = 101.245, p < 0.001) were identified as significant factors influencing cyst abundance in Placentia Bay (Fig. 3c,d), with *C. maenas* having higher metacercarial abundance than *C. irroratus*, and males having higher metacercarial abundance than females. The interaction between species and sex was not significant (F = 0.056, p = 0.813).

3.3. Haplotype network and phylogenetic analyses

We detected a total of 25 haplotypes in our genetic analyses (Table S1; GenBank acc. nos. MT025331-MT025355). Haplotype network analysis (Fig. 4) and a Bayesian phylogenetic tree (Fig. 5) identified 4 genetically distinct clades (A–D). Trematodes of Clades A and D were primarily hosted by *C. irroratus* (Clade A/D: 85/83% *C. irroratus*) and were rarer in *C. maenas*. In contrast, Clade B was split evenly between the 2 crab species (Clade B: 50% *C. irroratus*) and was the only microphallid trematode clade infecting the first-intermediate *Littorina* spp. snails. Thus, Clade B was cosmopolitan across locations, detected in all sampled regions (Newfoundland, USA, and Europe) and in all sampled organisms (both crab species and the *Littorina* spp. snails), while Clades A and D were primarily only found in microphallids infecting *C. irroratus*, with just a few occurrences in *C. maenas* (HAP11 in Clade A and HAP1 in Clade D). Finally, Clade C was only found in European *C. maenas*. Haplotypes within Clade A were 92–96% similar to haplotypes within Clade B, 91–93% similar to haplotypes within Clade C, and 94–97% similar to haplotypes within Clade D. Haplotypes within Clade B were 97–99% similar to haplotypes within Clade C, and 97–98% similar to haplotypes within Clade D. Haplotypes within Clade C were 96–98% similar to haplotypes within Clade D. In Newfoundland, Shannon diversity analyses revealed the greatest genetic diversity in Placentia Bay (1.82) where both crab species were present; the next highest was Placentia Bay for *C. irroratus* (1.56); then Placentia Bay for *C. maenas* (1.34); and finally Conception Bay for *C. irroratus* (0.66).

Among the haplotypes, HAP5 (Clade B) had the highest frequency across all locations and organisms. In the crabs, it was genetically matched to the trematode species *Microphallus similis* (James 1968). HAP6–10, 16, and 17 (also in Clade B) were several mutational steps away from HAP5 (Fig. 5), and similarities were 99–100% among them, thus likely representing genetic variants of *M. similis*. In contrast, HAP15 was found only in crabs from Europe (the Netherlands and Denmark) and had the closest match to a sequence from GenBank for the micro-

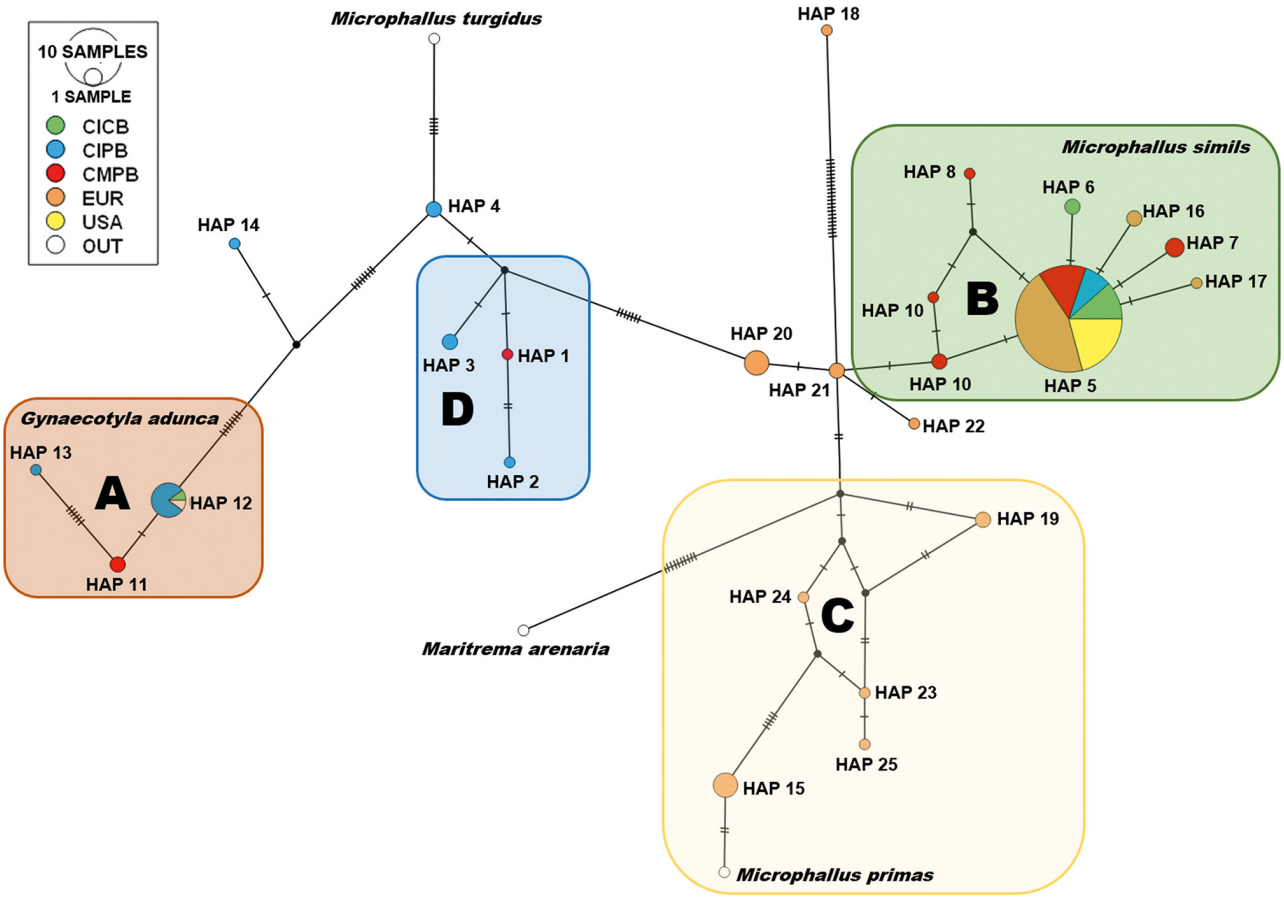

Fig. 4. TCS haplotype network of a 468 bp fragment of the 18S rRNA gene of microphallid trematodes infecting *Cancer irroratus* and *Carcinus maenas*. Circle size represents the number of occurrences for that haplotype (per key in left corner). Colors represent crab species and locations: CICB: *C. irroratus*, Conception Bay; CIPB: *C. irroratus*, Placentia Bay; CMPB: *C. maenas*, Placentia Bay; EUR: Europe (Netherlands, Denmark, and Ireland for microphallids infecting *C. maenas* and Sweden/Norway for microphallids infecting periwinkle snails); USA: US samples from Massachusetts and New Hampshire (infecting *C. maenas* and *C. irroratus*; see Table S1). All *Littorina* spp. snails occur in Clade B (see Table S1). White circles represent other microphallid trematodes including *Microphallus primas*, *M. turgidus*, *Gynaecotyla adunca*, and *Maritrema arenaria* (root). Letters (A–D) represent the 4 major clades detected in the analyses

phallid species *M. primas*. HAP19, 23, 24, and 25 were also part of the *M. primas* clade. HAP11–13 (in Clade A) matched most closely to the microphallid species *Gynaecotyla adunca*, which uses the mud snail *Tritia obsoleta* as its first-intermediate host (Hunter & Vernberg 1953). Of the remaining haplotypes, HAP1–3 (in Clade D) are multiple mutational steps away from the microphallids described above and do not have an identified match to known microphallid species. HAP4 is genetically closest (96% similarity) to a sequence for *M. turgidus*, which uses hydrobiid snails as first-intermediate hosts (Pung et al. 2008). HAP18 is a microphallid detected in 1 *L. saxatilis* snail in Sweden and is a microphallid taxon genetically distinct from all others detected in our analysis. HAP20–22 were sequences detected in Europe only (Ireland) in *C. maenas* (Fig. 4) but are outside of these 4 described clades, although they are genetically closest to the haplotypes in Clades B and C. Altogether, *M. similis* (Clade B) made up 71% of the 123 sequences included in genetic analyses; the remainder were 9% *Gynaecotyla adunca* (Clade A), 6% *M. primas* (Clade C), and 14% unknown/unidentified (Clade D, or outside the 4 clades) (Table S1).

4. DISCUSSION

Our study highlights the influence of non-native species on invaded communities through alterations in parasite life cycles. With the addition of novel hosts, native parasites may be able to host-switch and use native and non-native hosts alike, and this may positively or negatively alter the abundance and

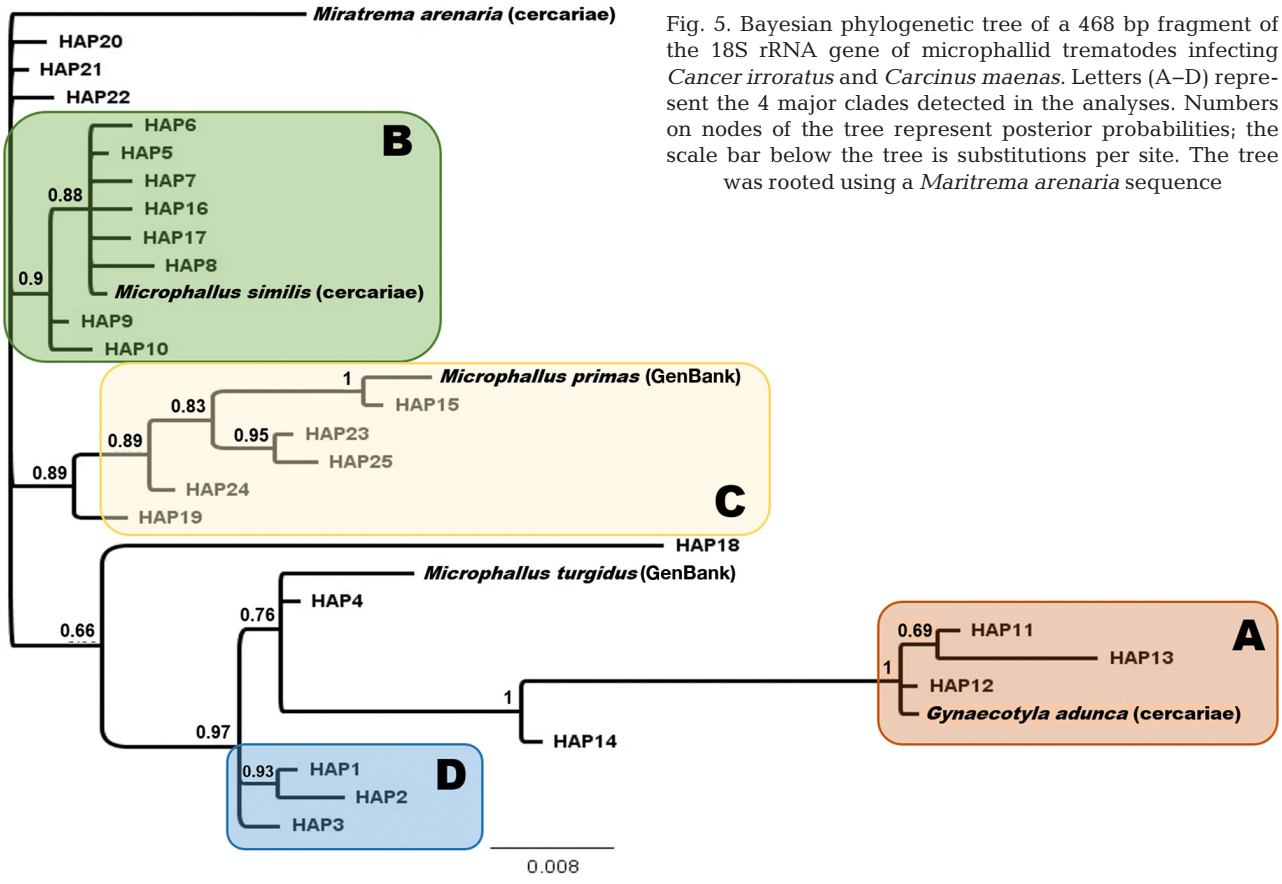

Fig. 5. Bayesian phylogenetic tree of a 468 bp fragment of the 18S rRNA gene of microphallid trematodes infecting *Cancer irroratus* and *Carcinus maenas*. Letters (A–D) represent the 4 major clades detected in the analyses. Numbers on nodes of the tree represent posterior probabilities; the scale bar below the tree is substitutions per site. The tree was rooted using a *Maritrema arenaria* sequence

diversity of parasites in invaded communities (Fig. 1). In this study, we examined the effect of invasive *Carcinus maenas* on trematode prevalence and abundance in first- and second-intermediate hosts (Fig. 2). The existence of proximally close sites differing in crab host composition provided an opportunity to detect host-switching in the invaded region and investigate the implications for native host communities. In our investigation, we found similar prevalence in the 2 crab species in Placentia Bay, and also between Conception and Placentia Bay for *Cancer irroratus*, but analyses of metacercarial cyst abundance demonstrated that crab species and bay strongly influenced host infection. Specifically, *C. maenas* had greater metacercarial cyst abundance than *C. irroratus* in Placentia Bay, while *C. irroratus* had lower cyst abundance in Placentia versus Conception Bay. Moreover, the presence of *C. maenas* enhanced microphallid genetic diversity in Placentia Bay. Taken together, these results suggest that while *C. maenas* has not indirectly affected the proportion of *C. irroratus* infected by microphallid trematodes in Placentia Bay, it has perhaps lessened the abundance of those cysts in the native crab while also enhancing the genetic diversity of its microphallids. Below, we further discuss these major findings by exploring the evidence for host-switching in the region, the implications of prevalence in first- and second-intermediate hosts, how host sex plays a role, and what evidence exists for signatures of parasite spillover, spillback, and/or dilution in the invaded and uninvaded communities (Hall et al. 2009, Kelly et al. 2009, Goedknegt et al. 2016). Finally, we discuss the implications to Newfoundland communities given the continued spread of *C. maenas* throughout the region.

4.1. Evidence for host-switching

Genetic analyses demonstrated clear overlap in microphallid lineages between the crab species, indicating that they are serving as intermediate hosts for many of the same microphallid trematodes in the region. Although non-native species often escape parasites when they are introduced to new locations (e.g. Torchin et al. 2002), *C. maenas* is clearly serving as a competent host in Placentia Bay, including to native North American microphallids (see Section 4.4). In fact, 3 lineages were identified as being shared between the crabs (Clades A, B, and D; Figs. 4 & 5).

Clade B was the most commonly observed, and 1 haplotype (HAP5) had the highest frequency of all haplotypes and was also ubiquitous across crab species, *Littorina* spp. snails, and region (both Newfoundland bays, USA, and Europe; Fig. 4, Table S1). Using published keys of cercariae infecting *Littorina* spp. (James 1968, Werding 1969, Pohley 1976, Stunkard 1983) paired with genetic evidence, we identified HAP5 (along with its variants: HAP6–10, 16, 17) as *Microphallus similis*. This species is a common trematode found on both sides of the Atlantic and is one of the most prevalent parasites infecting *C. maenas* in both regions (James 1968, Elner & Raffaelli 1980, Blakeslee et al. 2009). Because *M. similis* is cosmopolitan across the Atlantic, including North Atlantic islands such as Iceland (Skirnisson & Galaktionov 2002), we cannot determine whether there has been trans-Atlantic gene flow either naturally via mobile definitive bird hosts (e.g. Miura et al. 2006), or anthropogenically as a result of the introduction of *C. maenas* to North America. However, if so, it is possible that the other native hosts in eastern North America (*L. obtusata*, *L. saxatilis* snail hosts, and shorebird hosts such as *Larus* spp. gulls) may be harboring genetic variants of *M. similis* that originated from Europe. The use of a less conserved marker or genome-wide approach could help resolve this question.

In contrast to the cosmopolitan Clade B, haplotypes from Clades A and D were only found in North America, primarily infecting the native crab *C. irroratus* and more rarely *C. maenas*. Given the preponderance of these lineages in the native crab host and their lack of detection in Europe, it seems likely that these trematodes are native to North America and have host-switched to *C. maenas*. In fact, Clade A matches with an identified microphallid species, *Gynaecotyla adunca* (A. M. H. Blakeslee unpubl.), which is only found in northeastern North America and uses the eastern mudsnail *Tritia obsoleta* as a first-intermediate host (Hunter & Vernberg 1953). The identification of this trematode species was confirmed based on cercarial morphology from infected *T. obsoleta* (Hunter & Vernberg 1953) and genetic data for both the cercariae/sporocysts from the snail and metacercarial cysts from crustacean intermediate hosts (A. M. H. Blakeslee unpubl.). While *T. obsoleta* has a broad North American range (from north Florida to Atlantic Canada), we could not find records of it in Newfoundland. However, a closely related mud snail, *T. trivittata,* is reported from Newfoundland (Malacolog 4.1.1, A Database of Western Atlantic Marine Mollusca, Academy of Natural Sciences; www.malacolog.org). Trematode surveys of *T. trivittata* are needed to confirm whether it hosts *G. adunca* in Newfoundland. Clade D, on the other hand, represents a microphallid lineage that has not yet been identified. In our phylogenetic analysis, the genetically closest trematode species to Clade D is *M. turgidus*. This trematode uses hydrobiid snails as first-intermediate hosts and numerous crustaceans as second-intermediate hosts (Pung et al. 2008, A. M. H. Blakeslee unpubl.). While to our knowledge *C. irroratus* has not been identified as a host of this trematode, very little parasite information exists for this crab generally. Again, comprehensive surveys and further genetic analyses could help resolve this issue.

The final trematode lineage (Clade C) we detected was only found in Europe, based on trematode surveys in *Littorina* snails and *C. maenas* collected from Denmark, the Netherlands, and Ireland. This lineage genetically matched the trematode *M. primas* (Stentiford & Feist 2005), a species that uses hydrobiid snails as first-intermediate hosts and *C. maenas* as a second-intermediate host (James 1968, Thieltges et al. 2009, Pina et al. 2011). We did not detect any evidence of *M. primas* in either *C. maena*s or *C. irroratus* in Newfoundland or the 2 USA locations; thus if there has been transfer of parasites with *C. maenas* to North America from Europe, this species does not seem to have been introduced, although greater genetic surveying throughout the introduced range of *C. maenas* is needed to confirm this.

4.2. Infection prevalence in snails versus crabs

We did not find any evidence for differences in snail infection prevalence between the Newfoundland bays. However, there is very little understanding of what microphallid parasite infection prevalence or diversity may have looked like prior to the introduction of *C. maenas* to Newfoundland. One study conducted prior to the crab's invasion (Blakeslee & Byers 2008) examined trematode infection prevalence in *Littorina* spp. snails in Conception Bay in 2003. That study found a 4% infection prevalence of microphallids in *Littorina* spp. versus a 1% infection prevalence in our 2016 data. Unfortunately, we could not find any prior evidence for trematode infection prevalence in *Littorina* spp. from Placentia Bay, where *C. maenas* is now established. We therefore cannot assess whether trematode prevalence has changed in first- or second-intermediate hosts over time. In our 2016 sampling, *Littorina* spp. prevalence ranged from 0–3% in Placentia Bay, and this overlaps with prevalence in Conception Bay (0–2%) where *C. maenas* is absent. Past

studies have shown that prevalence in snails (including *Littorina* spp.) can be remarkably spatiotemporally constant, which may reflect consistent habitat usage patterns in definitive hosts such as shorebirds (Thieltges et al. 2013, Byers et al. 2016). Additionally, strong links have been identified between first-intermediate (snail) host prevalence and richness with definitive host abundance (Hechinger & Lafferty 2005, Thieltges et al. 2013), but there may be less perceived influence from second-intermediate hosts in this system (Byers et al. 2008). Thus, if definitive host usage patterns have remained relatively consistent through time and with the introduction of *C. maenas*, this could help explain the similarity in prevalence we have observed in the snail first-intermediate hosts in Newfoundland.

Infection prevalence was approximately 41 times lower in the snail versus crab hosts (Fig. 2), with some sites at 100% infection prevalence in both crab species. Although a small proportion of snails may be infected at a site, the 'reproductive firepower' (Rohde 2005) that occurs at the snail stage, where the trematode goes through asexual reproduction and can produce thousands to potentially millions of cercariae over a snail's lifetime, can result in a very large number of parasite propagules being emitted into the water column. Many of these propagules become part of the energy flow of marine systems, but many are still likely to successfully infect second-intermediate hosts (Thieltges 2007, Thieltges et al. 2008). This likely explains the major differences in prevalence we observed between upstream and downstream hosts. In contrast, we do not believe these differences are due to sampling bias because the sample size was much higher for the snails than for the crabs (~100 snails versus ~15 crabs).

4.3. Influence of host sex

Interestingly, the sex of host crabs was identified as an important factor influencing metacercarial cyst abundance (Fig. 3). In general, males demonstrated higher cyst abundance than females regardless of species or bay. Male-biased infection intensity and prevalence has been observed in other organisms that possess an immunosuppressant effect of testosterone versus female hormones which may boost immunity (Grossman 1985, Klein 2004). Yet this same pattern was not observed in a meta-analysis of studies (n = 33) examining infection intensity and sex-bias in arthropod hosts that lack testosterone (Sheridan et al. 2000). For example, microphallid infection was not influenced by host sex in the amphipod *Paracalliope novizealandiae* in New Zealand (Bryan-Walker et al. 2007).

Recent evidence for *C. maenas* in Newfoundland has revealed that males mature at smaller sizes than females and are on average larger than females (Best et al. 2017). In our investigation, male *C. maenas* were on average 11 mm larger than females (males: 62.6 ± 14.8 mm; females: 51.5 ± 4.0 mm), and male *C. irroratus* were on average 14 mm larger than females (males: 91.5 ± 14.5 mm; females: 76.9 ± 8.2 mm). Although size was not an influential factor of microphallid prevalence in crabs, it could potentially play a role in resulting metacercarial cyst abundance, if infective cercariae home in on chemical cues of aggregated cysts (Fried 1986, Haas et al. 2002, Haas 2003, Neal & Poulin 2012). Alternatively, our results may reflect differences in sample sizes (females n = 30; males n = 102) and habitat usage between the sexes. Regarding the latter, our collection methodology relied on baited traps, which may influence sex-biased sampling if males are more likely to be captured by traps than females, or if the sex ratio in the vicinity of the traps was biased towards males. Few studies have yet uncovered the mechanisms leading to sex-biased infection in crustaceans, thus requiring further study in this system and more generally.

4.4. Spillover, spillback, or dilution effect?

Our data appear to support a parasite dilution effect (Fig. 1) following the introduction of *C. maenas* to Newfoundland. This is because *C. irroratus* demonstrated lower abundance of microphallid cysts in Placentia Bay compared to Conception Bay where *C. maenas* is absent. Prior studies have shown that enhanced community diversity, including the addition of new species, can mediate infection levels in organisms within those systems, sometimes resulting in an indirect positive effect on native individuals by reducing their parasite loads (Johnson & Thieltges 2010). In this system, the presence of *C. maenas* may be the cause of reductions in infection abundances in *C. irroratus* in the bay where the crabs overlap, versus where it is absent. A few mechanisms may explain this result. (1) The population explosion of *C. maenas* in the region (e.g. a mitigation harvest removed 23 t of *C. maenas* in fall 2017; C. H. McKenzie & K. Matheson unpubl.) may have made it the more prominent host for microphallid cercariae to locate and infect, thus enhancing the use of *C. maenas* as a host in the region. (2) Related to this point, evidence

suggests that *C. maenas* has displaced *C. irroratus* from the lower intertidal and shallow subtidal waters where *C. maenas* is abundant, pushing the native crab into deeper subtidal waters (C. H. McKenzie & K. Matheson unpubl.). Because first-intermediate snail hosts are located within the intertidal zone, the displacement of *C. irroratus* to lower waters may expose the species to fewer trematode propagules than *C. maenas*. (3) The recent introduction of *C. maenas* may make it more susceptible to infection due to its naivety to native North American microphallids; such a phenomenon has been observed in other invasive crustacean hosts (Keogh et al. 2017). (4) Inherent ecological differences may exist between the 2 bays, such that *C. irroratus* is naturally less infected in Conception versus Placentia Bay. A potential argument against this latter explanation is that prevalence of microphallid infection in first-intermediate snail hosts was observed to be fairly similar between the bays.

Our results may also reveal some evidence of parasite spillover, thus demonstrating that multiple mechanisms may be at play in the region. Based on our genetic evidence, host-switching has occurred, particularly the utilization of *C. maenas* as a host for North American microphallid lineages. It is unclear, however, whether there has been any movement of trematode genotypes or taxa from *C. maenas* to *C. irroratus* since we cannot detect gene flow across the Atlantic with the genetic marker used here. Yet, it is interesting that genetic diversity was highest in Placentia Bay where both crabs are present, possibly suggesting that along with a dilution effect, we may also be observing some signature of parasite spillover (Fig. 1), where the presence of *C. maenas* has enhanced trematode genetic diversity in the invaded bay, with its parasites spilling over to native hosts. Such a cryptic spillover (i.e. *C. maenas* transferring cryptic alleles/lineages upon introduction that host-switched to *C. irroratus*) may be possible, particularly for Clade B (notably HAP5), which is cosmopolitan across all hosts and regions, as discussed above (Fig. 4). Again, a less conserved marker or genome approach could resolve this question.

One final consideration is that definitive hosts are a vital component to trematode life cycles, and a suitable intermediate host could become a parasite sink if transmission to the definitive host is unsuccessful or the intermediate host is less suitable (Krakau et al. 2006, Koppel et al. 2011, Paterson et al. 2013). In the system we describe here, both crab species are readily consumed by *Larus* spp. gulls — highly prevalent definitive hosts for the microphallid trematodes described here (James 1968, Pohley 1976, Byers et al. 2008). However, evidence suggests that *Cancer* spp. crabs are a more preferable prey species for *Larus* spp. than *C. maenas* in northeastern North America, which may be due to greater avoidance and hiding behaviors by the non-native versus native crab (Ellis et al. 2012). This could suggest that microphallid trematodes are more likely to complete their life cycles in native *Cancer* spp. crabs than in *C. maenas*. Yet *C. maenas* is now one of the most abundant crabs in Placentia Bay, likely providing numerous opportunities for definitive host transmission; it also demonstrates greater metacercarial cyst abundance than *C. irroratus* in Placentia Bay (Fig. 3). Further sampling of North American bays with and without *C. maenas* would help determine whether our results are reflective of other invaded versus uninvaded populations. Unfortunately, without historical trematode infection data in *C. irroratus*, we cannot directly compare trematode prevalence, abundance, and diversity pre and post invasion in *C. maenas*.

4.5. Conclusions and significance

Our study enhances understanding of the role that species invasions have in affecting native communities, not just the free-living species but also those, like parasites, that are less visible but still highly impactful. This investigation also represents the first to demonstrate the magnitude of microphallid infection in crab hosts in Newfoundland, including the commercially valuable native crab *C. irroratus*, which is being strongly affected by the presence of *C. maenas* in locations where the crabs overlap. Such studies are vital to our understanding of species invasions and parasite life cycles, which can ramify through whole ecosystems. Moreover, studies like ours are important to consider for risk assessments of invasive species, given that non-native hosts could indirectly affect the ecology and health of native communities by altering parasite dynamics in the region. In fact, the spatial utilization of habitat by a native host may be altered by the introduction of a non-native competitor, thereby impacting the infection cycles of parasites with complex life cycles and changing the prevalence and infection intensities of competent hosts in the community. Such a scenario may have occurred in Placentia Bay, Newfoundland, where the presence of a non-native host may be diluting the infection abundance of the native host. Continued explorations of trematode diversity in Newfoundland would be highly valuable to understanding its impact across multiple scales, especially

as the invasive crab continues to spread throughout the island. In general, the continued spread of introduced species and their associated diseases and parasites to regions beyond their natural boundaries is of increasing concern, especially given the limited or lack of evolved defenses to non-native parasites and pathogens in many of these systems.

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