The role of helminth parasites in host invasion: a freshwater fish system

by

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Declaration

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Summary

The incidence of species invasions have increased steadily during the past century and is continuing to rise, becoming a major threat to the biodiversity and functioning of native ecosystems. Accordingly, research output on biological invasions has grown rapidly in recent decades but research on the role of parasites in invasions remains comparatively understudied. Parasites comprise a biodiverse and highly-connected component of ecosystems, capable of mediating the effects of invasive species on native hosts. Here, I use a freshwater fish study system of native brown trout (Salmonidae: *Salmo trutta*) and invasive common dace (Cyprinidae: *Leuciscus leuciscus*) at the core and front of the dace invasive range in Ireland to explore how biological invasion influences the helminth communities of invasive and sympatric populations in recently-invaded and long-invaded fish communities. My thesis combines helminth community surveys, systematic reviews, and stable isotope analysis to investigate enemy release and invasion impacts on native parasite dynamics. My results show that invasive dace has lost parasites in the process of invasion but has acquired native generalist parasites, thereby disrupting native host-parasite dynamics. I demonstrate that invasive dace likely continues to escape infection long after establishment due to low competency for native parasites and a lack of cyprinid-specific parasites in the invaded range, rather than through trophic niche separation with native trout hosts. Additionally, by updating existing knowledge and contributing new data on the helminth communities of two important fish species in previously understudied regions in Ireland, I provide vital baseline knowledge for assessing the parasite-mediated impacts of likely future fish invasions. I conclude that my thesis contributes valuable empirical data to advance our understanding of helminth parasite ecology in the invasion context and highlight important avenues for future investigation into the mechanistic drivers of parasite infection in biological invasions.

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Paths are made by walking – Franz Kafka

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1. Introduction

1.1 Invasive species

Invasive species are one of the leading threats to biodiversity and the second most common cause of species extinctions (Vitousek et al. 1997; International Union for the Conservation of Nature 2000; Convention on Biological Diversity 2020), as well as having negative impacts on the economy and on human health and well-being (Pimentel et al. 2001; Pejchar & Mooney 2009; Hulme 2014; Diagne et al. 2020). Although the precise definition of an invasive species varies somewhat between legal jurisdictions and scientific fields, most definitions are broadly in agreement that a non-native species refers to species introduced by human activity (intentionally or unintentionally) into a biogeographical range in which they would not naturally or historically occur (Mack et al. 2000; Copp et al. 2005; Blackburn et al. 2011). Invasive species are non-native species that have some associated deleterious effects on the environment, economy or human health (Mack et al. 2000; Kolar & Lodge 2001; Copp et al. 2005; Blackburn et al. 2011).

Biological invasions are best understood as a series of barriers a species overcomes (Richardson et al. 2000) or stages a species passes through in the process of invasion (Williamson & Fitter 1996). The unified framework by Blackburn et al. (2011) establishes four stages of invasion — transport, introduction, establishment and spread — during which potential invaders must initially overcome geographic barriers, occasionally followed by captivity barriers, then barriers to survival, reproduction and establishment, and finally the barrier of variable environments in order to successfully invade a novel range. The often-cited “tens rule” (Williamson 1996; Williamson & Fitter 1996) proposes that only ~10% of potential invaders overcome the barriers to introduction, ~10% of those subsequently establish, and ~10% of those “naturalised” species go on to have negative ecological or socioeconomic impacts. However, empirical tests suggest that the percentage of successful establishment is potentially much higher: in the order of 50-60% for vertebrates (García-Berthou et al. 2005; Jeschke & Strayer 2005; Jeschke & Pyšek 2018).

Increased globalisation and worldwide transport over the past century has facilitated introductions of non-native species at unprecedented rates (Vitousek et al. 1997; Levine & D'antonio 2003; Lenda et al. 2014; Essl et al. 2015). Despite advances in biosecurity policy and in detection and control methods for invasive species in recent decades (Armstrong & Ball 2005; Demertzis et al. 2018; Sutcliffe et al. 2018; Shannon et al. 2020), the incidence of biological invasions continues to increase and is not predicted to slow (Seebens et al. 2017, 2020). Aquatic systems are particularly vulnerable to biological invasions due to their role as pathways of human transport and the multitude of commercial and recreational vectors for aquatic introductions (Roman & Darling 2007; Goedknegt et al. 2016). Invasions in freshwater aquatic systems are more widespread (Gozlan et al. 2010; Nunes et al. 2015) and less reported (Bailey et al. 2020) than for terrestrial systems, with freshwater fish among the most commonly introduced of all taxa (Gozlan et al. 2010).

With the rate of introductions of invasive species continuing to rise, there is burgeoning interest in understanding the underlying mechanisms of biological invasions to better predict invasion outcomes and manage invasion impacts (Lee & Klasing 2004; Prenter et al. 2004; Cornet et al. 2016; Essl et al. 2020; Lenzner et al. 2020). Illuminating general patterns in biological invasions across ecosystems is challenging as the ecological impacts of invaders may be context-dependent and likely vary among taxa and biomes (Simberloff et al. 2013; Ricciardi et al. 2017; Essl et al. 2020; Grimm et al. 2020). Invasive species can negatively impact the biodiversity and functioning of ecosystems through various direct and indirect effects (White et al. 2006). Invasive species may prey on native species (Medina et al. 2011; Doherty et al. 2016), directly compete with native species for food or space (Jensen et al. 2002; Corbin & D'Antonio 2004; Kakareko et al. 2013; Gioria & Osborne 2014; Molina-Montenegro et al. 2019) or alter soil chemistry or abiotic processes such as nutrient cycling (Crooks 2002; Linders et al. 2019; Gray et al. 2020). Invaders may also indirectly affect native species by altering the interaction of the native species with a third species (White et al. 2006) as in cases of apparent competition (Carvalheiro et al. 2008; Dangremond et al. 2010), exploitative competition (Dick et al. 2013; Damas-Moreira et al. 2020), through trophic cascades (Kimbro et al. 2009; Walsh et al. 2016) or altered parasite or pathogen dynamics (Dunn et al. 2012; Telfer & Bown 2012).

1.2 Biological invasions and parasites

The role of parasites in invasions is receiving growing attention because parasites can act both as mediators of indirect effects and as invasive species themselves, directly affecting native hosts (Dunn et al. 2012; Lymbery et al. 2014; Médoc et al. 2017). In terms of abundance, biomass, and impact, parasites make up a remarkably large and important component of ecological communities in their own right (Hudson et al. 2006; Lafferty et al. 2006; Dallas & Cornelius 2015). Parasites are involved in host population regulation (Marcogliese 2004), ecosystem engineering (Thomas et al. 1998; Boze et al. 2012; Pascal et al. 2020), nutrient cycling (Vannatta & Minchella 2018; Williams et al. 2019), and bioaccumulation (Sures & Siddall 1999; Sures et al. 2003; Erasmus et al. 2020). They increase food web connectence and can indirectly alter consumer-resource and competitive interactions (Lafferty & Kuris 2012; Gopko et al. 2017; Giari et al. 2020). Due to the potential of parasites to influence host variables such as condition, diet, behaviour and physiology, ignoring the presence of parasites in ecological studies of wild hosts can bias analyses and impair the detection of ecological patterns (Timi & Poulin 2020). Taken together, the importance of parasite-mediated effects and the role of parasites in ecosystem functioning indicate the clear potential for parasites to influence interactions in the invasion context (Prenter et al. 2004; Dunn et al. 2012). However, there is much parasite biodiversity yet to be explored (De León & Poulin 2018; Pappalardo et al. 2020; Poulin et al. 2020) and the role of parasites in invasions remains understudied. While research output on biological invasions has increased exponentially in recent decades (Simberloff 2011; Lowry et al. 2013), new research on parasitism in the context of biological invasions has failed to keep up, with only 50 out of 4000 articles on invasion per year involving parasite interactions in invasions (Figure 1.1; Poulin 2017).

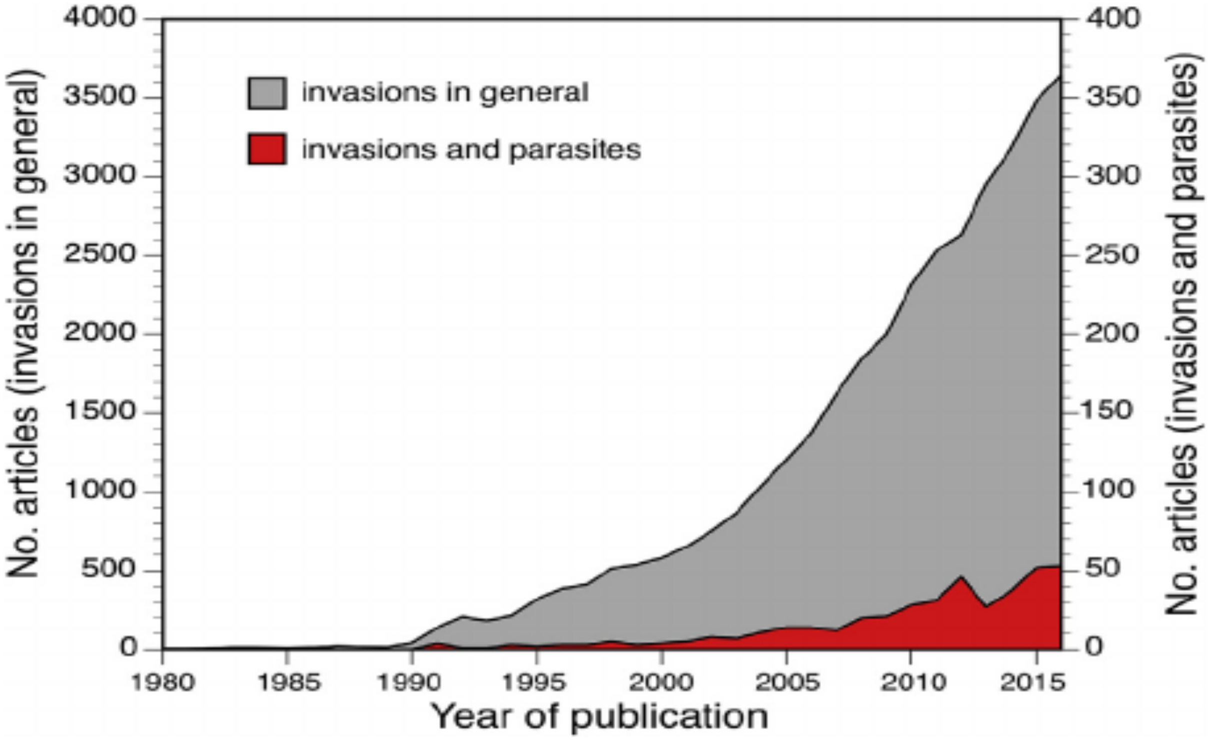

Figure 1.1. Number of articles published per year, between 1980 and 2016, on biological invasions in general and specifically on parasitism in the context of biological invasions (note different scales). Adapted from Poulin (2017).

From a parasitism point of view, invasive hosts can impact native species both by co-introducing non-native parasites and altering native host-parasite dynamics through the acquisition of native parasites (Figure 1.2; Poulin et al. 2011; Telfer & Bown 2012; Lymbery et al. 2014). Invasive species can also impact parasite dynamics in native species through trait-mediated indirect effects such as by causing behavioural changes that affect exposure or altering habitat characteristics that interact with infection (Paterson et al. 2011; Poulin et al. 2011). There are a number of key mechanisms through which invasive hosts are theorised to cause parasite mediated impacts on native species, however, the collection of empirical data often lags behind theory (Telfer & Bown 2012; White & Perkins 2012; Médoc et al. 2017). These key mechanisms can broadly be nested under the pathways of co-introduction of non-native parasites and acquisition of native parasites (Figure 1.2; Prenter et al. 2004; Hatcher & Dunn 2011; Telfer & Bown 2012; Dunn & Hatcher 2015; Goedknegt et al. 2016). Importantly, these mechanisms are not mutually exclusive; different mechanisms may operate over different time scales or stages of invasion, and an invader may interact differently with various parasites or native species (Goedknegt et al. 2016).

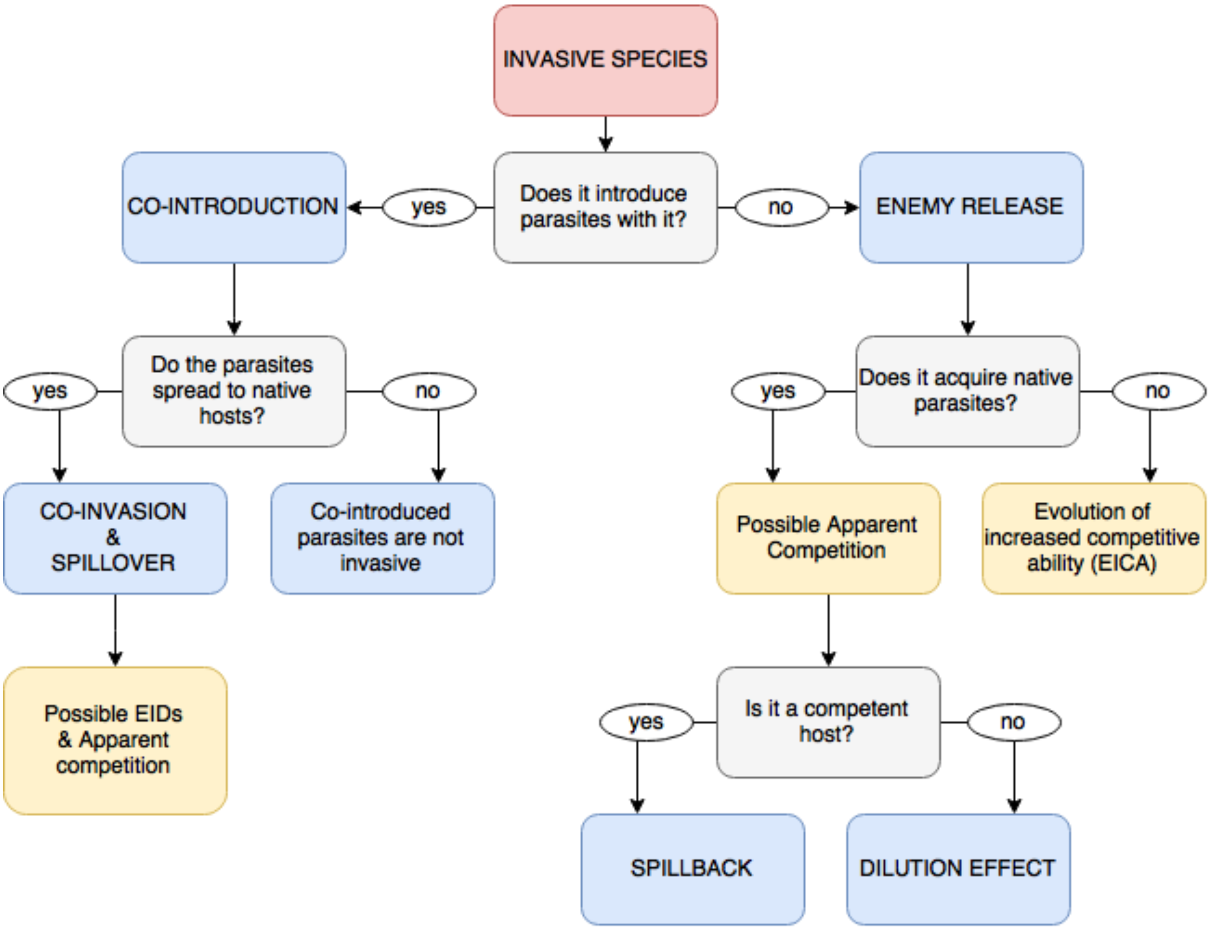

Figure 1.2. Conceptual diagram of potential parasite-mediated impacts of invasive species on native species.

1.2.1 Co-introduction, spillover and co-invasion

The translocation of non-native parasites into a novel biogeographical range along with their non-native host is termed co-introduction (Lymbery et al. 2014). Fish are especially common co-introducers of parasites; Lymbery et al.'s 2014 review of 98 studies found that 55% of hosts that co-introduced parasites were fish and 85% of these were freshwater fish. Some introduced parasites are capable of host switching to native hosts, resulting in the establishment of new parasite populations in native hosts (Gagne et al. 2016; Šimková et al. 2019; Sures et al.

2019; Wilson et al. 2019). These parasites are themselves considered invasive (Lymbery et al. 2014) and may form self-sustaining populations in the native host such as the invasive East Asian eel swim bladder nematode *Anguillicola crassus*, which has become an important parasite of European eel, *Anguilla anguilla*, after introduction to Europe in the 1980s (Weclawski et al. 2014; Hohenadler et al. 2018).

Invasive parasites can also “spill over” into native hosts from an invasive host that acts as a source (reservoir) for infection (Lymbery et al. 2014). Spillover of novel parasites into native hosts with which they have no co-evolutionary history may result in emerging infectious diseases, particularly if the invasive parasite affects native hosts more adversely than invasive hosts (Power & Mitchell 2004; Dunn & Hatcher 2015; Blackburn & Ewen 2017; Reid et al. 2019). The co-introduction of squirrel pox virus to the UK with grey squirrels, *Sciurus carolinensis*, and crayfish plague (caused by the fungal pathogen *Aphanomyces astaci*) into Europe with signal crayfish, *Pacifastacus leniusculus*, are two cases of spillover that resulted in high mortalities of native sympatric species, respectively the red squirrel, *S. vulgaris* (Tompkins et al. 2003), and the white clawed crayfish, *Austropotamobius pallipes* (Reynolds 1988; Bauer 1991). In cases where invasive and native hosts share a common parasite species but do not otherwise interact, apparent competition mediated by the parasite can promote extinction or co-existence between species, depending on the relative dominance and virulence of the parasite in the two host species (Dunn & Hatcher 2015). For example, models of the nematode *Heterakis gallinarum* shared by ring-necked pheasant (*Phasianus colchicus*) and grey partridge (*Perdix perdix*) populations indicate that parasite-mediated apparent competition contributes to exclusion of grey partridge where the ranges of the two gamebird species overlap in space (Tompkins et al. 2000).

1.2.2 Enemy release and evolution of increased competitive ability

Like their free-living hosts, parasites must overcome geographic barriers to introduction, growth and reproduction barriers to establishment, and dispersal barriers to range expansion before they are considered invasive. Parasites have the additional barrier of switching to novel native hosts (Lymbery et al. 2014) which may result in the loss of parasitic co-invaders, particularly those with multi-host life cycles[*] (Torchin et al. 2003). Furthermore, the small size of most founding host populations means that the parasite fauna of the invaders is likely to be only a small subset of that of the source population (Lettoof et al. 2013). The likelihood of an introduced parasite establishing also depends on host factors related to transmission efficiency like host body size and sociality (Macleod et al. 2010). For these reasons, invasive host species frequently lose parasites during the invasion process and tend to have lower parasite richness compared to their source population and to native sympatric species (Torchin et al. 2003; Torchin & Mitchell 2004; Dunn et al. 2012; Gendron et al. 2012; Sarabeev et al. 2017; Schoeman et al. 2019). The important review by Torchin et al. (2003) found that host populations in their introduced range had roughly half the number of parasite species compared to populations in the native range. This escape of invasive species from natural enemies such as predators, competitors and parasites when translocated into a novel habitat is known as enemy release (Mitchell & Power 2003). The enemy release hypothesis posits that the loss of natural enemies is likely to give introduced species a competitive advantage over native sympatric species and contribute to their invasion success (Mitchell & Power 2003).

Release from the deleterious effects of parasites could constitute a fitness benefit for an invasive host in the short term (regulatory release; Colautti et al. 2004). Over evolutionary time, the reduced need to defend against parasites should favour the reallocation of resources to traits that increase the host's invasion potential, such as growth and reproduction, or a shift towards less costly defence mechanisms such as dampening the Th1 immune response pathway in favour of upregulating Th2 responses (White & Perkins 2012; Cornet et al. 2016). This is

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[*] Many helminth parasites have indirect life cycles requiring stages of the parasite to transmit through a sequence of host species to complete a generation. The host in which the adult stage of the parasite undergoes sexual reproduction is termed the definitive host. An intermediate host is an obligate host in which a larval stage of the parasite grows but does not reproduce. A paratenic host is a facultative host in which no parasite growth occurs, that can harbour the parasite stage infective to the definitive host and transmits the parasite if ingested by a definitive host (Parker et al. 2009).

termed compensatory release or evolution of increased competitive ability (EICA) (Blossey & Notzold 1995; Colautti et al. 2004; Lee & Klasing 2004; White & Perkins 2012). Enemy release and EICA are expected to be more pronounced at the leading edge of the invasive range (i.e. the invasion front) where parasites are more likely to be lost as a consequence of reduced host density, and environmental and demographic stochasticity (Phillips et al. 2010; Kelehear et al. 2012; White & Perkins 2012; Chalkowski et al. 2018). Parasites also evolve adaptations at the invasion front, particularly adaptations that increase transmission despite low host density. For example, Kelehear et al. (2012) found that edge populations of *Rhabdias pseudosphaerocephalus*, a nematode lungworm of the invasive cane toad, *Rhinella marina*, had a number of adaptations to maximise transmission such as a shorter prepatent period and larger eggs.

However, while robust conceptual frameworks exist and the loss of parasites during invasions has become a well-documented phenomenon, the practical challenge of testing mechanisms of parasite loss and invasion success has resulted in little empirical evidence to date that causally links parasite loss to invasion success (Keane & Crawley 2002; Liu & Stiling 2006; Heger & Jeschke 2014, 2018; Blackburn & Ewen 2017).

1.2.3 Parasite acquisition, spillback and dilution

Invasive species gain parasites from native sympatric fauna over time (Poulin & Mouillot 2003; Kvach & Stepien 2008; Kvach & Winkler 2011; Gendron et al. 2012) and are more likely to acquire native parasites than to co-introduce non-native parasites (Torchin et al. 2003). The acquisition of native parasites by invasive hosts can modify native host-parasite interactions through a range of parasite-mediated indirect effects on the native host. The competency of hosts is often key to determining these effects (Telfer & Bown 2012; Paterson et al. 2013a; Lymbery et al. 2014; Loxton et al. 2016). Competency refers to a host's ability to support the development and transmission of the relevant stages of the parasite (Telfer & Bown 2012). Competent invasive hosts can amplify a parasite population by acting as parasite reservoirs or as ‘super-spreaders’ of infective stages which can then “spill back” into the native population (Kelly et al. 2009; Goedknegt et al. 2016). For example, in Britain, invasive American mink, *Neovison vison*, has a higher intensity of the digenean *Pseudamphistomum truncatum* than native otters, *Lutra lutra*, and likely contributes to native infection by spreading three times more *P. truncatum* eggs into the environment than otters (Sherrard-Smith et al. 2015). In contrast, an invader with low competency can act as an infection sink, producing a dilution effect which reduces parasite burden in the native host (Telfer et al. 2005; Thieltges et al. 2009; Dunn & Hatcher 2015; Loxton et al. 2017; Young et al. 2017; Stuart et al. 2020). Higher species diversity in an ecosystem can reduce parasite transmission by creating a mosaic of competent and incompetent hosts (Johnson & Thieltges 2010). Incompetent invasive hosts may also reduce infection rates of native hosts by acting as a dead end for infective stages of the parasite, for example, infective stages of native *Rhabdias* lungworms, which infect native Australian frogs, are taken up by cane toads and killed by the toads’ immune system (Lettoof et al. 2013).

1.3 Study system

1.3.1 Invasions in Ireland

The present project uses a freshwater teleost study system of invasive common dace (Cyprinidae: *Leuciscus leuciscus*) and native brown trout (Salmonidae: *Salmo trutta*) in Ireland to study the role of helminth parasites in a biological invasion. Freshwater fish are globally important invaders (Gozlan et al. 2010) and fish systems are particularly suitable models for studying parasite ecology in vertebrates due to the relative ease of sampling that allows for sufficiently high sample sizes to detect rare species and achieve statistical power (Kennedy 2009). Cut off by sea from continental Europe since the last glaciation event, Ireland has a depauperate native fauna but also has experienced fewer biological invasions than continental Europe due to its geographic isolation (Griffiths 1997; Stokes et al. 2004). However, non-native species introductions to Ireland are increasing and Ireland may be particularly susceptible to invasion because of its island biogeography and low native diversity of flora and fauna (Mooney

& Drake 1989; Stokes et al. 2004). Ireland’s depauperate freshwater fish fauna results in relatively unusual patterns of parasite occurrence due to the typical preferred hosts being absent or occurring at low density in Ireland (Byrne et al. 2004; Kennedy 2009). For example, Ireland is the only range in which the acanthocephalan *Pomphorhynchus laevis* uses brown trout as its preferred definitive host (Molloy et al. 1993). Kennedy (2009) noted that Ireland’s species-poor fauna benefits parasite ecology studies as the simpler host communities allow for easier detection of patterns in host-parasite interactions.

1.3.2 Invasive dace

Common dace, *L. leuciscus*, is a relatively small fish, native to Central Europe but currently with a distribution ranging across Europe (Wheeler 1977; Caffrey et al. 2007). Dace was first introduced to Ireland into the Munster Blackwater in the south-west of the country in 1889. Dace of Great British origin along with roach, *Rutilus rutilus,* were accidentally released by British pike anglers who were using the fish as live bait (Went 1950). Such a precise account of introduction is unusual in biological invasions as the detection of most invasive species lags behind the actual date of introduction (Bailey et al. 2020). Dace did not spread further than the Blackwater for about a century, before expanding their range during the 1980s, invading the Shannon catchment, and later, the River Barrow in the east of the country in the 1990s and 2000s (Figure 2.1; Caffrey et al. 2007). Recent fish assessments conducted by Inland Fisheries Ireland as part of the Water Framework Directive place the edge of dace’s invasive range at the upper River Barrow, where it is the most abundant of all recorded fish species (Kelly et al. 2015; Delanty et al. 2017). Here, I leverage the spread of dace to compare parasite-mediated impacts of invasion at the core of the invasive range in the Munster Blackwater which has been invaded for over 120 years to the invasion front in the Upper River Barrow where dace has been present for less than ten years. In doing so, I am able to harness the invasive range of dace to investigate the effects of time since invasion on helminth parasite interactions.

1.3.3 Native brown trout

Brown trout, *S. trutta*, is one of the most well-studied freshwater fish in Ireland with regards to parasite fauna and represents a native sympatric species with which to compare invasive dace. While brown trout and dace are presumed to compete and some spatial segregation between the two species occurs, the interaction between brown trout and dace is not well understood (Delanty et al. 2017). Dace and trout share similar food and habitat preferences so there is considerable potential for niche overlap (Caffrey et al., 2007) and for parasite spillback or spillover between the two species.

1.3.4 Existing knowledge on helminths of dace and brown trout

While the helminth parasite fauna of brown trout is relatively well studied in Ireland (e.g. see Conneely & McCarthy 1988; Molloy et al. 1993, 1995; Byrne et al. 2000, 2002, 2003; Kennedy & Hartvigsen 2000), the parasite fauna of dace in Ireland remains largely unknown. The only record of a helminth parasite of dace in Ireland is of the eel acanthocephalan, *Acanthocephalus clavula*, dating from 1966 (Kane 1966). Checklists of the helminth parasite fauna of Irish trout *S. trutta* (Holland & Kennedy 1997) and of dace *L. leuciscus* in Great Britain (the source population of Irish dace) (Kennedy 1974) reveal that, if the helminth community of dace in Britain reflects those species likely to infect dace in Ireland, the two fish species have the potential to share several species of parasites in Ireland (Table 1.1). Five species of acanthocephalan are common to both British dace and Irish brown trout: *A. clavula*, *Acanthocephalus lucii, Echinorhynchus truttae, Neoechinorhynchus rutili* and *Pomphorhynchus laevis*. Also present on both checklists are one nematode species (*Raphidascaris acus*), two species of digenean (*Diplostomum spathaceum* and *Sphaerostoma bramae*) and an unidentified species of the monogenean genus *Gyrodactylus*.

Table 1.1. Checklist of the helminth species recorded to infect brown and sea trout, *Salmo trutta*, in Ireland and dace, *Leuciscus leuciscus*, in Great Britain. Helminth species common to both lists are highlighted in bold. Adapted from Holland & Kennedy (1997) and Kennedy (1974).

***Salmo trutta* – Ireland**	***Leuciscus leuciscus* – Great Britain**
Phylum Platyhelminthes	Phylum Platyhelminthes
Class Monogenea	Class Monogenea
Discocotyle sagittata	*Dactylogyrus cordus*
Gyrodacrylus derjavini	*Dactylogyrus tuba*
Gyrodactylus truttae	*Dactylogyrus* sp.
***Gyrodactylus* sp.**	*Diplozoon paradoxum*
	***Gyrodactylus* sp.**
Class Digenea	Class Digenea
Bunodera lucipercae	*Allocreadium isoporum*
Crepidostomum farionis	*Bucephalus polymorphiis*
Crepidostomum metoecus	***Diplostomum spathaceum***
Diplostomum spathaceum	*Diplostomum* sp
Diplostomum gasterostei	*Posthodiplostomum cuticola*
Phyllodistomum conostomum	*Sanguinicola volgensis*
Sphaerostomum bramae	***Sphaerostoma bramae***
Tetracotyle sp.	*Tylodelphys clavata*
Class Cestoda	Class Cestoda
Diphyllobothrium dendriticum	*Caryophyllaeides fennica*
Diphyllobothrium ditrenum	*Taryophyllaeus fimbriceps*
Diphyllobothrium sp.	*Caryophyllaeus laticeps*
Eubothrium crassum	*Ligula intestinalis*
Schistocephalus solidus	*Proteocephalus torulosus*
	Proteocephalus sp.
Phylum Nematoda	Phylum Nematoda
Anisakis sp.	***Raphidascaris acus***
Camallanus lacustris	
Capillaria sp.	
Cucullanus truttae	
Cystidicola farionis	
Raphidascaris acus	
Rhabdochona sp.	
Salmonema ephemeridarum	
Phylum Acanthocephala	Phylum Acanthocephala
Acanthocephalus anguillae	***Acanthocephalus clavula***
Acanthocephalus clavula	***Acanthocephalus lucii***
Acanthocephalus lucii	***Echinorhynchus truttae***

Echinorhynchus truttae	***Neoechinorhynchus rutili***
Neoechinorhynchus rutili	***Pomphorhynchus laevis***
Pomphorhynchus laevis	

While existing knowledge on the ecology and parasitology of brown trout and invasive dace in Ireland indicate a role for helminth parasites in mediating the interspecific interactions between the two species, many questions on the parasite dynamics of both species remain unanswered. The parasite community of brown trout has not previously been studied in the River Barrow or Munster Blackwater and this thesis represents the first study, to my knowledge, to provide any data on the helminth parasites infecting brown trout in the east of Ireland. This work also represents the first study of the helminth parasite community of dace in an invaded range and the first to study the effects of an invader on parasite dynamics in Irish freshwater fish. As well as describing, for the first time, the helminth communities of dace and brown trout in the two study rivers, I contribute valuable empirical data of parasite-mediated invasion interactions, building on the theoretical predictions on the role of parasites in biological invasions.

1.4 Thesis structure

1.4.1 Chapter 2. Invasive freshwater fish (*Leuciscus leuciscus*) acts as a sink for a parasite of native brown trout *Salmo trutta*

This chapter explores native and invasive host competency, invasion impacts on native host-parasite dynamics, and dilution effects in invaded systems. In this chapter, I investigate the factors affecting infection (prevalence, abundance, intensity, aggregation) with the dominant generalist acanthocephalan parasite *Pomphorhynchus tereticollis* in native brown trout and invasive common dace, highlighting differences in infection at the core and front of the dace invasive range. I evaluate the relative host competency of dace and brown trout for this native helminth by comparing field data on infection parameters, relative proportions of adult worms, and sexual development of female worms. Finally, I evaluate the extent to which low host competency of invasive dace for *P. tereticollis* may lead to diluted infection in native brown trout.

1.4.2 Chapter 3. Evidence for enemy release in invasive common dace *Leuciscus leuciscus* in Ireland: a helminth community survey and systematic review

This chapter combines a helminth community survey and systematic literature review to explore the loss of helminth parasite diversity in a freshwater fish between its native and invasive ranges, providing support for the enemy release hypothesis. I use a systematic review approach to compile a comprehensive checklist of the helminth parasites recorded in common dace in its native British and continental European ranges. I then compare the number of species infecting dace in its native range to the helminth communities of two invasive populations of dace at the front and core of its invasive range in Ireland to evaluate whether and to what extent invasive dace has escaped from helminth parasites in the process of invasion.

1.4.3 Chapter 4. Structure and composition of helminth communities in brown trout (*Salmo trutta*) and dace (*Leuciscus leuciscus*) in Ireland

This chapter describes the similarities and differences in the structure and composition of helminth communities of invasive dace and brown trout in Ireland, and discusses the likely drivers of the observed variation in helminth communities. I firstly investigate the factors that influence the prevalence, abundance and intensity of the helminth species infecting two riverine populations of native brown trout, in the Munster Blackwater and River Barrow. Secondly, I compare the helminth community diversity of invasive dace to sympatric native brown trout to evaluate enemy release in the invader and the potential for overlap in the helminth communities of invasive dace and native brown trout. Finally, I compare my data on the helminth communities of brown trout to the past 40 years of data on brown trout helminths in Ireland, with a focus on how the environmental context of a river or lake system may influence the structure and composition of freshwater fish helminth communities.

1.4.4 Chapter 5. Trophic niche overlap in invasive common dace and native brown trout

This chapter utilises stable isotope analysis to evaluate the similarity in the trophic niches of native brown trout and invasive dace and investigate whether infection with trophically-transmitted helminths is associated with trophic niche breadth in these two species. I compare isotopic niche size, trophic position and isotopic niche overlap in brown trout and dace at the core and front of dace's invasive range in order to investigate invasion-induced trophic niche separation, constriction or diversification in invasive and native species. Furthermore, I explore the effect of trophic niche on trophically-transmitted helminth parasitism by assessing differences in niche size, trophic position and niche specialisation between infected and uninfected cohorts of brown trout and dace, and by investigating the association of niche breadth with helminth species richness.

1.4.5 Chapter 6. Discussion

This chapter brings together the evidence presented in Chapters 2 to 5 on the factors driving enemy release in invasive dace and the impacts of dace invasion on native helminth parasite dynamics in brown trout. I discuss the significance of my results for the management of invasive species including the importance of establishing baseline knowledge of helminth parasite communities and potential ecosystem-wide impacts of altered parasite dynamics. Finally, I discuss promising future directions for research on parasite ecology in the invasion context.

1.5 Additional work

In addition to the chapters enclosed in this thesis, my PhD provided me the opportunity to contribute to two other research projects which resulted in published research papers, listed below:

1.5.1 Invasive species horizon scan

I contributed to the expert discussion of freshwater invasive species in a horizon scan of invasive species which identified the most likely invasive alien species to invade the island of Ireland in the decade 2017-2027. I contributed revisions to the resulting manuscript.

Lucy, F.E., Davis, E., Anderson, R., Booy O., Bradley, K., Britton, J.R., Byrne, C., Caffrey, J.M., Coughlan, N.E., Crane, K., Cuthbert, R.N., Dick, J.T.A., Dickey, J.W.E., Fisher, J., Gallagher, C., Harrison, S., Jebb, M., Johnson, M., Lawton, C., Lyons, D., Mackie, T., Maggs, C., Marnell, F., McLoughlin, T., Minchin, D., Monaghan, O., Montgomery, I., Moore, N., Morrison, L., Muir, R., Nelson, B., Niven, A., O'Flynn, C., Osborne, B., O'Riordan, R.M., Reid, N., Roy, H., Sheehan, R., Stewart, D., Sullivan, M., **Tierney, P.**, Treacy, P., Tricarico, E., Trodd, W. (2020) Horizon scan of invasive alien species for the island of Ireland. Management of Biological Invasions 11: 155–177. https://doi.org/10.3391/mbi.2020.11.2.01

1.5.2 Helminth parasites of pink salmon

Following the unprecedented widespread occurrence of non-native pink salmon *Oncorhynchus gorbuscha* in Ireland in 2017, I obtained 16 pink salmon specimens from Inland Fisheries Ireland which I examined with a undergraduate student for helminth infection. The helminth parasite findings were included in the below paper, for which my contribution was acknowledged.

Millane, M., Walsh, L., Roche, W.K., & Gargan, P.G. (2019). Unprecedented widespread occurrence of Pink Salmon *Oncorhynchus gorbuscha* in Ireland in 2017. Journal of Fish Biology 95: 651-654. https://doi.org/10.1111/jfb.13994

2. Invasive freshwater fish (*Leuciscus leuciscus*) acts as a sink for a parasite of native brown trout *Salmo trutta*

Status: This manuscript has been published in *Biological Invasions* (2020) 22: 2235-2250. Published paper available at https://doi.org/10.1007/s10530-020-02253-1

Co-authors: Joe M. Caffrey, Sebastian Vogel, Sharon M. Matthews, Emy Costantini, Celia V. Holland

Author contributions: PAT conducted host sampling in 2017, laboratory dissections, data analysis and wrote the manuscript. JMC, CVH and PAT conceived the study design. JMC assisted in co-ordinated of host sampling. SMM conducted host sampling in 2015. EC conducted laboratory dissections. SV conducted genetic barcoding of worms and wrote the Methods paragraph on genetic identification. CVH supervised the project. All authors commented on the manuscript.

Ethical statement: Electrofishing and euthanasia of fish was carried out by trained Inland Fisheries Ireland staff. This work was approved by the Trinity College Dublin School of Natural Science's Research Ethics Committee.

2.1 Introduction

The introduction of non-native species into new biogeographical ranges enhances the potential for novel host-parasite interactions. However, while the scientific literature on biological invasions expand, studies that incorporate the invasion impacts on parasite dynamics are lacking (Poulin 2017). Advances in theory, combining parasite and invasion ecology, have generated strong hypotheses on how invasive species can impact native host-parasite dynamics but the need for empirical evidence remains (Prenter et al. 2004; White & Perkins 2012; Dunn

& Hatcher 2015). Invasive species are known to lose parasites in the process of invasion and are left with fewer parasites than native sympatric species (Torchin et al. 2003; Goedknegt et al. 2016; Sarabeev et al. 2017) but they also acquire parasites from native host communities (Dunn & Hatcher 2015; Goedknegt et al. 2016) Given the context dependency of invasion impacts (Ricciardi et al. 2017), empirical evidence of this loss and gain of parasites in invasions in different ecological contexts is crucial.

Part of this variable context is host competency for a given parasite: the ability of a host to support parasite growth and development (Kelly et al. 2009). The competency of an invasive host for acquired parasites is important in predicting the potential impacts on native host-parasite dynamics (Kelly et al. 2009; Poulin et al. 2011; Paterson et al. 2013a). The impacts of host competency on parasite dynamics are mediated by the abundance of a host species relative to other hosts; transmission to and from a rare invasive host may have negligible impact on native parasite dynamics (e.g. Paterson et al. 2013b) whereas a relatively abundant invasive species is likely to have a strong impact on transmission dynamics (e.g. Gendron & Marcogliese 2017). An abundant competent invasive host for native parasites is likely to increase transmission and result in increased infection of native hosts, known as spillback (Kelly et al. 2009; Sherrard-Smith et al. 2014). Alternatively, an abundant but incompetent invasive host may act as an infection sink and dilute infection in native hosts (Thieltges et al. 2009; Paterson et al. 2013a; Gagne et al. 2016; Gendron & Marcogliese 2016, 2017).

In this study I use an acanthocephalan and freshwater fish model system to explore the impact of invasive host competency and parasite population biology within an invasive host on native parasite dynamics. Acanthocephalans are especially useful for investigations of host competency as, unlike other helminth taxa, development of eggs in female parasites can be assessed, which provides a reliable indicator of host suitability along with parasite size (Brown et al. 1986; Lyndon & Kennedy 2001; Kennedy 2006). Location of the acanthocephalan within the host can also give an indication of host suitability as parasites have been observed to penetrate through the intestinal wall and encyst extra-intestinally in unsuitable fish hosts, e.g., see Taraschewski (1985, 1989), Dezfuli et al. (2011).

The acanthocephalan *Pomphorhynchus laevis sensu lato* (*s.l.*) (Zoega in Müller 1779) is among the most common and widespread helminth parasites of freshwater fish in Europe, utilising gammarid species as intermediate hosts and freshwater fish as definitive hosts (Molloy et al. 1995a; Holland & Kennedy 1997; Byrne et al. 2002; Perrot-Minnot 2004). *Pomphorhynchus laevis s.l.* is the only species of *Pomphorhynchus* recorded in Ireland and previous ecological, morphological and molecular studies suggest that *P. laevis s.l.* from Irish freshwaters represents a strain distinct from Great Britain and Continental Europe (Kennedy et al. 1989; Holland & Kennedy 1997; O'Mahony et al. 2004a, b). Recent taxonomic and biogeographic work resurrected *Pomphorhynchus tereticollis* (previously a synonym of *P. laevis*) as a separate species to *P. laevis* within the *Pomphorhynchus laevis* complex (Špakulová et al. 2011; Perrot-Minnot et al. 2018) and suggests that the freshwater strains previously described as *P. laevis* native to Ireland and Great Britain are likely to belong to the resurrected *P. tereticollis.* As *Pomphorhynchus tereticollis* has been commonly recorded as *P. laevis* in previous parasitological work (Špakulová et al. 2011), I refer to *P. laevis s.l.* in those cases where the species identity is not certain.

The host-parasite dynamics of *P. laevis s.l.* in Ireland are relatively unique due the absence of chub (*Leuciscus cephalus*) and barbel (*Barbus barbus*) which are the preferred definitive hosts in Great Britain and continental Europe (Hine & Kennedy 1974a; Kennedy et al. 1989; Kennedy 1996, 2006). Neither chub nor barbel are native to Ireland and, following the recent eradication of chub from the only Irish river system to which it had been introduced (River Inny) (Caffrey et al. 2018), neither species are now present in Ireland. In the absence of chub and barbel, brown trout is the preferred definitive host of *P. laevis s.l.* in Ireland (Kane 1966; Fitzgerald & Mulcahy 1983; Kennedy et al. 1989; Byrne et al. 2003; Kennedy 2009).

Invasive common dace (*Leuciscus leuciscus*) has been present in Ireland since 1889 when bait fish of British origin escaped into the Munster Blackwater river in the south-west of Ireland (Went 1950) (Figure 2.1). While the spread of the species was initially slow, dace invaded the River Barrow in the east of Ireland in the 1990s (Caffrey et al. 2007) and are currently widespread and abundant in the main River Barrow channel (Delanty et al. 2017). As of 2015, when this study began, the limit of dace's range was in the upper subcatchments of the River Barrow (Delanty et al. 2017). Dace have similar habitat and feeding preferences to native brown trout. Both favour gravelly fast-flowing rivers for spawning and predominantly feed on aquatic macro-invertebrates (Caffrey et al. 2007). Very little is known about the parasitology of invasive dace in Ireland. Only one record of a single parasite species (Kane 1966) exists from dace in Ireland. Given that brown trout is an ecologically and economically important species (National Strategy for Angling Development 2015), the recent invasion success of dace has prompted a need for studies on the interactions between dace and brown trout in Ireland (Caffrey et al. 2007; Delanty et al. 2017).

The distribution of dace provides the opportunity to compare the population biology of a native helminth species, *P. tereticollis*, in a native and an invasive species at the core and edge[†] of its range and to investigate the effect of long-established and recently established invasive host populations on parasite dynamics in native brown trout. Following conceptual and empirical studies that suggest that parameters of parasite infection will be lower in an invasive species at the invasion edge (Kelehear et al. 2012; White & Perkins 2012), I hypothesise that dace will be less infected at the edge of their invasive range in the River Barrow. Survey and experimental data from Great Britain indicate that dace become infected with *P. laevis s.l.* at similar rates to brown trout and host competency for *P. laevis s.l.* is low but similar for both fish species (Hine & Kennedy 1974a, b). However, as the Irish strain of *P. tereticollis* is adapted to utilising brown trout as its definite host and parameters of parasite infection tend to be lower

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[†] Invasion edge and invasion front are synonymous terms. Invasion edge is predominantly used in this chapter and invasion front is predominantly used in the other chapters; however, the meaning is the same.

in invasive species (Loxton et al. 2016), I hypothesise that the competency of dace as a host for *P. tereticollis* will be low in Ireland and thus, may confer some benefit to native brown trout in the form of parasite dilution and mitigated infection risk. However, it is possible that *P. tereticollis* may adapt to the availability of a novel host where that host has been established for a long period of time, as has been observed in other acanthocephalan species encountering invasive hosts (Gendron & Marcogliese 2016).

2.2 Methods

2.2.1 Study sites

Dace and brown trout were collected by electrofishing in 2015 and 2017 from the core of the dace invasive range on the Munster Blackwater and at the edge of the invasive range on the upper River Barrow (Figure 2.1). Sampling in 2015 collected 77 brown trout and 71 dace from three sites on the Munster Blackwater (Fermoy main channel, River Funsion at Glanworth and River Funsion at east Fermoy) and 41 brown trout and 81 dace from three sites on the upper River Barrow (Portarlington, Monasterevin and River Slate at Rathangan) in July and August, respectively. In August 2017, I collected 60 brown trout and 48 dace from two sites on the Munster Blackwater (Fermoy main channel and River Funsion at east Fermoy) and 71 brown trout and 87 dace from two sites on the upper River Barrow (Portarlington and Monasterevin). Subsites varied between years due to the availability of fish in these rivers.

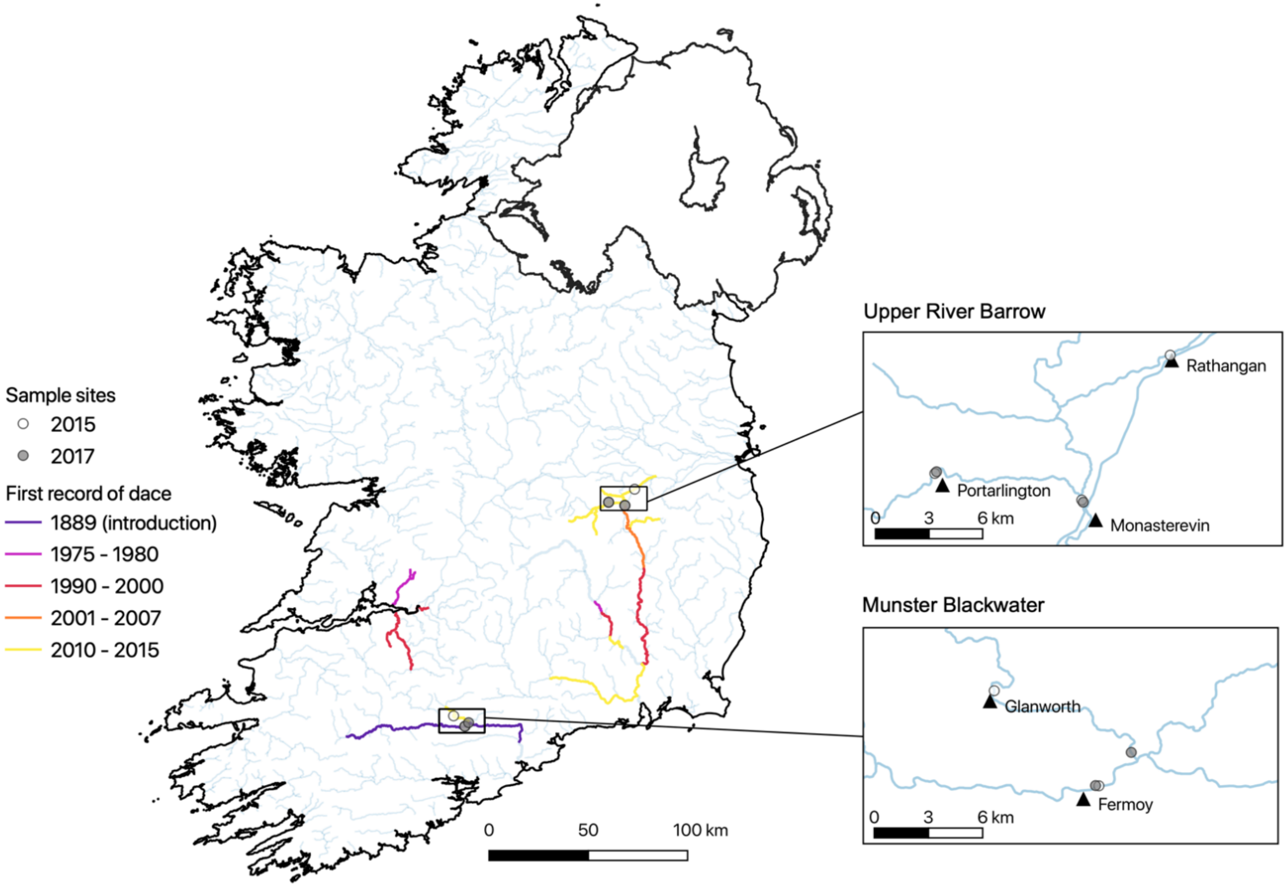

Figure 2.1. Rivers of Ireland showing the date range in which dace were first recorded in each invaded river system and the sample sites used in this study. White and grey circles represent the sites sampled. Black triangles represent towns. Dace distribution data from Went (1950); Moriarty & Fitzmaurice (2000); Caffrey et al. (2007); Kelly et al. (2011), (2015); Delanty et al. (2017). River data reproduced courtesy of Ordnance Survey Ireland.

2.2.2 Fish processing

Fish were euthanised on-site using anaesthesia by eugenol oil or percussive stunning and decerebration. Fish were bagged and transported on ice to the laboratory where they were frozen and stored at -20°C before dissection. In the laboratory, the fish were defrosted overnight (or as appropriate for their size) and the weight (g), standard length (mm) and sex were recorded. The alimentary canal was removed from the fish, cut longitudinally and washed with 0.9% saline. The intestine, mesenteries, and body cavity were examined for helminth parasites. Acanthocephalans were, where necessary, dissected away from the intestinal wall and relaxed in tap water for 24 hours to evert the proboscis before storing in 70% ethanol and 4% glycerol solution.

2.2.3 Parasite identification

Acanthocephalans found were recorded as either adult worms which occurred in the lumen of the intestine or immature cyst-like subadult worms which had penetrated through the intestinal wall and encysted extra-intestinally in the mesenteries (Taraschewski 1989; Emde et al. 2014; Gendron & Marcogliese 2016). All adult acanthocephalans found were identified as *P. tereticollis* by morphological characteristics as described by Špakulová et al. (2011).

To confirm the morphological identification, seven randomly chosen adult worms (four from the invasion core population and three from the invasion edge) were used to carry out genetic barcoding. DNA was extracted according to Grabner et al. (2015). The following primers based on mitochondrial COI were used: PT/PL-COI-forward (5-ATGGGGTTTTCTATAAGRCTA) and PT/PL-COI reverse (5-CAAATTACGATCCATCAAAAGCA). PCR was conducted using OneTaq® 2X Standard Buffer Master Mix (New England Biolabs) with 0.5 µM of each Primer and 1 µl of template DNA in a total volume of 20 µl per reaction. PCR cycle conditions were set as follows: initial denaturation at 94°C for 3 mins, followed by 40 cycles of 94°C for 40s (denaturation), 51°C for 40s (annealing) and 68°C for 45s (elongation). Final elongation was 68°C for 3 mins. PCR products were purified with MicroElute ® Cycle-Pure Kit (OMEGA) and send for sequencing to GATC Biotech using the forward primer.

The received sequences were compared with those of *P. tereticollis* collected from flounder (*Platichthys flesus*) from the Baltic Sea, Rostock, Germany, and specimens of *P. tereticollis* and *P. bosniacus* collected from barbel and chub from the Upper Rhine, Karlsruhe, Germany (Vogel 2018). The sequences were aligned using “MEGA 7” and subsequently a maximum likelihood tree was constructed. Additionally, each sequence was BLAST searched against NCBI GenBank. Both methods confirm the identification of the Irish strain investigated in this study as *P. tereticollis*.

The characteristic hooks on the proboscis were, in most cases, not sufficiently developed in the subadult (extra-intestinal) worms to allow for species identification, however, the characteristic *Pomphorhynchus* bulb at the base of the proboscis was visible in some subadult specimens. As there were no *Pomphorhynchus* species nor acanthocephalan species other than *P. tereticollis* identified among the 521 adult parasites, I assume that the subadults were also *P. tereticollis.*

2.2.4 Parasite processing

Analysis of worm size and maturity was carried out on adult worms only. I sexed, weighed (mg) and measured the length (mm) of all adult worms using scaled photographs and ImageJ image processing software (Schneider et al. 2012). Female worms were dissected using fine forceps to release the contents of the ovaries and assigned to one of three sexual maturity stages according to Brown et al. (1986): stage 1 in which ovarian balls are present but acanthors are absent (immature worms), stage 2 in which ovarian balls and unshelled acanthors are present (immature worms), and stage 3 in which ovarian balls, shelled and unshelled acanthors are present (mature worms).

2.2.5 Calculations and statistical analyses

Calculations of parasite infection and statistical analyses were carried out in RStudio Version 1.2.5019 (R Core Team 2018). I calculated prevalence, mean abundance and mean infection intensity of *P. tereticollis* for dace and brown trout, grouped by invasion region (core and edge) and sample year, according to Bush et al. (1997). I calculated Clopper-Pearson exact 95% confidence intervals for prevalence data using the R package PropCIs (Scherer 2018). I calculated aggregation of *P. tereticollis* within the host populations at the core and edge of the invasive range using variance to mean ratios (variance divided by mean) (Wilson et al. 2002).

I modelled adult and subadult *P. tereticollis* infections separately, using prevalence and intensity as parameters of infection. The reasons for modelling adults and subadults separately were two-fold. Biologically, adults and subadults represent functionally different forms of the parasite and it is necessary to interpret them separately. Statistically, due to the difference in intensity range between the two forms, combined data of adults and subadults were so aggregated as to be unable to be fitted to negative binomial models. I modelled prevalence using binomial generalised linear mixed models (GLMMs) and infection intensity using negative binomial GLMMs from the glmmTMB package (Brooks et al. 2017). I also modelled proportion of adult worms of total worms using binomial GLMMs from glmmTMB. Initial models included year and site as random effects and host species, invasion core or edge and host length as fixed effects with two-way interactions between species and invasion core/edge, and species and length. I used backwards stepwise deletion and AIC values to select the most parsimonious model. The fit of the models was validated by examining residual deviance of the selected model. In order to test whether *P. tereticollis* infection in brown trout is diluted by the long-standing presence of dace in the invasion core, I tested the effect of being in the invasion core compared to the edge on total *P. tereticollis* abundance in brown trout (subadults and adults combined) using the Wilcoxon-Mann-Whitney test as a negative binomial model could not be fitted to the total worm abundance data.

I modelled worm weight using a GLMM with a Gamma distribution incorporating worm sex, host species and host length as fixed effects, and observer, host ID, site nested within invasion region and year as random effects. Models could not be satisfactorily fitted to the data for worm length or worm maturity stage so a Wilcoxon-Mann-Whitney test was used to test the main effects.

2.3 Results

In total, I recorded 472 *P. tereticollis* individuals in brown trout and 558 *P. tereticollis* individuals in dace. In brown trout, 452 (96%) were adults and 20 (4%) were subadults. In dace, 69 (12%) were adults and 489 (88%) were subadults.

Parameters of *P. tereticollis* infection (prevalence, mean abundance, mean infection intensity) are presented in Table 2.1. The highest total prevalence was 76% (CI95% 60-88), recorded in brown trout from the invasion edge in 2015. The lowest parameters of parasite infection for both fish species were recorded in the invasion edge in 2017, when no *P. tereticollis* infection was recorded in dace and only a single adult worm was recorded in brown trout.

2.3.1 Aggregation

Overall *P. tereticollis* infection was highly aggregated in dace and less aggregated in brown trout. When core and edge populations were combined, I found that in dace, 100% of infections were carried by 8% of fish. In brown trout, the most heavily infected 20% of fish carried 84% of infections. The aggregated distribution of *P. tereticollis* in dace was driven by highly aggregated subadult worms. In contrast, the variance to mean ratios of adult worms were similar between dace and brown trout within each invasion region (Table 2.2).

Table 2.1. Indices of *Pomphorhynchus tereticollis* infection across host species and sample year in the Munster Blackwater and upper River Barrow

Host	Invasion region	Year	n	Loc		n worms	n hosts	Prev (CI)	Ab (SE)	Int (SE)
Brown trout	Core (Blackwater)	2015	77	IN	Adults	112	47	61 (49-72)	1.45 (0.19)	2.38 (0.24)
				M	Subadults	3	2	3 (0-9)	0.04 (0.02)	1.5 (0.05)
					Total	115	47	61 (49-72)	1.49 (0.2)	2.45 (0.24)
		2017	61	IN	Adults	65	24	39 (27-53)	1.07 (0.29)	2.71 (0.61)
				M	Subadults	5	4	7 (2-16)	0.08 (0.04)	1.25 (0.25)
					Total	70	27	44 (32-58)	1.15 (0.29)	3.87 (0.85)
	Edge (Barrow)	2015	41	IN	Adults	274	30	73 (57-86)	6.68 (1.13)	9.13 (1.29)
				M	Subadults	12	7	17 (7-32)	0.29 (0.13)	1.71 (0.47)
					Total	286	31	76 (60-88)	6.98 (1.19)	9.23 (1.35)
		2017	71	IN	Adults	1	1	1 (0-8)	0.1 (0.01)	1
				M	Subadults	0	0	0 (0-5)	0	0
					Total	1	1	1 (0-8)	0.01 (0.01)	1
Dace	Core (Blackwater)	2015	71	IN	Adults	31	12	17 (9-28)	0.44 (0.15)	2.58 (0.61)
				M	Subadults	69	13	18 (10-29)	0.97 (0.34)	5.31 (0.028)
					Total	100	19	27 (17-39)	1.41 (0.41)	5.26 (1.14)
		2017	48	IN	Adults	19	10	21 (10-35)	0.40 (0.13)	1.9 (0.28)
				M	Subadults	295	23	48 (33-63)	6.15 (1.9)	12.83 (3.5)
					Total	314	22	46 (31-61)	6.54 (1.94)	14.14 (3.64)
	Edge (Barrow)	2015	81	IN	Adults	19	2	2 (0-9)	0.23 (0.17)	9.5 (0.5)
				M	Subadults	125	3	4 (1-10)	1.54 (1.33)	41.67
					Total	144	3	4 (1-10)	1.78 (1.48)	48 (35.25)
		2017	87	IN	Adults	0	0	0 (0-4)	0	0
				M	Subadults	0	0	0 (0-4)	0	0
					Total	0	0	0 (0-4)	0	0

n = sample size, Loc = location within host, IN = intestine, M = mesenteries, n worms = number of parasites, n hosts = number of infected hosts, Prev = percentage prevalence of infection, CI = 95% confidence interval, Ab = abundance, SE = standard error, Int = infection intensity

Table 2.2. Aggregation of *P. tereticollis* in brown trout and dace by invasion region and parasitic form (adult or subadult *P. tereticollis*), measured by mean to variance ratio ($\sigma^2/\bar{x}$).

Host species	Invasion region	Parasitic form	$\sigma^2/\bar{x}$
Brown trout	Core	Adult	3.08
		Subadult	1.45
		Total	2.97
	Edge	Adult	12.0
		Subadult	2.41
		Total	12.7
Dace	Core	Adult	3.05
		Subadult	26.3
		Total	24.5
	Edge	Adult	9.47
		Subadult	93.7
		Total	99.5

2.3.2 Adult worms

2.3.2.1. Adult prevalence

The overall prevalence of adult *P. tereticollis* was 41% (CI95% 35-47) in brown trout and 8% (CI95% 5-12) in dace. Brown trout were significantly more likely to be infected with adult worms than dace (GLMM, host species: family = binomial, *Z* = 5.6, *P* = <0.001; Appendix Table A1). The effect of host species on prevalence was marginally altered by invasive region (core or edge) (GLMM, family = binomial, species:region: *Z* = -2.0, *P* = 0.046) but for both dace and brown trout, prevalence was lower at the invasion edge compared to the core (Figure 2.2a). Host standard length significantly changed the effect of host species on prevalence (GLMM, family = binomial, species:length: *Z* = -5.4, *P* = <0.001). In both host species, fish with a longer standard length were more likely to be infected with *P. tereticollis*, however, in dace, likelihood of infection increased more steeply with length than in brown trout (Figure 2.2c). Brown trout were infected across the entire size range studied (79-360 mm) while all dace below 100 mm were uninfected and 96% of infected dace were over 130 mm in standard length (size range: 67-241 mm).

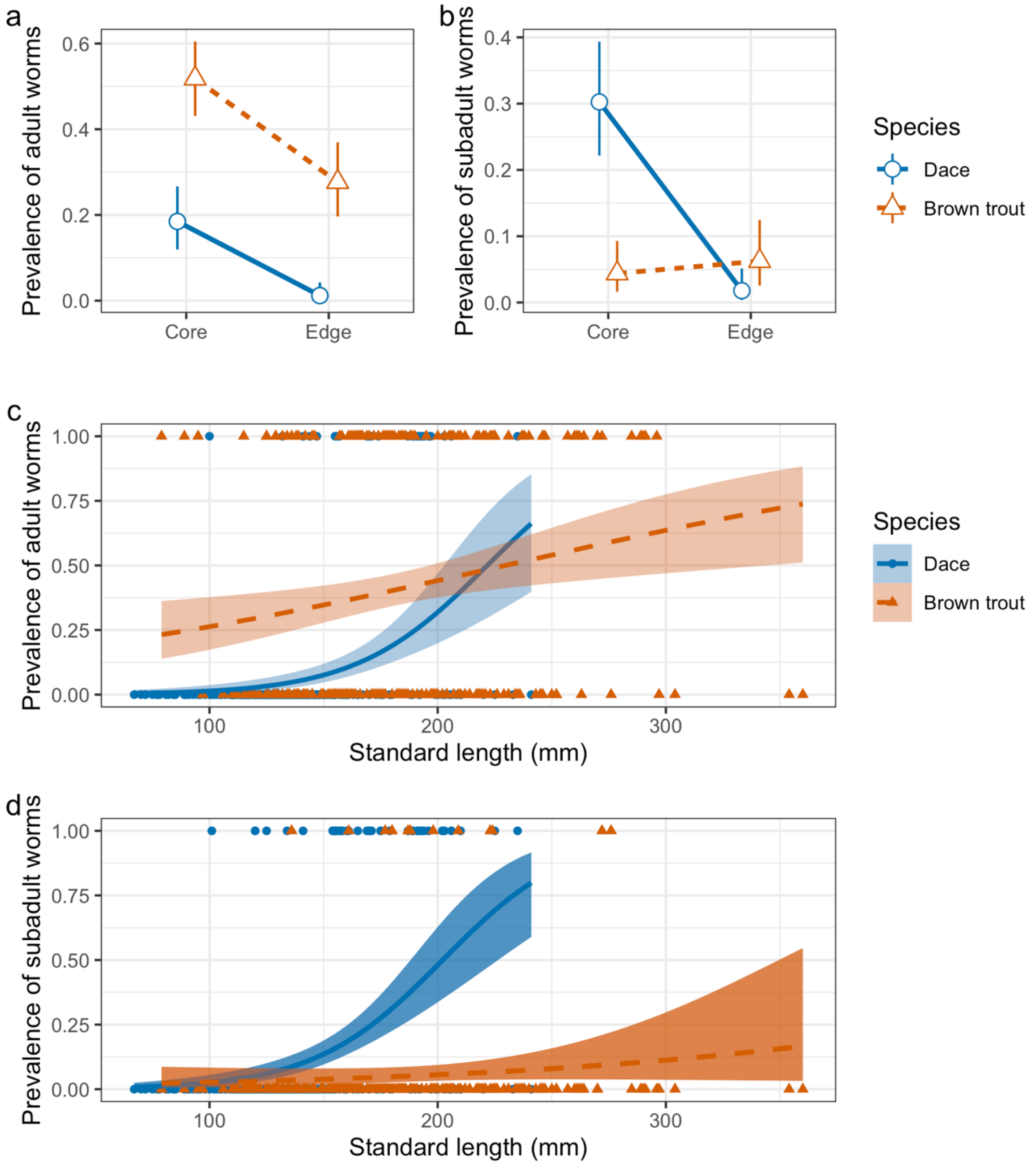

Figure 2.2. Factors influencing prevalence of adult and subadult *P. tereticollis*: (a) prevalence of adult worms by host species and invasion core or front; (b) prevalence of subadult worms by host species and invasion core or front; (c) prevalence of adult worms against host standard length (mm); (d) prevalence of subadult worms against host standard length (mm). Observations of infection (1 = infected, 0 = uninfected) are represented by points. Regression lines represent binomial GLMs and shaded areas represent 95% confidence intervals. Figure represents data pooled from the two sampling years.

2.3.2.1. Adult intensity

Host species did not affect the intensity of adult *P. tereticollis* infection (GLMM, family = negative binomial, host species: *Z* = -0.04, *P* = 0.962; Appendix Table A2). Intensity was significantly higher at the invasion edge compared to the core (GLMM, family = negative binomial, invasion region: *Z* = -2.2, *P* = 0.029) (Figure 2.3).

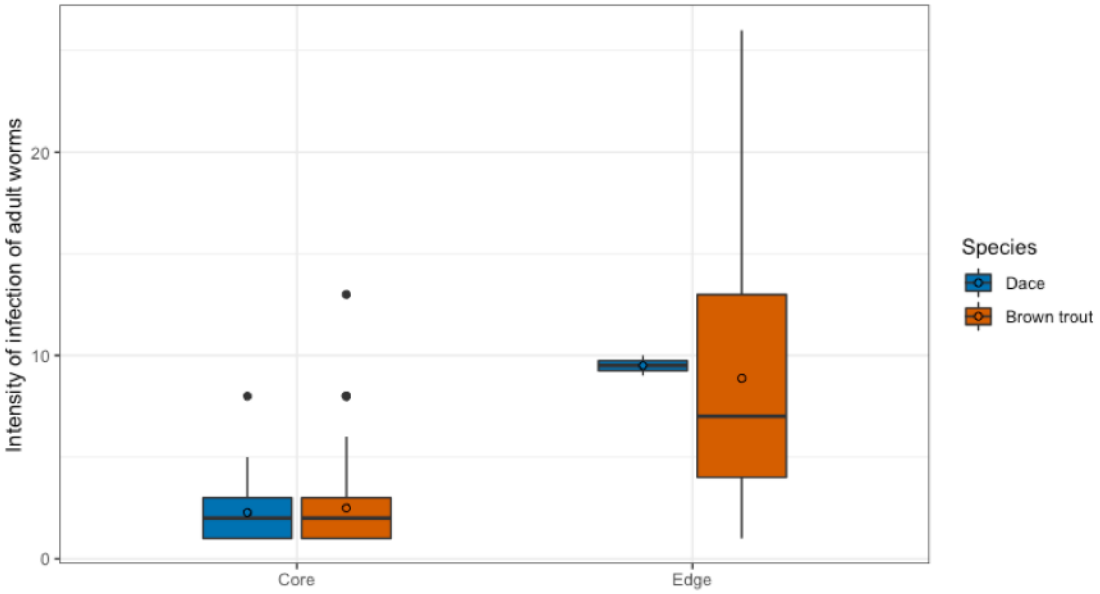

Figure 2.3. Infection intensity of adult *P. tereticollis* at the invasion edge and core by host species. Medians represented by solid lines and means represented by open circles. Figure represents data pooled from the two sampling years.

2.3.3 Subadult worms

2.3.3.1. Subadult prevalence

Overall, 14% (95%CI 10-18) of dace and 5% (95%CI 3-9) of brown trout were infected with subadult *P. tereticollis.* Region and host species interacted significantly to influence prevalence (GLMM, family = binomial, species:region: *Z* = -2.8, *P* = 0.005; Appendix Table A3); brown trout were more likely to be infected with subadult *P. tereticollis* than dace at the invasion edge whereas in the invasion core, dace were more likely to be infected than trout (Figure 2.2b).

Host standard length also altered the effect of host species on subadult *P. tereticollis* prevalence (GLMM, family = binomial, species:length: *Z* = -4.8, *P* < 0.001) (Figure 2.2d). Increasing host length increased the likelihood of infection in both fish species but the effect was stronger in dace: for each one millimetre increase in standard length, the log odds of *P. tereticollis* infection increases by 0.06 in dace and 0.004 in brown trout.

2.3.3.2. Subadult intensity

There was a significant effect of host species on subadult intensity which was dependent on host body length (GLMM, family = negative binomial, species:length: *Z* = -4.6, *P* < 0.001; Appendix Table A4); in dace subadult intensity increased in response to increasing host length while in brown trout, intensity remained low across size classes (Figure 2.4). Invasion region did not significantly affect subadult intensity (GLMM, family = negative binomial, invasion region: *Z* = 1.6, *P* = 0.111).

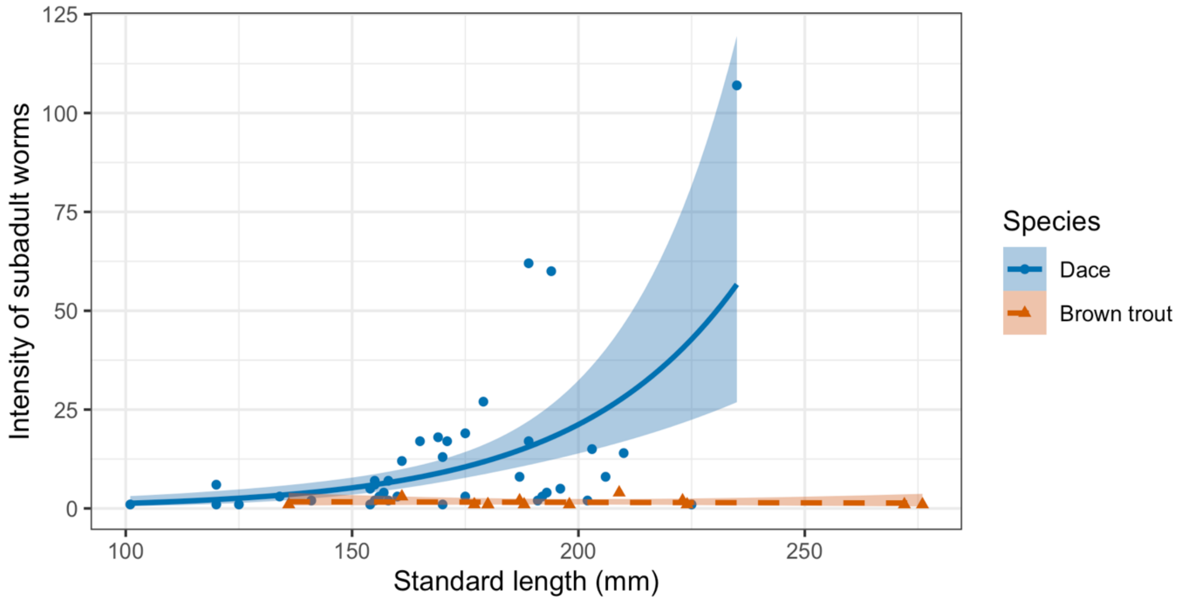

Figure 2.4. Intensity of subadult *P. tereticollis* in response to host standard length (mm) and host species. Regression lines represent negative binomial GLMs and shaded areas represent 95% confidence intervals. Figure represents data pooled from two sampling years.

2.3.4 Proportion of adult worms

The proportion of adult worms was significantly higher in brown trout compared to dace (GLMM, family = binomial, species: $Z = 9.3$, $P < 0.001$; Appendix Table A5) (Figure 2.5). Host length had a significant negative effect on the proportion of adult worms with larger fish having a small proportion of adult worms relative to total worms. The proportion of adult worms was not affected by region.

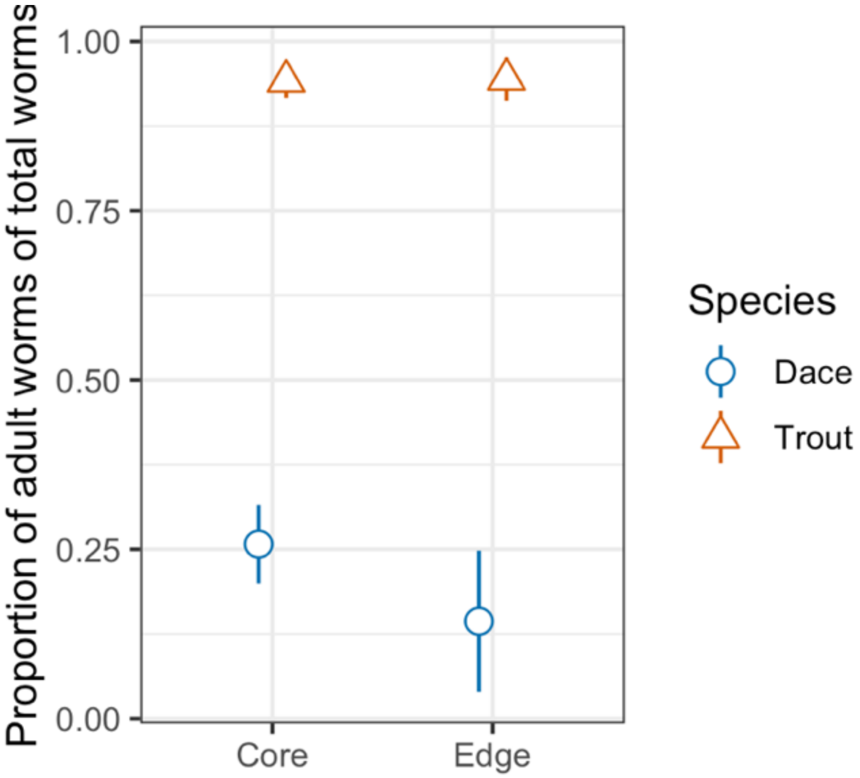

Figure 2.5. The proportion of adult *P. tereticollis worms* relative to total worms in dace and brown trout

2.3.5 Total worm abundance (adults and subadults combined)

Within brown trout, mean abundance ± standard error of combined adult and subadult *P. tereticollis* was 1.35 ± 0.17 at the invasion core and 2.56 ± 0.53 at the edge (Figure 2.6). The Wilcoxon-Mann-Whitney test also indicated combined abundance in brown trout was lower in the invasion core than the invasion edge (Wilcoxon-Mann-Whitney, core: $W = 6470$, $P < 0.001$, edge: $W = 8994$, $P = 0.02$). Within dace, mean abundance ± standard error of combined adult and subadult *P. tereticollis* was 3.48 ± 0.85 at the invasion core and 0.86 ± 0.71 at the edge (Figure 2.6).

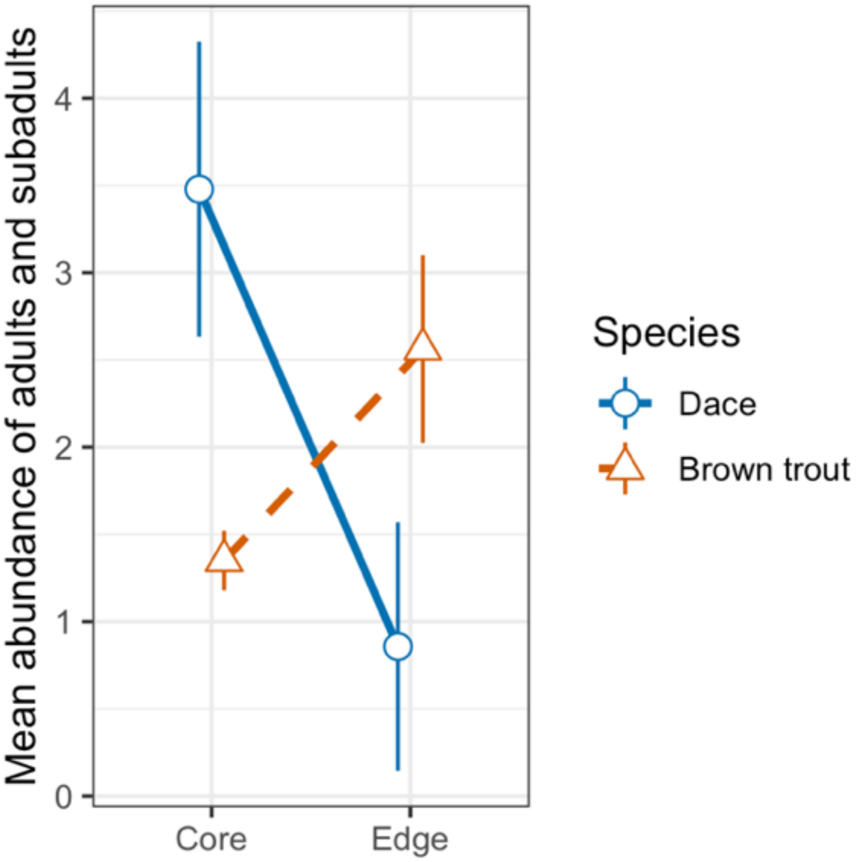

Figure 2.6. Mean abundance of adult and subadult *P. tereticollis* combined at the invasion edge and core. Error bars represent standard error. Figure represents data pooled from two sampling years

2.3.6 Worm size and maturity

Worms from brown trout were significantly larger in weight than those from dace (GLMM, family = Gamma, $Z = 2.49$, $P = 0.013$; Appendix Table A6) (Figure 2.7) but worm length did not significantly differ between host species (Wilcoxon-Mann-Whitney: $W = 11251$, $P = 0.409$). Both parasite weight and length were influenced by the sex of the parasite with males being significantly smaller than females (Weight: GLMM, family = Gamma , $Z = -5.21$, $P < 0.001$; Length: Wilcoxon-Mann-Whitney: $W = 21542$, $P < 0.001$). All of the female parasites collected from dace hosts were stage 1 or stage 2 *i.e.* none were reproductively mature (Table 2.3). In brown trout, 16% of female parasites were stage 3 mature females.

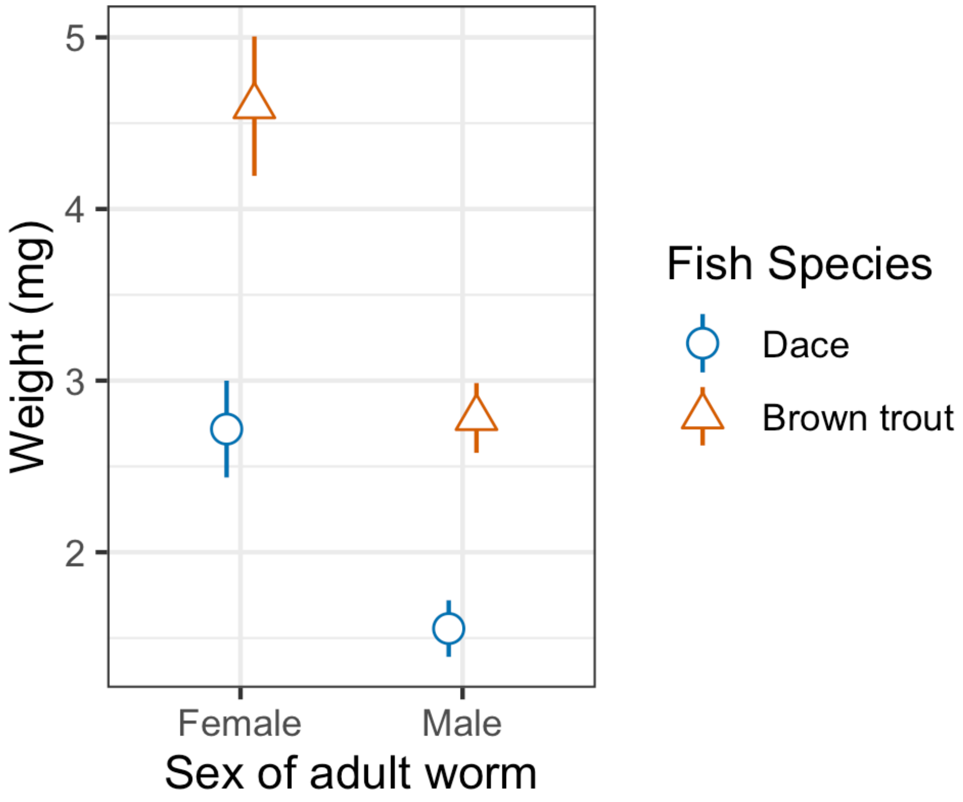

Figure 2.7. Mean weight (mg) of adult *P. tereticollis* worms in dace and brown trout hosts. Error bars represent standard error. Figure represents data pooled from two sampling years and two invasion regions.

Table 2.3. Weights and lengths of male and female adult *P. tereticollis* by host species. The number of male worms by host species is given. For female worms, the sample size and percentage of total females is given for each maturity stage: stage 1 = ovarian balls present and acanthors absent, stage 2 = ovarian balls and immature acanthors present but shelled acanthors absent, stage 3 = ovarian balls, immature and mature acanthors present.

Host species	Sex	Length (SE)	Weight (SE)	Maturity stage	n	%
Brown trout	Male	7.15 (0.17)	2.78 (0.20)	-	188	
	Female	8.08 (0.22)	4.60 (0.41)	1	180	77
				2	17	7
				3	37	16
Dace	Male	7.07 (0.39)	1.65 (0.16)	-	28	
	Female	8.34 (0.38)	2.72 (0.28)	1	21	95
				2	1	5
				3	0	0

2.4 Discussion

Our findings on *P. tereticollis* infection, size and maturity in invasive dace indicate that this fish species is an unsuitable host for this dominant parasite in Ireland. Dace acquired adult infections of *P. tereticollis* but at significantly lower levels than brown trout, which is the preferred definitive host in Ireland (Byrne et al. 2003). Prevalence and abundance of adult *P. tereticollis* were lower in dace than in brown trout at both the invasion core and edge, as was the proportion of adults relative to total worms. This reduced infection in dace compared to a sympatric definitive host species from the same sites provides evidence that dace is an incompetent host for *P. tereticollis* as indicated by its infrequent occurrence and low numbers (Hine & Kennedy 1974a).

Traits of adult *P. tereticollis* also indicate poor competency in dace. Worms that established as adults in dace were smaller in weight than those from trout, suggesting slow growth of *P. tereticollis* in dace consistent with descriptions by (Hine & Kennedy 1974a). In Great Britain, dace and brown trout are both regarded as unsuitable hosts for *P. laevis s.l.* – as parasites occur in low numbers and females "rarely" become gravid – but capable of maintaining populations in localities where more preferred definitive hosts are absent (Hine & Kennedy 1974a; Chubb 1982; Kennedy 2003, 2006). In the English rivers studied, between 4.2% and 30%

mature females were reported from dace hosts and between 15% and 71% from brown trout hosts, depending on the river (Kennedy 2006). At 16%, the proportion of mature stage 3 females in brown trout in the current study was lower than previous studies at similar time of year in brown trout from the Burishoole lake system in Ireland which found 28% stage 3 females in June/July (Molloy et al. 1995b) and 78% stage 3 females in August (Byrne et al. 2002). Previous studies on host-acanthocephalan specificity note that acanthocephalan species infecting unsuitable hosts show little reproduction, e.g. *Acanthocephalus clavula* in brown trout in Ireland (Byrne et al. 2004), or no reproduction, e.g. *P. laevis* in grayling *Thymallus thymallus* in England (Hine & Kennedy 1974a). The lack of gravid females in invasive dace in the current study, especially given that the host sample sizes used were an order of magnitude greater than those in the aforementioned English studies, provide strong evidence for and are consistent with dace as an incompetent host for *P. tereticollis*.

The majority of the *P. tereticollis* individuals infecting dace were extra-intestinal subadults, a parasitic form that was comparatively rare in brown trout. Subadult *P. tereticollis* demonstrated an almost opposite pattern to adults with respect to host species, infecting dace at a higher intensity than brown trout in both invasion regions and at higher prevalence and abundance at the invasion core. This high infection with subadults in dace gives a further indication of their poor host competency; *P. tereticollis* and other fish acanthocephalans are known to establish extra-intestinally in unsuitable hosts. Experimental infections of carp (*Cyprinus carpio*), goldfish (*Carassius auratus*) and three-spined stickleback (*Gasterosteus aculeatus*) with the acanthocephalan of eel and chub, *Acanthocephalus anguillae*, resulted in most acanthocephalans encysting outside of the intestine (Taraschewski 1985, 1989). Sures et al. (2003) reported that *P. laevis* had similar overall rates of establishment in experimentally infected chub and goldfish but 21% of worms established intra-intestinally in goldfish compared to 100% in chub. Médoc et al. (2011) found that, following experimental infection of minnow (*Phoxinus phoxinus*) with *P. laevis* cystacanths, 2.9% of *P. laevis* established intra-intestinally as adults and 33.3% established extra-intestinally. Perrot-Minnot et al. (2019) reported extra-intestinal *P. laevis* and *P.tereticollis* in wild minnow, gudgeon (*Gobio gobio*) and black bullhead catfish (*Ameiurus melas*) and extra-intestinal *P. laevis* in three-spined stickleback (*Gasterosteus aculeatus*). In natural infections of invasive round gobies (*Neogobius melanostomus*), both *Pomphorhynchus bosniacus* (Emde et al. 2012; Reier et al. 2019) and *Neoechinorhynchus tenellus* (Gendron & Marcogliese 2016) were observed to penetrate the intestine and encyst in the viscera.

Extra-intestinal subadults may play a role in post-cyclic or paratenic transmission (see Odening 1976) in other freshwater fish-acanthocephalan systems (Kennedy 2006; Médoc et al. 2011b; Gendron & Marcogliese 2016), however, I find it unlikely that dace in Ireland is involved in paratenic transmission of *P. tereticollis* to the preferred brown trout definitive host. In piscivorous brown trout, the mean length of prey fish tends to be approximately a third of the length of the predator (L'Abée-Lund et al. 1992; Jonsson et al. 1999). In the current study, while small fish (mainly sticklebacks) were observed in some brown trout stomachs, no dace parts were observed in brown trout stomach contents. Moreover, as no dace under 100 mm in standard length were infected, infected dace would be too large a prey for any of the brown trout in the size range captured in this study. Therefore, the presence of subadult *P. tereticollis* in dace is likely a dead end for the parasite as opposed to a route for paratenic transmission.

I observed a highly aggregated distribution of subadult worms in dace, with particularly high aggregation of subadults at the invasion edge. Interestingly adults in dace were not as highly aggregated, nor were subadults in brown trout. My finding of higher overall aggregation differs from previous work on aggregation in invasive species which reported lower aggregation of acquired helminths in invasive fish with respect to native fish (Sarabeev et al. 2017). Aggregated distributions are typical in macroparasite populations and may be driven by many processes that influence host exposure and parasite establishment such as heterogeneity in environmental factors, host immunity and parasite infectivity (Gourbière et al. 2015; Warburton & Vonhof 2018; Tinsley et al. 2019). Given that adults and subadults show strikingly different patterns of aggregation in dace but both forms arise from the same infection pathway, i.e.

exposure to cystacanths from ingested amphipods, it is likely that the observed aggregated distribution of subadults arises from processes that influence parasite establishment rather than variation in exposure.

For both adult and subadult *P. tereticollis*, I observed that infections were absent in small dace but increased steeply in fish greater than 130 mm standard length. This is in contrast to brown trout in which infections were acquired gradually across size classes or were unaffected by host length. As length is a reliable indicator of fish age (Britton 2007), this implies that young dace avoid infection in a way that brown trout do not. Limited exposure due to diet may in part explain the absence of infection in young dace. While the diet of juvenile brown trout in Ireland is dominated by invertebrates (Kennedy & Fitzmaurice 1971; Kelly-Quinn & Bracken 1990), vegetation and detritus form a large part of the diet of juvenile dace (Mann 1974). If this is true for invasive dace in Ireland, the steep increase in parasite acquisition in larger dace may represent a switch to a more invertebrate-rich diet as dace age. Indeed, the high infection intensities of subadults in older dace from the invasion core show that they are feeding intensively on the infected intermediate host, *Gammarus duebeni.* In light of this, it is likely that the low adult prevalence and abundance in this long-established dace population is due to the parasite's failure to successfully establish intra-intestinally as opposed to lack of exposure.

I hypothesised that dace would be more likely to be infected and have higher worm burdens at the invasion core where they had been established longest. However, since prevalence of adult worms was higher in the core for both host species, other intrinsic factors such as intermediate host abundance in addition to invasion history may be responsible for the observed differences in prevalence between the two river systems. Previous work on the acanthocephalan *Echinorhynchus truttae* in brown trout has suggested that differences in intermediate host presence and/or abundance can drive significant differences in parasite prevalence between adjacent catchments (Couso-Pérez et al. 2018).

These findings posit that, particularly at the invasion core, dace consume infected intermediate hosts of *P. tereticollis* and acquire adult infections but do not themselves become infective, neither by shedding infective eggs nor by acting as paratenic hosts. Since *P. tereticollis* that infect dace are not capable of completing their life cycle, the presence of *P. tereticollis* in dace may act as a sink for parasite infective stages, inhibiting transmission to and diluting infection in other hosts, particularly in the invasion core where *P. tereticollis* infection of dace is high (see Johnson & Thieltges 2010). My finding that the total abundance of *P. tereticollis* in the preferred definitive trout host is reduced at the invasion core compared to the invasion edge supports this dilution hypothesis. Given that I observed reduced infection in brown trout only in the invasion core where dace have been established for over 120 years, my findings imply that the parasite-mediated effects of invasive species are likely to be dynamic over time and that important effects like parasite dilution may only emerge or become apparent in the later stages of invasion.

Dilution of helminths due to invasive species has been previously proposed in several fish systems (e.g. Paterson et al. 2013a; Gagne et al. 2016; Gendron & Marcogliese 2017) and previously in Ireland in small mammals (Telfer et al. 2010; Loxton et al. 2017; Stuart et al. 2020). My finding is based on data from both sampling years combined. However, when 2017 is considered alone, total abundance of *P. tereticollis* in brown trout is higher in the invasion core than at the edge, contrary to my expectation that a dilution effect occurring in the invasion core would become more pronounced over time. This may be explained by an apparent anomalous period for *P. tereticollis* in 2017 in the River Barrow (invasion edge). *P. tereticollis* infection was notably low in both fish species in this region in 2017: 100% of dace and 99% of brown trout were uninfected. I observed during parasitological examination that many gastrointestinal tracts from this sample, particularly in dace, were either empty or contained mostly plant matter so the low prevalence observed at the invasion edge in 2017 may be a result of lack of availability and/or consumption of infected prey. Studies in other fish-helminth systems have also found that infection of some parasite species can be highly dynamic across time (Poulin & Valtonen

2002; Young & MacColl 2017). My observations of the potential temporal variability of *P. tereticollis* infection further underline the importance of multi-year sampling when examining patterns in parasite infection. Furthermore, while my study focussed only on the dynamics of *P. tereticollis* in definitive fish hosts, parasite dilution over a long period of time as result of a “dead end” fish host would also be observable in the parasite prevalence of intermediate hosts. Temporal monitoring of intermediate host density and prevalence in addition to definitive would be advantageous in assessing how the transmission dynamics of *P. tereticollis* is altered throughout the parasite life cycle.

To conclude, while *P. tereticollis* is present in invasive dace in Ireland, dace are unlikely to contribute to maintaining *P. tereticollis* populations as most of the infections occur as subadults encysted extra-intestinally in the mesenteries, adult abundance is low and female worms fail to mature. I propose that invasive dace in Ireland act as incompetent hosts for *P. tereticollis* and, as a result, may dilute infection of this important fish helminth in the preferred definitive host, brown trout.

3. Evidence for enemy release in invasive common dace *Leuciscus leuciscus* in Ireland: a helminth community survey and systematic review

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Author contributions: PAT conducted host sampling in 2017, laboratory dissections, data analysis and wrote the manuscript. JMC, CVH and PAT conceived the study design. JMC assisted in co-ordination of host sampling. SMM conducted host sampling in 2015. EC conducted laboratory dissections. CVH supervised the project. All authors commented on the manuscript.

Ethical statement: Electrofishing and euthanasia of fish was carried out by trained Inland Fisheries Ireland staff. This work was approved by the Trinity College Dublin School of Natural Science's Research Ethics Committee.

3.1 Introduction

Invasive species are recognized globally as one of the largest threats to biodiversity as well as to human health, the environment and the economy (International Union for the Conservation of Nature 2000; Pimentel et al. 2001; Hulme 2014; Lucy et al. 2016). The success of invasive species may be partially explained by their capacity to escape natural enemies such as competitors, predators and parasites in the process of invasion, known as enemy release (Keane & Crawley 2002; Colautti et al. 2004; Liu & Stiling 2006). Evidence is mixed as to whether and to what extent enemy release confers a competitive advantage to invasive species (Blossey & Notzold 1995; Lacerda et al. 2013; Heger & Jeschke 2018). However, there is strong evidence that invasive species host fewer parasite species compared to conspecifics in their native range (Torchin et al. 2003; Goedknegt et al. 2016; Sarabeev et al. 2017; Schoeman et al. 2019).

Parasites are lost in the process of invasion due to a combination of failure to arrive with invading hosts and failure to persist in the new environment (Macleod et al. 2010). Non-native species must overcome a number of barriers to invasion: geographic barriers in order to be introduced to a novel range, growth and reproduction barriers in order to establish new populations, and dispersal barriers in order to invade new ranges (Blackburn et al. 2011). Parasites must overcome the same barriers to invasion as free-living organisms, often with the additional barrier of switching to native hosts in the introduced range, especially if introduced parasites have multi-host life cycles (Lymbery et al. 2014). Moreover, small founding populations of non-native hosts are likely to harbour only a subset of the parasite fauna of the source population (Hatcher & Dunn 2011; Lymbery et al. 2014). Among invaders that co-introduce parasites, freshwater fish are the most common and account for over half of documented cases, probably reflecting the high incidence of invasive fish in freshwater ecosystems (Lymbery et al. 2014). Parasites that are successfully co-introduced alongside their invasive hosts may go on to establish infections in native hosts, known as parasite spillover (Prenter et al. 2004; Dunn & Hatcher 2015; Sures et al. 2019).

While parasites of invasive species are typically lost during the invasion process, invaders often acquire novel parasites as they are exposed to the local parasite community in their invasive range (Sheath et al. 2015; Gendron & Marcogliese 2016; Loxton et al. 2016). Invasive species usually do not accumulate a sufficient number of local parasite species to replace the lost parasite diversity (Torchin & Mitchell 2004; Loxton et al. 2016; Schoeman et al. 2019; Stuart et al. 2020) (although see Gendron et al. 2012; Lacerda et al. 2013). This may be due to low competency of invasive hosts for native parasites (Paterson et al. 2013a; Tierney et al. 2020b) or the absence of host-specific parasites in the invaded range (Kennedy & Bush 1994; Torchin & Mitchell 2004). However, it has been suggested that invasive species are more likely to be infected with parasite species that are directly transmitted (e.g. Monogenea, Myxosporea, Crustacea) or generalist parasite species that can utilize many species of intermediate hosts (Dobson & May 1986; Bauer 1991; Torchin & Mitchell 2004).

Parasite diversity of an invasive host varies across its invasive range and is expected to be lowest at the expanding edge of the range (the invasion front) (White & Perkins 2012; David et al. 2018; Stuart et al. 2020). Host density at an invasion front is typically low which inhibits parasite transmission and parasites are likely to lag behind the establishment of their invasive hosts or be lost along the invasion corridor due to stochastic events or transience of hosts (Phillips et al. 2010). Lags in parasite establishment along an invasion gradient has been observed in fish (Gaither et al. 2013; David et al. 2018), amphibian (Phillips et al. 2010) and small mammal invasions (Stuart et al. 2020). This decrease in parasite infection along an invasion corridor leads to enhanced enemy release at the invasion front compared to the core of the invasive range (site of first introduction and expansion) (White & Perkins 2012).

The cyprinid fish, common dace *Leuciscus leuciscus* (Linnaeus 1758), is native to continental Europe with the exception of Ireland, Spain, Italy and Greece (Welcomme 1988; Kottelat & Freyhof 2007). Common dace was first introduced to Ireland from Great Britain in 1889 by the accidental release of bait fish into the Munster Blackwater River, Co. Cork (Went 1950). Common dace invaded the lower River Barrow in 1992 (Caffrey et al. 2007) and by 2015 their range had spread approximately 90 km upstream (Delanty et al. 2017). Two helminth species (both native to Ireland) have previously been recorded in common dace in Ireland: the eel acanthocephalan *Acanthocephalus clavula* (Kane 1966) and the generalist acanthocephalan *Pomphorhynchus tereticollis* (Chapter 2; Tierney et al. 2020b). The current study is the first survey of the helminth community of invasive common dace to be conducted in Ireland.

The distribution of invasive common dace in Ireland provides an opportunity to study helminth parasite community dynamics of an invasive species at the core and front of its invasive range. By sampling from the invasion core on the Munster Blackwater (over 120 years post-introduction) and from the invasion front on the upper River Barrow (less than five years post-colonization), I can utilize a “space for time” substitution (e.g. Kołodziej-Sobocińska et al. 2018) to study how the helminth parasite community of invasive common dace has changed over time since introduction. In the absence of longitudinal data across the invasion history of an invasive species, a “space for time” substitution method allows one to infer the timing of events in an invasion by studying spatially separate populations at different stages of invasion. I then supplement my own data with information on the helminth species of common dace across their European range to assess the helminth parasite community between the native and invasive range of common dace.

I aim to answer three main research questions: 1) Is there evidence for enemy release i.e. is the parasite diversity of common dace in Ireland reduced compared to common dace in its native range? 2) Have common dace replaced lost diversity by acquiring local parasites in its invasive range? 3) Is there evidence for enhanced enemy release at the invasion front?

At the macro scale, the recorded parasite diversity of a species is likely to vary across its native range due to variation in the diversity of other hosts and heterogeneity in research effort (Poulin et al. 2020). Host diversity is known to drive parasite diversity (Hechinger & Lafferty 2005; Thieltges et al. 2011; Kamiya et al. 2014; Johnson et al. 2016). Due to species extirpations caused by glaciation events, northern and western Europe have a lower native biodiversity of freshwater fish than central and Mediterranean European regions (Reyjol et al. 2007). As islands, Great Britain and Ireland have yet lower native freshwater fish biodiversity than continental Europe (Wheeler 1977). Combining the effect of enemy release and low host biodiversity, I expect helminth parasite diversity in invasive common dace to be lowest in Ireland, at an intermediate level in Great Britain and highest in Continental Europe.

3.2 Methods

Invasive common dace were caught by electrofishing in July and August 2015 and 2017, from the core of the common dace invasive range on the Munster Blackwater and at the invasion front on the upper River Barrow (see Tierney et al. 2020b). Recent fish community surveys have recorded 11 species of fish in the Munster Blackwater comprising six native fish (brown trout *Salmo trutta*, Atlantic salmon *Salmo salar*, European eel *Anguilla anguilla*, lamprey *Lampetra* sp., three-spined stickleback *Gasterosteus aculeatus* and European flounder *Platichthys flesus*) and five non-native species (common dace, Eurasian minnow *Phoxinus phoxinus*, roach *Rutilus rutilus,* stoneloach *Barbatula barbatula* and gudgeon *Gobio gobio*) (Kelly et al. 2014). In the River Barrow, recent large-scale surveys recorded 12 species comprising five native (brown trout, Atlantic salmon, European eel, lamprey and three-spined stickleback) and seven non-native species (common dace, Eurasian minnow, roach, stoneloach, gudgeon, European perch *Perca fluviatilis* and Northern pike *Esox lucius*), along with one non-native hybrid (bream x roach) (Delanty et al. 2017). Native twaite shad *Alosa fallax* and non-native freshwater bream *Abramis brama*, rudd *Scardinius erythrophthalmus* and tench *Tinca tinca* are also likely be present in the River Barrow (Delanty et al. 2017). Over the two sampling years, a total of 168 common dace were collected from the River Barrow and 119 common dace from the Munster Blackwater (Table 3.1). Subsite sample size varied between years due to the distribution and availability of common dace in these rivers. The size range of the sampled dace were similar between regions (Gaussian GLM: df = 285, coefficient ± SE = -0.02 ± 0.03, t = -0.6, P = 0.55) (Table 3.1).

Table 3.1. Sample size and size range (standard length) of common dace *Leuciscus leuciscus* sampled (n = 287).

		2015			2017			Both years		
Location	Site	n	Length range (mm)	Mean length (mm) ± SD	n	Length range (mm)	Mean length (mm) ± SD	n	Length range (mm)	Mean length (mm) ± SD
River Barrow (invasion front)	Monasterevin	40	67-130	110 ± 16	5	89-125	106 ± 17	45	67-130	109 ± 16
	Portarlington	33	100-241	163 ± 45	82	89-206	144 ± 18	115	89-241	150 ± 29
	River Slate	8	115-235	148 ± 42	-	-	-	8	115-235	148 ± 42
	Total	**81**	**67-241**	**135 ± 41**	**87**	**89-206**	**142 ± 20**	**168**	**67-241**	**138 ± 32**
Munster Blackwater (invasion core)	Fermoy	33	98-150	117 ± 13	48	72-194	137 ± 36	81	72-194	129 ± 31
	Glanworth	38	107-210	152 ± 32	-	-	-	38	107-210	152 ± 32
	Total	**71**	**98-210**	**136 ± 31**	**48**	**72-194**	**137 ± 36**	**119**	**72-210**	**136 ± 33**

Table 3.2. Parameters of component and infracommunity structure in invasive common dace *Leuciscus leuciscus* from the front (Upper River Barrow) and core (Munster Blackwater) of its invasive range in Ireland.

	Front			**Core**			**All sites**
	2015	2017	Both years	2015	2017	Both years	
n	81	87	168	71	48	119	287
Mean taxonomic richness ± SD	0.04 ± 0.19	0	0.02 ± 0.13	0.35 ± 0.54	0.54 ± 0.54	0.43 ± 0.55	0.19 ± 0.42
Max. taxonomic richness	1	0	1	2	2	2	2
Total number of taxa	1	0	1	3	2	4	4
Mean Brillouin index ± SD	0	0	0	0.01 ± 0.07	0.001 ± 0.01	0.01 ± 0.05	0.003 ± 0.03
Max. Brillouin index	0	0	0	0.46	0.07	0.46	0.46
Simpson’s index	0	0	0	0.14	0.01	0.04	0.03

Fish were euthanized on-site using anaesthesia by eugenol oil or percussive stunning and decerebration. Fish were bagged and transported on ice to the laboratory where they were frozen and stored at -20°C until dissection. The eyes, gills, alimentary tract, swim bladder and body cavity were examined for helminth parasites. Keys by Yamaguti 1963, Brown et al. 1986, Moravec 1994, Gibson et al. 2002, Jones et al. 2005 and Bray et al. 2008, and the paper by Špakulová et al. 2011 were used for parasite identification. The identification of *Pomphorhynchus tereticollis* was additionally confirmed with molecular analysis (see Tierney et al. 2020b). Five digenean trematode specimens and three nematode specimens found in common dace were unidentifiable beyond Class (Digenea) and Phylum (Nematoda), respectively, because the characteristic features in these specimens were not sufficiently clear to allow for morphological identification.

Statistics were conducted using R (R Core Team 2018). Helminth community structure was analysed at the component and infracommunity levels (Bush et al. 1997) following Kennedy & Hartvigsen (2000). I described component community structure using total number of taxa and Simpson's Index of Diversity calculated as

$$\boxed{D = 1 - \frac{\sum n_i(n_i - 1)}{N(N - 1)}}$$

where n_i is the total number of individuals of taxon i and N is the total number of individuals of all taxa, using the R package vegan (Oksanen et al. 2019). The value of D ranges from zero to one and increases with increased diversity and evenness (Pielou 1966; DeJong 1975).

To describe infracommunity structure, I calculated mean taxonomic richness, maximum taxonomic richness, prevalence, mean intensity (Bush et al. 1997), aggregation (mean to variance ratio; $\sigma^2/\bar{x}$ (Wilson et al. 2002)) and mean and maximum Brillouin's Index of Diversity (Pielou 1966). Brillouin's Index was calculated in R using the formula

$$HB = \frac{\ln(N!) - \sum \ln(n_i!)}{N}$$

where N is the total number of parasite individuals in a host and n_i is the number of individuals of taxon i. Brillouin's index measures the diversity of a fully cencussed collection where HB increases with increased diversity (Pielou 1966).

Prevalence of the acanthocephalan, *Pomphorhynchus tereticollis,* was modelled with a binomial Generalised Linear Mixed Model (GLMM) and intensity of *P. tereticollis* was modelled with a negative binomial GLMM using the R package glmmTMB (Brooks et al. 2017). Data on *P. tereticollis* combined intra-intestinal adult and extra-intestinal subadult parasite individuals (see Tierney et al. 2020b). Models incorporated invasion region (front or core) and host standard length as fixed effects, and subsite and year as random effects. Model fit was validated using the R package DHARMa (Hartig 2019). All other helminth taxa detected in common dace occurred at too low prevalence and intensity to be fitted to a model.

A systematic literature search of helminth parasites of common dace across their European range was conducted in Web of Science using the search string (dace OR "*Leuciscus leuciscus*") AND (parasit* OR helminth) and in Google Scholar using search terms "*Leuciscus leuciscus*" "dace" "parasite" "helminth". Additional searches were conducted by replacing "*Leuciscus leuciscus"* with "freshwater fish". Records of non-helminth parasites, experimental infections and records of the subspecies Siberian dace, *Leuciscus leuciscus baicalensis,* were excluded. The Natural History Museum Host-Parasite database (Gibson et al. 2005) and references in the collected literature were also checked for additional relevant material that had not been captured in the literature search.

3.3 Results

3.3.1 Helminth community survey

Four helminth taxa were recorded in common dace across their invasive range in Ireland: one acanthocephalan species, one monogenean species, and a number of nematode and digenean trematode individuals which were unidentifiable to species level. All four taxa were present in common dace at the invasion core (although during different years) and one species was recorded at the invasion front. At the invasion core, I recorded three and two helminth taxa in 2015 and 2017, respectively (Table 3.2). At the invasion front, I recorded one helminth species in 2015 but did not detect any helminths in 2017. Helminth community diversity in dace was greater at the invasion core than the invasion front, as measured by taxonomic richness, total number of taxa, Brillouin's Index and Simpson's Index (Table 3.2). The maximum taxonomic richness per fish was two, which was recorded in both years at the invasion core. Here, Brillouin's and Simpson's Diversity indices were lower in 2017 than 2015 but mean taxonomic richness increased from 2015 to 2017.

The majority of common dace (82%) were uninfected. Among the four helminth taxa recorded in common dace, the acanthocephalan *Pomphorhynchus tereticollis* was the most prevalent (Table 3.3). The prevalence of *P. tereticollis* ranged from zero at the front in 2017 to 52% (95% confidence interval: 37 - 67) at the core in 2017. At the invasion core, prevalence of *P. tereticollis* was significantly higher than at the front (Binomial GLMM: df = 282, coefficient ± SE = 6.02 ± 1.49, Z = 4.05, P < 0.001). Intensity of *P. tereticollis* infection did not differ significantly between the invasion core and front (Negative binomial GLMM: df = 41, coefficient ± SE = 0.83 ± 1.30, Z = 0.63, P = 0.53). Both prevalence (Binomial GLMM: df = 282, coefficient ± SE = 0.07 ± 0.01, Z = 5.71, P < 0.001) and intensity (Negative binomial GLMM: df = 41, coefficient ± SE = 1.05 ± 0.17, Z = 6.35, P < 0.001) of *P. tereticollis* increased with host size (see also Tierney et al. 2020b). *P. tereticollis* infection at the front was highly aggregated with a small number of infected fish (3.7%) hosting high worm burdens (mean intensity ± SD = 48 ± 35) (Table 3.3). *P. tereticollis* was the only helminth that infected common dace populations at the invasion front. The other three helminth taxa all occurred in few hosts and in low numbers (prevalence less than 6% and intensity less than 2; Table 3.3). The monogenean *Discocotyle sagittata* was represented by only a single individual, recorded in the core in 2017. The three nematode individuals and five digenean trematode individuals found in common dace were all recorded from the invasion core in 2015.

Table 3.3. Population biology parameters of helminth taxa recorded in common dace *Leuciscus leuciscus* at the front (Upper River Barrow) and core (Munster Blackwater) of their invasive range. n represents the number of infected hosts. N represents the number of helminth individuals.

				Front					Core				
Helminth	Taxon	Host tissue(s)	Year	n	N	Prevalence (95% CI)	Intensity ± SE	$\sigma^2/\bar{x}$	n	N	Prevalence (95% CI)	Intensity ± SE	$\sigma^2/\bar{x}$
Pomphorhynchus tereticollis	Acanthocephala	Intestine, mesenteries	2015	3	144	3.7 (1-10)	48.0 ± 35.2	99.2	19	100	26.8 (16-39)	1.1 ± 1.3	8.4
			2017	0	0	0	-	-	25	314	52.1 (37-67)	12.6 ± 3.3	27.5
Discocotyle sagittata	Monogenea	Gills	2015	0	0	0	-	-	0	0	0	-	-
			2017	0	0	0	-	-	1	1	2.1 (0-11)	1	1
Unidentified nematodes	Nematoda	Intestine	2015	0	0	0	-	-	2	3	2.8 (0-10)	1.5 ± 0.5	1.6
			2017	0	0	0	-	-	0	0	0	-	-
Unidentified digenean trematodes	Digenea	Intestine	2015	0	0	0	-	-	4	5	5.6 (2-13)	1.3 ± 0.3	1.4
			2017	0	0	0	-	-	0	0	0	-	-

3.3.2 Systematic review

The literature search yielded 443 records of 109 helminth species from 20 European countries (full dataset available at https://doi.org/10.1017/S0022149X20000759). For the purposes of this study, locations were categorized into four broad biogeographical ranges: Ireland, Great Britain, Nordics, and Continental Europe (excluding Nordics) (adapted from Reyjol et al. 2007). I combined my own survey data for helminth species present with the previous record for *A. clavula* in common dace in Ireland to give a total of three helminth species recorded in invasive common dace in Ireland, representing two acanthocephalan species and one monogenean species. This number was low compared to the number of helminth species in the native range of common dace in Great Britain, the Nordics and Continental Europe (Figure 3.1). Digenean trematodes are the most commonly recorded helminth parasite species of common dace in Great Britain, the Nordics and Continental Europe (Figure 3.1). In the Nordics and Continental Europe, monogeneans are the next most common, while in Great Britain, similar numbers of acanthocephalan, cestode and monogenean species are recorded.

Although every effort was made to be comprehensive, it was not possible to gather information relating to specific site and sample size for every record due to some data being drawn from checklists that did not report such details. In total, I obtained site data for 163 records. The number of papers that recorded helminth species in common dace (as a rough proxy for sample effort) varied between countries and sites (Appendix Table B1). I note that this approach is likely to underestimate sample effort where records came from existing checklists, which were coded as one paper. Additionally, some papers included in the systematic review specifically studied only a single parasite species or taxon including Thomas & Ollevier (1992) on *Anguillicola crassus* (Belgium), Zrnčić et al. (2009) on *Posthodiplostomum cuticula* in Croatia, Kirk & Lewis (1994) on *Sanguilicola* spp. in Great Britain (Anglian and Thames regions) and Perrot-Minnot et al. (2019) on *Pomphorhynchus laevis* and *P. tereticollis* in France. Where relatively comprehensive surveys of the helminth community have been conducted in common dace, the number of helminth species in the component community is similar across the native range. For example, in England, a total of 10 species of helminth have been recorded in common dace in the River Avon (Kennedy 1974), 14 species in the River Kivijoki system in Finland (Ieshko et al. 1997), nine species in the River Morava in the Czech Republic (Gelnar et al. 1994) and 10 species in the Tamis River region in Serbia (Djikanovic et al. 2012) (Appendix Table B1).

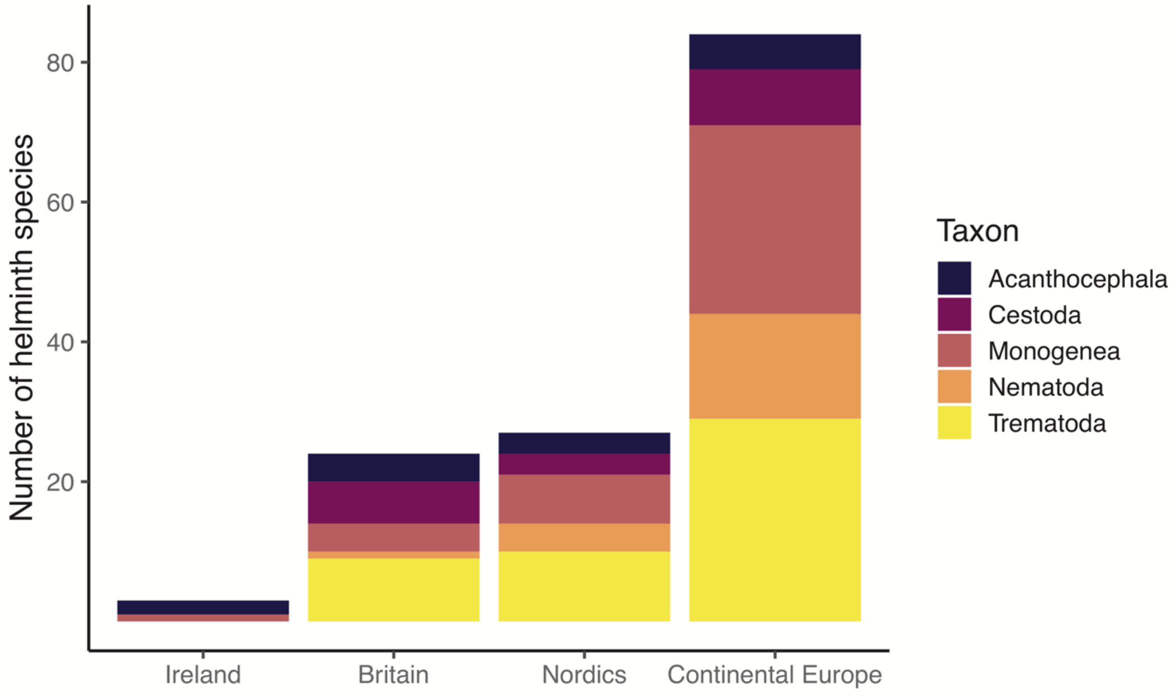

Figure 3.1. Total number of helminth species recorded in common dace *Leuciscus leuciscus* in its invasive range in Ireland and its native range in Great Britain, the Nordics and Continental Europe.

3.4 Discussion

Invasive common dace in Ireland had a lower diversity of helminth parasites compared to common dace in their native range and a lower diversity of helminth taxa at the invasion front compared to the invasion core. The total number of species recorded in dace was markedly lower in Ireland compared to Great Britain, the Nordics and Continental Europe where common dace are native. These findings support the hypothesis that common dace have lost parasites in the process of invasion and have experienced release from helminth parasites. Populations of common dace at the long-established invasion core had higher component and infracommunity diversity than those at the recently colonized invasion front. This supports the hypotheses that enemy release is enhanced at the invasion front and more recently established populations host less diverse parasite communities.

The lower number of total helminth species in common dace in Ireland compared to Great Britain is as expected. Holland & Kennedy (1997) found a reduced parasite fauna in most freshwater fish in Ireland compared to Great Britain and demonstrated that the helminth parasites found in Ireland are a subset of the British parasite fauna. In the literature review, I found fewer helminth parasites in Britain compared to Continental Europe but the difference in scale and in sample effort between these areas must be noted. It would be expected that a geographically smaller area would have lower biodiversity (Rosenzweig 1995). Moreover, there are fewer papers reporting helminths in common dace in Great Britain than in Continental Europe, probably by virtue of fewer researchers specialising in fish parasitology. Nonetheless, when I compared Great Britain and Europe at a similar scale, the parasite community of common dace at the population level has similar richness across the native range. For example, the River Avon in England and the River Morava in the Czech Republic have both been well-studied (see Kennedy 1974; Moravec 2001) and have similar numbers of recorded helminth species. My finding of enemy release in invasive common dace compared to Great Britain remains valid when examined at the scale of river/host population. Esch et al. (1988) studied three English rivers and found between eight and nine helminth species infecting common dace populations. This is in contrast to my findings of zero to four helminth taxa in total at the population level in Irish rivers. These considerations highlight the limitations of producing checklists of species: that biases are likely to exist in sampling effort, geographic coverage and possible inclusion of accidental infections (Holland & Kennedy 1997; Poulin et al. 2015, 2020; Poulin 2019). Helminth parasite species richness is likely to have been underestimated in some regions due to lack of study and, in some cases (e.g. France (Perrot-Minnot et al. 2019) and Croatia (Zrnčić et al. 2009)), the available records focussing on a given helminth species or taxon rather than the complete helminth community.

I did not find evidence that common dace co-introduced novel helminth species to Ireland when introduced from Great Britain. *P. tereticollis* is considered native to Europe, including the British Isles (Perrot-Minnot et al. 2018). It is hypothetically possible that genetically distinct strains of *P. tereticollis* could have been introduced from Great Britain with the introduction of common dace (O'Mahony et al. 2004a, b). However, given that the Irish strain of the parasite is considered to be widespread and any founder population of genetically distinct strains co-introduced with dace would have been small, such an introduction would have been unlikely to impact the native *P. tereticollis* strain. *D. sagittata* is similarly widespread throughout Ireland and Great Britain (Kennedy 1974; Molloy et al. 1993; Holland & Kennedy 1997; Byrne et al. 2002). As the nematode and digenean trematode specimens recovered from common dace were unidentifiable, I cannot state whether they represent novel species to Ireland. However, considering that the prevalence and intensity of these taxa were so low, it is unlikely that these would represent co-introduced species maintained by the invasive population of common dace. *D. sagittata*, nematodes and digeneans were rare in common dace and each taxon was present only in one locality in one year, respectively. This could be an indication that these were accidental infections of parasites maintained by other hosts and not regular components of the dace parasite community (e.g. as with accidental infections of *Crepidostomum* sp. in eel in Thomas (1958)).

I found notably few monogeneans and digenean trematodes in common dace in Ireland, although they are among the most frequently reported taxa in the native range. The generalist digenean eye flukes of the family *Diplostomidae* are frequently acquired parasites of invasive fish species (Ondračková et al. 2009; Francová et al. 2011; Lacerda et al. 2013; Tyutin et al. 2013; Gendron & Marcogliese 2017). In Ireland, there was a notable absence of eye flukes in dace, despite eight species of the family *Diplostomidae* having been previously recorded in introduced cyprinids in Ireland (Holland & Kennedy 1997). Given that diplostomids are known to detrimentally impact the foraging ability and anti-predator response of their fish host (Crowden & Broom 1980; Seppälä et al. 2011; Lacerda et al. 2013; Gopko et al. 2017), the release from eye flukes in Ireland may be advantageous for invasive common dace. Monogeneans, being directly transmitted, theoretically should be less likely to be lost during invasion since additional intermediate hosts are not required for their persistence. Host-specific monogenean parasites have been found to have been successfully co-introduced in a number of invasions and capable of host switching to native fish (Galli et al. 2007; Sarabeev et al. 2018; Šimková et al. 2019). Conversely, several studies have refuted that directly transmitted parasites make especially successful invaders. Lymbery et al. (2014) found no association between parasite life cycle and host switching, and Lyndon & Kennedy (2001) found that the indirectly-transmitted acanthocephalans are the most successful parasite colonizers of the British Isles. Holland & Kennedy (1997) also noted that monogeneans and digeneans are both poorly represented in the Irish helminth parasite fauna compared to that of Great Britain. The success of acanthocephalans as colonizers was mirrored in the present study; two of the three species recorded in common dace in Ireland were acanthocephalans. In contrast, the Acanthocephala make up 19% of total parasite species in common dace in Great Britain and 6% of total parasite species in common dace in Continental Europe. Moreover, the acanthocephalan *P. tereticollis* was the only helminth to infect common dace at the invasion front.

Helminth diversity in common dace was particularly low at the invasion front. This population of common dace had effectively no parasite diversity, being infected with only one species, the generalist acanthocephalan *P. tereticollis* (see Tierney et al. 2020). Moreover, that species was present in only one year at low prevalence and intensity, raising the possibility that stochastic events may have caused it to disappear from the front population in the latter of the two sampling years. Additionally, given that parasites tend to be spatially aggregated in their environment (Sherrard-Smith et al. 2015), it is possible that differences in subsite sample sizes between 2015 and 2017 resulted in differences in detection between years. Nonetheless, my findings of low helminth diversity at the invasion front support theoretical predictions (Phillips et al. 2010; White & Perkins 2012) and other empirical studies (Gendron et al. 2012; Loxton et al. 2016; David et al. 2018; Stuart et al. 2020) that demonstrate that parasite diversity is lowest in most recently invaded parts of the range. These findings also fit the colonization time hypothesis that areas recently colonized by host species have the lowest diversity of parasites (Guégan & Kennedy 1993).

I expected that invasive species acquire local parasites over time (Kvach & Winkler 2011; Emde et al. 2012; White & Perkins 2012). However, parasite diversity in common dace was low even in the invasion core where common dace have been established for over 120 years, especially considering the possibility that dace may be merely an accidental host for some of the recorded taxa. The acquisition of local parasite species by invasive common dace seems markedly slow compared to other fish invaders e.g. Ponto-Caspian gobies. Gendron et al. (2012) found that, following an initial period of parasite release, invasive round goby *Neogobius melanostomus* in the Great Lakes accumulated native parasites and had similar parasite diversity to native species by 15 years post-colonization. Francová et al. (2011) found that round goby acquired native parasites and reported no difference between the parasite species richness of native and invasive round goby populations in the River Danube. In contrast, Kvach & Stepien (2008), studying invasive round goby and tubenose goby *Proterorhinus semilunaris* in the Great Lakes, found lower parasite species richness compared to the native Ponto-Caspian range and that most parasite species were rare, but observed no increase in parasite richness over 10 years. Both Kvach & Stepien (2008) and Gendron et al. (2012) recorded that the helminth parasite communities of invasive gobies were composed entirely of acquired native parasites with no evidence of goby specialists or helminths co-introduced by gobies.

The low helminth diversity of common dace in Ireland may be similarly due to a lack of co-introduced cyprinid-specific parasites and an absence of suitable parasites in the invaded range. Ireland has a depauperate freshwater fish fauna, dominated by salmonids and other anadromous fish (Wheeler 1977; Fahy 1989). Furthermore, Ireland has no native cyprinids, although a number of cyprinid species such as bream, rudd and roach have been introduced prior to the 20th century (Fitzmaurice 1984; Fitzsimons & Igoe 2004). The River Barrow has a relatively rich community of other non-native cyprinid species (Delanty et al. 2017) which one might expect would promote greater parasite diversity in common dace. However, I observed markedly low parasite diversity in this river. Given that Ireland's cyprinid fauna are the result of introductions, and presumably underwent parasite loss over the course of their invasions, there may be few cyprinid-specific helminth parasite species present in the local parasite community adapted to infect common dace. If cyprinid specialist parasites are present, their transmission may be diluted by the presence of dominant native hosts such as salmonids. Adaptation of local parasites to introduced common dace may take some time. For example, the parasite community of introduced bream in Ireland comprises 16 species, similar to the total number of helminth species in its native British range (Holland & Kennedy 1997). However, this may be explained by the length of time since colonization; unlike common dace, bream are thought to have been present in Ireland since around the 5th century (Hayden et al. 2010). Furthermore, in the ecological context of Ireland where biodiversity is known to be low, it may not be possible for an invasive species to regain similar parasite richness to its native parasite diversity without the occurrence of subsequent invasion and co-introduction events to introduce suitable and/or specific parasites.

3.5 Conclusions

Invasive common dace in Ireland are infected with a considerably less diverse parasite community than common dace in their native ranges in Britain and Continental Europe, supporting the hypothesis that this invasive species has undergone enemy release. The generalist acanthocephalan *P. tereticollis* was the only helminth parasite species detected in the common dace populations at the invasion front and all helminth taxa found at the invasion core, other than *P. tereticollis* were rare, despite this population being established for over 120 years. These findings provide evidence that not only do recently established populations host less diverse parasite communities, but that enemy release may persist in invasive populations long after establishment. This apparent slow acquisition of local parasites by common dace may be explained by the biogeographical and ecological context of Ireland where biodiversity is low and native cyprinids are absent.

4. Structure and composition of helminth communities in brown trout (*Salmo trutta*) and dace (*Leuciscus leuciscus*) in Ireland

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Author contributions: PAT conducted host sampling in 2017, laboratory dissections, data analysis and wrote the chapter. JMC, CVH and PAT conceived the study design. JMC assisted in co-ordinated of host sampling. SMM conducted host sampling in 2015. CVH supervised the project.

Ethical statement: Electrofishing and euthanasia of fish was carried out by trained Inland Fisheries Ireland staff. This work was approved by the Trinity College Dublin School of Natural Science's Research Ethics Committee.

4.1 Introduction

Freshwaters are biodiverse ecosystems, both in terms of free-living and parasitic organisms. Freshwaters (rivers, lakes and reservoirs) occupy just 2% of Earth's surface, yet contain over 9% of earth's animal biodiversity (Reid et al. 2019). Contrastingly, marine environments cover 71% of the Earth's surface but have similar species richness of ray-finned fishes (Actinopterygii) to freshwaters (Vega & Wiens 2012). It has been proposed that the fragmented nature of freshwater ecosystems, leading to heterogeneity and geographic isolation of freshwater habitats, has driven the diversification of freshwater free-living fauna and, in turn, their parasites (Poulin 2016). Even so, the parasitic communities of freshwater fish populations are often species-poor and isolationist in nature compared to other host taxa, exhibiting a depauperate parasite fauna with little interspecific interaction between parasites (Kennedy

2009). For example, Kennedy et al. (1986) found that freshwater fish communities were low in species richness and diversity compared to similar communities of aquatic birds.

In recent decades, efforts have been made to explain the structuring of helminth parasite communities and, in particular, to describe the processes that lead to variation in parasitic communities (Poulin & Valtonen 2002). Parasite communities of hosts are shaped by many factors including host vagility, the breadth of the host diet, sequence of infection and time since the host colonised that environment (Kennedy et al. 1986; Kennedy & Bush 1994; Karvonen et al. 2019). Within a host species, parasite communities can be unpredictable over space and time, due to stochastic local or temporal effects (Esch et al. 1988; Dezfuli et al. 2001; Poulin & Valtonen 2002; Kennedy 2009).

The composition and structure of parasite communities within a host population may also be influenced by the presence of co-occurring, diverse host species, including invasive hosts. For example, a native host population may acquire novel parasites introduced by a co-occurring invasive host (Lymbery et al. 2014). Additionally, in cases where parasite species are present in the parasite communities of both invasive and native hosts, invasion-related effects on parasite dynamics such as spillback (Kelly et al. 2009; Hohenadler et al. 2019) and dilution (Gendron & Marcogliese 2017; Tierney et al. 2020b) can occur. Invasive populations often have species-poor parasite communities compared to sympatric native populations, as predicted by the enemy release hypothesis (Keane & Crawley 2002; Torchin et al. 2003).

Nested patterns are often observed in the community ecology of free-living organisms, where species-rich assemblages tend to contain many species specific to that assemblage whereas species-poor assemblages tend to be composed of a generalist subset of the larger biota (Wright et al. 1998). Parasite communities also contain species that are generalist (infecting a wide range of hosts) and specialist (specific to one or few hosts) (Valtonen et al. 2001). Parasite communities are likely to exhibit similar pattern of nestedness, where depauperate parasite communities, such as those of invaders, are composed of few generalist parasite species that are shared with the richer parasite communities of native species (Poulin et al. 2001; Poulin & Valtonen 2002).

The spread of invasive dace *Leuciscus leuciscus* in Ireland, in recent years, has raised concerns over the potential impacts on co-occurring native freshwater fish, particularly brown trout, *Salmo trutta* (Caffrey et al. 2007; Delanty et al. 2017). Although common dace and brown trout represent different fish families (Cyprinidae and Salmonidae respectively), competition for food and habitat between these species in invaded rivers in Ireland is not unlikely (Caffrey et al. 2007; Barry et al. 2020). Invasive dace and native brown trout in Ireland have been found to share habitat and have similar spatial ecology, despite having different life histories (Barry et al. 2020). This raises the possibility that dace and brown trout may be exposed to, and interact with, a shared community of helminth parasites through preying on infected freshwater macroinvertebrates, in the case of trophically transmitted helminths (Acanthocephala, Cestoda, Nematoda, adult Dignenea), or through sharing habitat, in the case of helminths acquired passively through the environment (Monogenea, some larval Digenea).

Brown trout are common and widespread in freshwater systems in Ireland (Kelly et al. 2015) and the helminth community of brown trout has received more research attention than most, if not all, other freshwater fish species in Ireland. Previous studies on brown trout helminth communities in Ireland have found that the helminth communities are isolationist (Kennedy & Hartvigsen 2000), variable in composition (Byrne et al. 2002) and dominated by autogenic species (Molloy et al. 1995a). Autogenic species are those helminth species that utilise fish as definitive hosts, as opposed to allogenic species that utilise birds and mammals (Esch et al. 1988). However, much of the previous work on brown trout helminth communities has focussed on lentic (lake) systems and taken place in the west of Ireland (e.g. see also Conneely & McCarthy 1984; Molloy et al. 1993; Byrne et al. 2003). The helminth communities of brown trout in lotic (riverine) systems and in the south and east of the country have been largely neglected in the literature to date. Lentic and lotic ecosystems differ fundamentally in terms of environmental variables such as flow velocity (Marsh & Fairbridge 1999) and the environmental variables that characterise lentic and lotic systems are known to have a strong influence on the distribution of aquatic macroinvertebrates (Buffagni 2021). Since many of the helminth parasites recorded in brown trout in Ireland to date are trophically-transmitted through the consumption of macroinvertebrate intermediate hosts (Byrne et al. 2002), the environmental context of lentic or lotic systems may be important in structuring the helminth communities of brown trout.

In this chapter, I firstly describe the helminth parasite community of the native freshwater fish, brown trout *Salmo trutta,* over two sampling years in two previously unstudied river systems in Ireland: the Munster Blackwater in the southwest of Ireland and the upper River Barrow in the east of Ireland. Secondly, I compare the helminth community of brown trout to that of invasive common dace *Leuciscus leuciscus,* an abundant co-occurring non-native freshwater fish, and assess the degree of overlap in their helminth communities and potential for parasite acquisition and spillback. Finally, I compare the results of my study of brown trout to the previous 40 years of helminth surveys of brown trout in Irish freshwater and discuss how the characteristics of lentic and lotic systems and distribution of aquatic invertebrate intermediate hosts offer explanations for the differences in helminth parasite community composition among brown trout populations.

4.2 Methods

Samples of 249 brown trout and 287 common dace were collected by electrofishing from two rivers in Ireland in July and August 2015 and 2017: the Munster Blackwater (at Fermoy 52°08'26.4"N 8°16'15.9"W, River Funsion 52°09'25.3"N 8°14'30.5"W, Glanworth 52°11'17.0"N 8°21'12.0"W) and the upper River Barrow (at Portarlington 53°09'43.4"N 7°11'34.2"W, Monasterevin 53°08'44.6"N 7°04'13.2"W and River Slate 53°13'11.0"N 6°59'45.0"W) . Subsites varied between years due to the distribution and availability of fish (Table 4.1). A total of 137 brown trout and 119 common dace were collected from the Munster Blackwater and 112 brown trout and 168 common dace from the River Barrow. Fish were euthanized on-site using anaesthesia by eugenol oil or percussive stunning and decerebration.

Fish were transported on ice to the laboratory where they were frozen and stored at -20°C until dissection. Fish were weighed (g), standard length was measured and sex was recorded. Sex was recorded as undetermined for fish without visibly developed gonads. Upon dissection, the eyes, gills, alimentary tract and swim bladder were removed, washed with 0.9% saline and examined for helminth parasites. The body cavity was also washed and examined. Keys by Brown et al. (1986); Moravec (1994); Gibson et al. (2002); Jones et al. (2005); Bray et al. (2008) and Yamaguti (1963) and the paper by Špakulová et al. (2011) were used for parasite identification.

Statistics were conducted using R (R Core Team 2018). I analysed helminth community structure at the component and infracommunity levels as described by Bush et al. (1997) following Kennedy & Hartvigsen (2000) and Behnke et al. (2001). The measures of component community structure used were total number of helminth species, the Berger-Parker Dominance Index, and Simpson's Index of Diversity. The Berger-Parker Dominance Index was calculated as $d = Nmax/N$ where $Nmax$ is the number of individuals of the most abundant species and N is the total number of helminth individuals in the sample. Note that the value of d increases with increased dominance (i.e. a reduction in diversity). Simpson's Index was calculated as $D = 1 - (\Sigma n_i(n_i - 1)/N(N - 1))$ where n is the total number of individuals of species i and N is the total number of individuals of all species, using the R package vegan (Oksanen et al. 2019). The value of D ranges from zero to one and increases with increased diversity and evenness (Pielou 1966; DeJong 1975).

The measures of infracommunity structure calculated were mean species richness per fish, maximum species richness per fish, mean and maximum Brillouin's Index of Diversity (Pielou 1966), mean number of helminth individuals per fish, prevalence, mean abundance and mean intensity of individual helminth species. Brillouin's Index was calculated as $HB = (\ln(N!) -$

$\Sigma\ln(n_i!))/N$ where N is the total number of parasite individuals in a host and n_i is the number of individuals of species i. Brillouin’s index measures the diversity of a known collection where HB increases with increased diversity (Pielou 1966).

Table 4.1. Sample sizes, length ranges and sex ratio of brown trout and common dace used in this study, grouped by sample site.

			2015				2017			
Species	Region	Site	n	Length range (mm)	Mean length ± SD	Sex ratio (M:F)	n	Length range (mm)	Mean length ±SD	Sex ratio (M:F)
Brown trout	Munster	Fermoy	39	129-360	202 ± 44	5:17	35	112-231	174 ± 30	11:20
(*Salmo trutta*)	Blackwater	Funsion	-	-	-	-	26	120-214	165 ± 28	5:14
		Glanworth	37	138-297	227 ± 40	3:8	-	-	-	-
		Total	76	129-360	214 ± 44	14:41	61	112-231	170 ± 29	8:17
	River Barrow	Monasterevin	2	183-202	193 ± 13	1:1	1	204	204	1:0
		Portarlington	6	177-252	216 ± 26	1:3	70	106-204	144 ± 25	17:44
		River Slate	33	79-354	204 ± 64	11:14	-	-	-	-
		Total	41	79-354	205 ± 58	13:18	71	106-204	145 ± 25	9:22
Common dace	Munster	Fermoy	33	98-150	117 ± 13	1:1	48	72-194	137 ± 36	15:17
(Leuciscus	Blackwater	Glanworth	38	107-210	152 ± 32	4:3	-	-	-	-
leuciscus)		Total	71	98-210	136 ± 31	14:11	48	72-194	137 ± 36	15:17
	River Barrow	Monasterevin	40	67-130	110 ± 16	6:1	5	89-125	106 ± 17	1:0
		Portarlington	33	100-241	163 ± 45	9:8	82	89-206	144 ± 18	31:34
		River Slate	8	115-235	148 ± 42	1:0	-	-	-	-
		Total	81	67-241	135 ± 41	17:9	87	89-206	142 ± 20	33:34

Prevalence, abundance and intensity of each helminth species were modelled using Generalised Linear Mixed Models (GLMMs) from the R package glmmTMB (Brooks et al. 2017). Helminth species occurring at less than 3% prevalence were not modelled because of insufficient data. Prevalence was modelled with a binomial distribution. Abundance and intensity were modelled with negative binomial distributions with log link functions. Initial models included subsite as a random variable and host standard length, host sex (male, female and sex unidentified), region (Munster Blackwater and River Barrow) and year (2015 and 2017) as fixed effects. Models were simplified using stepwise selection and the fit of the final models were validated using diagnostics from the R package DHARMa (Hartig 2019). GLMMs did not satisfactorily fit to the data for species richness so the effects of region and host length were, respectively, tested using the non-parametric tests, Wilcoxon-Mann-Whitney U test and Kendall's Tau correlation (gives a correlation coefficient τ between -1 and 1).

Data on the composition of brown trout component communities in other Irish rivers and lakes were collected from ten previous helminth surveys conducted in the last 40 years (Fitzgerald & Mulcahy 1983; Conneely & McCarthy 1984; Molloy et al. 1993, 1995a; Byrne et al. 2000, 2002, 2004; Mc Carthy & Mc Carthy 2004; Faherty & Mc Carthy 2006; Maguire 2018). Studies older than 40 years were generally inaccessible. The above is comprehensive of the published literature in past 40 years apart from one survey (Conneely & McCarthy 1988) which was inaccessible. This yielded data on 16 brown trout populations from 12 study sites (nine lakes, two rivers and one mixed river and lake system). Jaccard dissimilarity coefficients were calculated for each pairwise combination of helminth communities using the vegan package in R, computed as $(A+B-2J)/(A+B-J)$ where A and B are the respective numbers of species in compared communities, and J is the number of species that occur in both compared communities. Jaccard dissimilarity measures the dissimilarity in composition of two communities as a value between 1 (no overlap between community composition) and 0 (identical community composition) (Poulin 2003).

4.3 Results

4.3.1 Component community structure

Brown trout were infected with more species of helminth than dace in both the Munster Blackwater and River Barrow. Over the two study years, nine and seven species of helminth were recorded in brown trout in the Munster Blackwater and River Barrow, respectively, compared to four and one in common dace (Figure 4.1, Table 4.3a). In brown trout, a total of ten species of helminth were recorded, comprising six nematodes, two trematodes, one acanthocephalan and one monogenean (Table 4.2). Three species, *Rhabdochona* sp., *Diplostomum* sp. and *Diplozooan paradoxum*, were detected only in brown trout in the Munster Blackwater. One nematode species, *Raphidascaris acus*, was represented by only a single individual recorded in brown trout in the River Barrow. I collected a small number of digenean trematodes from the gastrointestinal tract of brown trout which I was unable to identify to species level. I was also unable to identify the small number of Nematoda and Digenea recovered from dace (see Tierney et al. 2020a). For the purposes of comparing community diversity parameters between and among host species and localities, each of these three unidentified taxa was treated effectively as a species.

The helminth species of dace are recorded in Chapter 3, Table 3.3 (see also Tierney et al. 2020a). For the benefit of comparision with the brown trout helminth community, the list of helminth species recorded in dace is included in Table 4.2 and measures of component and infra-community richness are reported in Table 4.3. I detected no cestode species in either host fish species.

Table 4.2. Helminths recovered from brown trout and common dace by taxon, host tissue, life cycle, allogenic or autogenic status, and host species.

Taxon	Species	Host tissue	Life cycle	Allogenic/ autogenic	Host
Acanthocephala	*Pomphorhynchus tereticollis*	IN, M	Indirect	Autogenic	Bt, Cd
Nematode	*Cucullanus truttae*	IN, PC	Indirect	Autogenic	Bt
Nematode	*Cystidicola farionis*	SB	Indirect	Autogenic	Bt
Nematode	*Rhabdochona* sp .	IN	Indirect	Autogenic	Bt
Nematode	*Salmonema ephemeridarum*	ST, IN	Indirect	Autogenic	Bt
Nematode	Capillariidae gen. sp.	IN	Indirect	Autogenic	Bt
Nematode	*Raphidascaris acus*	IN	Indirect	Autogenic	Bt
Nematode	Unidentified nematodes	IN	Indirect	unknown	Cd
Monogenean	*Diplozoon paradoxum*	G	Direct	Autogenic	Bt
Monogenean	*Discocotyle sagittata*	G	Direct	Autogenic	Cd
Trematode	*Diplostomum* sp.	E	Indirect	Allogenic	Bt
Trematode	Unidentified digeneans	ST, IN	Indirect	unknown	Bt, Cd

Host tissues are indicated by IN, intestine; M, mesenteries; PC, pyloric caeca; ST, stomach; G, gills and E, eye lens. Bt, Brown trout; Cd, common dace.

Brown trout had a higher Simpson's diversity than sympatric dace in both the Munster Blackwater and River Barrow during both study years (Table 4.3a). All values for the total number of helminth species and Simpson's index were higher in brown trout than in the sympatric population of dace (Table 4.3a). Lower Berger-Parker Dominance indices in brown trout indicated that the brown trout helminth community is more even and, thus, more diverse, than that of common dace.

Within host species, the structure of the helminth component communities varied in space (between river systems) and time (between years). In both host species, more helminth species were recorded in total in the Munster Blackwater than the River Barrow and component community diversity was greater in 2015 than 2017 (Table 4.3a).

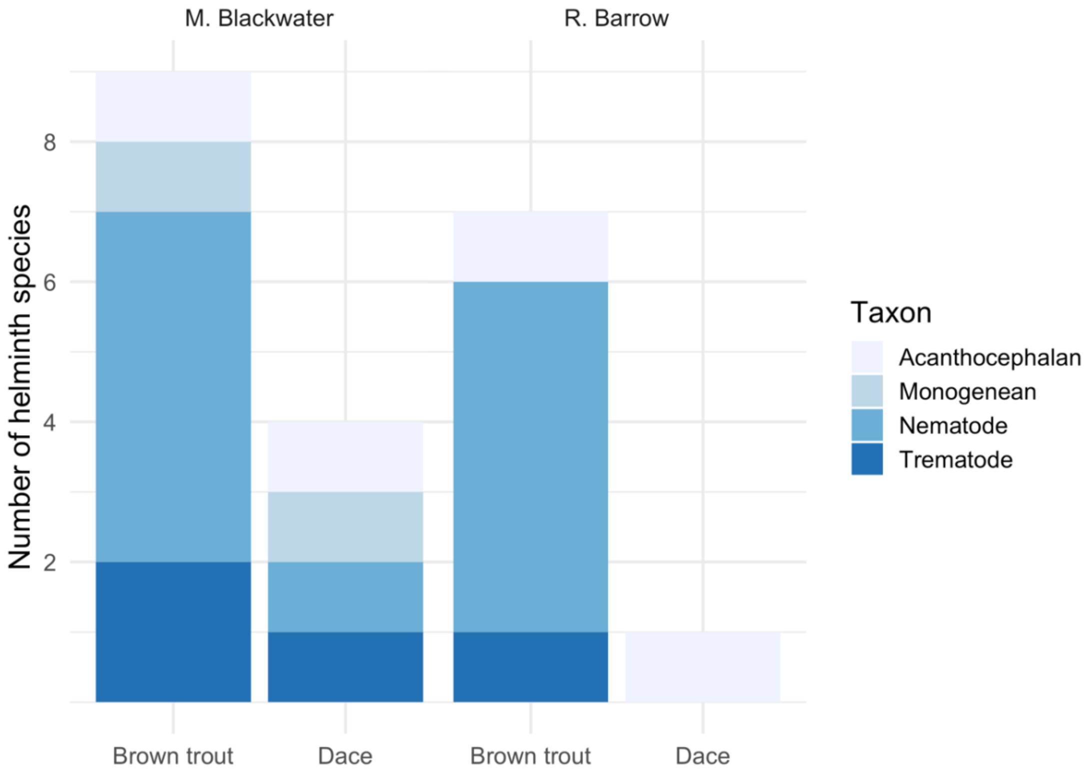

Figure 4.1. Total number of helminth species, grouped by helminth taxa, in native brown trout and invasive common dace in the Munster Blackwater and River Barrow in Ireland.

4.3.2 Infracommunity structure

At the infracommunity level, low helminth diversity was again apparent in common dace compared to brown trout. Brown trout had a maximum of five helminth species per fish in the Munster Blackwater and four in the River Barrow. Dace had a maximum of two in the Munster Blackwater and one in the River Barrow (Table 4.3b). Overall, 82% of dace were uninfected compared to 11% of brown trout (Figure 4.2).

Table 4.3. Parameters of helminth (a) component community and (b) infracommunity richness in brown trout *Salmo trutta* and common dace *Leuciscus leuciscus*.

Table 4.3a. Component community richness.

	Year	Dace			Brown trout		
		Munster Blackwater	River Barrow	All sites	Munster Blackwater	River Barrow	All sites
n	2015	71	81	152	76	41	117
	2017	48	87	135	61	71	132
	Both years	119	168	287	137	112	249
Total no. of species	2015	3	1	3	9	6	10
	2017	2	0	2	6	4	6
	Both years	4	1	4	9	7	10
Berger-Parker Dominance Index (dominant species)	2015	0.93 (*P.t*)	1 (*P.t*)	0.97 (*P.t*)	0.90 (*S.e*)	0.75 (*P.t*)	0.87 (*S.e*)
	2017	0.99 (*P.t*)	-	0.99 (*P.t*)	0.94 (*S.e*)	0.99 (*S.e*)	0.95 (*S.e*)
	Both years	0.98 (*P.t*)	1 (*P.t*)	0.98 (*P.t*)	0.91 (*S.e*)	0.72 (*S.e*)	0.89 (*S.e*)
Simpson's Index	2015	0.14	0	0.06	0.19	0.41	0.24
	2017	0.01	0	0.01	0.12	0.01	0.1
	Both years	0.042	0	0.031	0.17	0.42	0.2

P.t. denotes *Pomphorhynchus tereticollis* and *S.e* denotes *Salmonema ephemeridarum*.

Table 4.3b. Infracommunity richness.

	Year	Dace			Brown trout		
		Munster Blackwater	River Barrow	All sites	Munster Blackwater	River Barrow	All sites
Mean species richness ± SD	2015	0.35 ± 0.54	0.04 ± 0.19	0.18± 0.42	2.43 ± 1.07	1.29 ± 0.78	2.03 ± 1.12
	2017	0.54 ± 0.54	0	0.19 ± 0.41	1.46 ± 0.74	0.79 ± 0.50	1.10 ± 0.71
	Both years	0.43 ± 0.55	0.02 ± 0.13	0.19 ± 0.42	2.00 ± 1.06	0.97 ± 0.66	1.54 ± 1.04
Max. species richness	2015	2	1	2	5	4	5
	2017	2	0	2	3	2	3
	Both years	2	1	2	5	4	5
Mean no. of helminths per fish ± SD	2015	1.52 ± 3.45	1.78 ± 13.28	1.66 ± 9.95	131.07 ± 164.16	9.34 ± 7.88	88.41 ± 144.38
	2017	6.56 ± 13.5	0	2.33 ± 8.6	48.80 ± 62.22	10.87 ± 15.06	28.40 ± 47.48
	Both years	3.55 ± 9.27	0.86 ± 9.23	1.98 ± 9.33	94.44 ± 135.10	10.31 ± 12.88	56.60 ± 108.82
Mean Brillouin Index ± SD	2015	0.01 ± 0.07	0	0.01 ± 0.05	0.31 ± 0.28	0.13 ± 0.23	0.25 ± 0.27
	2017	0.001 ± 0.01	0	0.001 ± 0.01	0.14 ± 0.21	0.01 ± 0.06	0.07 ± 0.16
	Both years	0.01 ± 0.05	0	0.003 ± 0.03	0.24 ± 0.26	0.06 ± 0.16	0.15 ± 0.24
Max. Brillouin Index	2015	0.46	0	0.46	1.09	1.05	1.09
	2017	0.07	0	0.07	0.67	0.31	0.67
	Both years	0.46	0	0.46	1.09	1.05	1.09

SD = standard deviation.

In brown trout, mean species richness was highest in the Munster Blackwater in 2015 (2.43 ± 1.07) and lowest in the River Barrow in 2017 (0.79 ± 0.50). Median species richness in brown trout was one in the Blackwater and two in the Barrow, which were significantly different as indicated by a Wilcoxon-Mann-Whitney test (U = 3120.5, Z = -8.26, P < 0.001, r = 0.52). All brown trout infected with more than two helminth species were over 129 mm in standard length and Kendall's Tau correlation test indicated a strong correlation with species richness and host length (Z = 6.37, P < 0.001, τ = 0.31). In dace, the highest recorded mean species richness was in the Munster Blackwater in 2017 (0.54 ± 0.54) and the lowest was zero in the River Barrow in 2017. A Wilcoxon-Mann-Whitney test indicated that species richness in dace was significantly different between the two regions (U = 6138, Z=-8.40, P<0.001, r = 0.50) and a Kendall's Tau correlation test indicated that species richness was also correlated with host length (Z=6.55, P < 0.001, τ = 0.32). All dace below 100 mm were uninfected and, of the three dace infected with two helminth species, one was 147 mm in length and other two over 190 mm. The highest values of mean and maximum species richness in dace did not exceed even the lowest values in brown trout for any population (Table 4.3b).

Similarly, values of mean and maximum Brillouin's index were higher in brown trout than the sympatric populations of dace (Table 4.3a). The highest values of mean Brillouin's index for both host species were recorded in the Munster Blackwater in 2015 (trout: 0.31 ± 0.28, dace: 0.01 ± 0.07). Similarly to the component communities, infracommunities of both host species were generally more diverse in the Munster Blackwater and in the sampling year 2015 (Table 4.3b). I calculated Pearson correlation coefficients for all pairwise associations of abundance but found no strong negative or positive association between helminth species in brown trout in either the Munster Blackwater or River Barrow.

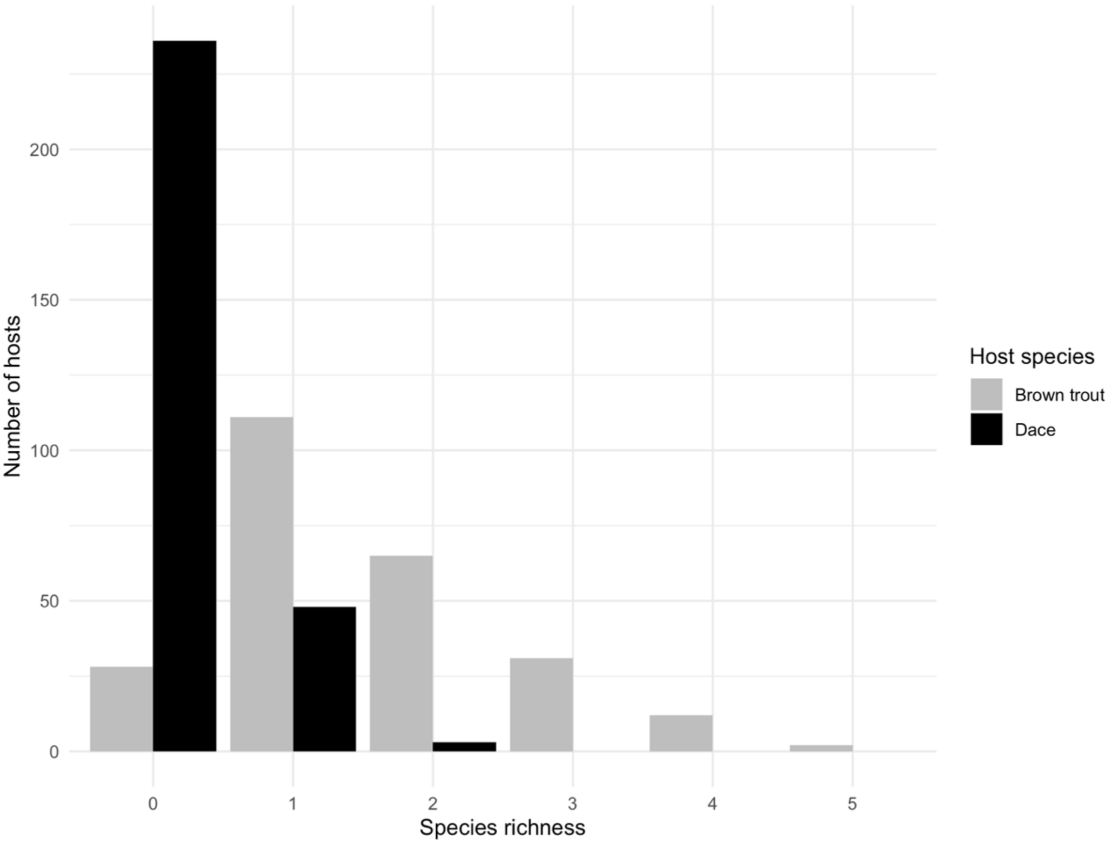

Figure 4.2. Frequency distribution of helminth species in dace and brown trout (Munster Blackwater and River Barrow sites combined).

4.3.3 Brown trout helminth community composition

The salmonid stomach nematode, *Salmonema ephemeridarum* (Linstow, 1872) occurred in all populations of brown trout and was the most dominant helminth species in all except the River Barrow population in 2015 (Table 4.3a, 4.4). I recorded 95% and 80% prevalence of *S. ephemeridarum* in the Munster Blackwater in 2015 and 2017, respectively. In the River Barrow, prevalence of *S. ephemeridarum* was 24% in 2015 and 75% in 2017. *S. ephemeridarum* occurred at particularly high intensities in the River Barrow: 124 ± 19 in 2015 and 57 ± 9 in 2017 (Table 4.4). The prevalence, abundance and intensity of *S. ephemeridarum* were significantly higher in the Munster Blackwater than in the River Barrow (Table 4.5, Appendix Table D1). The effect of year on prevalence, abundance and intensity of *S. ephemeridarum* was significantly altered by region (Appendix Table D1). In the Munster Blackwater, prevalence, abundance and intensity of *S. ephemeridarum* were higher in 2015 than in 2017 and in the River Barrow, 2015 was lower than 2017. Abundance and intensity of *S. ephemeridarum* significantly increased with increasing host length (Appendix Table D1). *S. ephemeridarum* was previously recorded in three out of nine lakes studied in Ireland and in one of two previously studied rivers, indicating that it may be more common in lotic systems (Table 4.6, Table 4.7).

The generalist acanthocephalan, *Pomphorhynchus tereticollis* (Rudolphi, 1809), was the next most common helminth species of brown trout, occurring in all of my study populations, and was the only acanthocephalan species recorded in brown trout. Both intra-intestinal adult and extra-intestinal subadult specimens of *P. tereticollis* were recorded with adult worms comprising 96% of the worms recorded (see Tierney et al. 2020b). Adult and subadult forms were combined for all statistical analyses. Prevalence of *P. tereticollis* was highest in the Munster Blackwater in 2015 at 62%. The lowest prevalence recorded was 1% in the River Barrow in 2017, where the parasite was detected in only one host (Table 4.4). Prevalence and abundance of *P. tereticollis* significantly decreased with increasing host length (Appendix Table D1) and was significantly lower in the year 2017 (Table 4.5, Appendix Table D1). Intensity of *P. tereticollis* was significantly higher in the River Barrow compared to the Blackwater (Table 4.5, Appendix Table D1). *P. laevis sensu lato* (*s.l.*), which includes *P. tereticollis* within the *P. laevis* complex is the third most frequently reported helminth of brown trout in Ireland after the cestode, *Euthbothrium crassum* and the monogenean, *Discocotyle sagittata* (Table 4.6). *P. laevis s.l.* was recorded in both of the other two rivers which have previously been investigated in Ireland and occurs in 22% of the previously surveyed lakes (Table 4.6, 4.7).

Cucullanus truttae (Fabricius, 1794), a nematode parasite of the pyloric caeca of salmonids, was found predominantly in brown trout in the Munster Blackwater. In the River Barrow, it was detected only in a single host in 2017 (Table 4.4). Prevalence, abundance and intensity of *C. truttae* were significantly higher in the Munster Blackwater than the River Barrow (Appendix Table D1) and significantly higher in 2017 compared to 2015 (Appendix Table D1). Prevalence and abundance of *C. truttae* significantly increased with host length (Table 4.5,

Appendix Table D1). This is the second record of *C. truttae* in brown trout in Ireland after Conneely & McCarthy (1988).

The salmonid swim bladder nematode, *Cystidicola farionis* (Fischer, 1798), was detected at low prevalence in brown trout in the Munster Blackwater in 2015 (4%), 2017 (3%) and in the River Barrow in 2017 (2%) (Table 4.4). It was not detected in the River Barrow in 2015. Prevalence of *C. farionis* was not influenced by region, year, host length or host sex (Table 4.5). Intensity of *C. farionis* was higher in the Munster Blackwater at 7.67 ± 2.73 and 9 ± 1 in 2015 and 2017, respectively, compared to 5 ± 0 in the River Barrow. However, a GLMM could not be fitted to the intensity data as there were too few data points. *C. farionis* has previously been recorded in one river, four lakes and one river and lake system in Ireland (Table 4.7).

Nematodes of the genus *Rhabdochona* were detected only in brown trout in the Munster Blackwater in 2015, but occurred at the highest abundance of any helminth other than *S. ephemeridarum* (Table 4.3b). The species of *Rhabdochona* is most probably *R. hellichi* (Srámek, 1901), given that the eggs are filamented, tail tips are pointed, and the long spicules measure between 0.55 and 0.6 mm in length and appear bifurcate (Moravec 1994; Moravec, *pers. comms*). Intensity of *Rhabdochona* sp. increased significantly with increasing host length (Appendix Table D1). All factors were non-significant in models fitted to *Rhabdochona* sp. prevalence and abundance (Table 4.5). *Rhabdochona* sp. has previously been recorded in two lakes and one river in Ireland (Table 4.7).

Table 4.4. Population biology parameters of helminth parasites detected in brown trout in the Munster Blackwater in 2015 (n = 76) and 2017 (n = 61) and in the River Barrow in 2015 (n = 41) and 2017 (n = 71).

Helminth	Loc	Year	Munster Blackwater				River Barrow			
			N	Prev. (95%CI)	Ab. ± SE	Int. ± SE	N	Prev. (95%CI)	Ab. ± SE	Int. ± SE
Pomphorhynchus tereticollis	IN, M	2015	47	61.84 (0.3-0.47)	1.51 ± 0.2	2.45 ± 0.24	31	75.61 (0.31-0.55)	6.98 ± 1.19	9.23 ± 1.35
		2017	27	44.26 (0.21-0.41)	1.15 ± 0.29	2.59 ± 0.54	1	1.41 (0-0.07)	0.01 ± 0.01	-
Cucullanus truttae	IN, PC	2015	10	13.16 (0.06-0.2)	0.45 ± 0.21	3.40 ± 1.32	0	ND	-	-
		2017	9	14.75 (0.06-0.23)	1.52 ± 0.55	10.33 ± 1.94	1	1.41 (0-0.07)	0.03 ± 0.03	2 ± 0
Cystidicola farionis	SB	2015	3	3.95 (0.01-0.11)	0.30 ± 0.19	7.67 ± 2.73	1	2.44 (0-0.13)	0.12 ± 0.12	5 ± 0
		2017	2	3.28 (0-0.11)	0.30 ± 0.21	9 ± 1	0	ND	-	-
Rhabdochona sp .	IN	2015	33	43.42 (0.22-0.4)	10.42 ± 3.69	24.00 ± 7.96	0	ND	-	-
		2017	0	ND	-	-	0	ND	-	-
Salmonema ephemeridarum	IN, ST	2015	72	94.74 (0.4-0.57)	117.55 ± 18.28	124.08 ± 19.01	10	24.39 (0.1-0.33)	1.54 ± 0.61	6.30 ± 1.84
		2017	49	80.33 (0.35-0.54)	45.80 ± 7.99	57.02 ± 9.28	53	74.65 (0.34-0.52)	10.82 ± 1.79	14.49 ± 2.18
Capillariidae gen. sp.	IN	2015	13	17.11 (0.08-0.24)	0.51 ± 0.19	3.00 ± 0.82	7	17.07 (0.06-0.28)	0.34 ± 0.15	2.00 ± 0.58
		2017	1	1.64 (0-0.09)	0.02 ± 0.02	1 ± 0	1	1.41 (0-0.07)	0.01 ± 0.01	1 ± 0
Raphidascaris acus	IN	2015	0	ND	-	-	1	2.44 (0-0.13)	0.02 ± 0.02	1 ± 0
		2017	0	ND	-	-	0	0 (0-0.05)	0	-
Diplozoon paradoxum	G	2015	1	1.32 (0-0.07)	0.01 ± 0.01	1 ± 0	0	ND	-	-
		2017	0	ND	-	-	0	ND	-	-
Diplostomum sp.	E	2015	1	1.32 (0-0.07)	0.07 ± 0.07	5 ± 0	0	ND	-	-
		2017	0	ND	-	-	0	ND	-	-
Unidentified Digenea	IN, ST	2015	5	6.58 (0.02-0.14)	0.23 ± 0.17	3.60 ± 2.36	3	7.32 (0.01-0.18)	0.34 ± 0.29	4.67 ± 3.67
		2017	1	1.64 (0-0.08)	0.01 ± 0.01	1 ± 0	0	ND	-	-

Loc = location in host, N = number of infected hosts, Prev. = prevalence, 95%CI = 95% confidence interval, Ab. Abundance,, SE = standard error, Int. = intensity of infection ND = not detected, IN = intestine, ST = stomach, PC = pyloric caeca, SB = swim bladder, M = mesenteries, G = gills, E = eyes.

Nematodes of the family Capillariidae were found in brown trout in the Munster Blackwater and the River Barrow in both 2015 and 2017. The capillariids could not be reliably identified to species level due to a lack of male specimens with sufficiently clear anterior and posterior regions. However, the specimens most probably belong to the species *Pseudocapillaria* (*Ichthyocapillaria*) *salvelini* (Polyansky, 1952), a common parasite of salmonids in Eurasia and North America (Moravec *pers. comm.*). Capillariidae gen. sp. have not previously been recorded from brown trout in Ireland.

Digenean trematodes were recovered from the gastrointestinal tract (intestines and stomachs) of brown trout in the Munster Blackwater and River Barrow (Table 4.4). Digeneans were found in intestines of brown trout in 2015 only and, in stomachs, digeneans were found in the Munster Blackwater in 2017 and in the River Barrow in 2015, each in only a single host.

Three helminth species were recovered from only a single brown trout host each. These were the intestinal nematode *Raphidascaris acus* (Bloch, 1779), detected in the River Barrow in 2015, the monogenean gill parasite, *Diplozoon paradoxum* (von Nordmann, 1832), in the Munster Blackwater in 2015 and a digenean eye fluke of the genus *Diplostomum*, detected in the Munster Blackwater in 2015 (Table 4.4).

Table 4.5. Summary of factors significantly affecting the prevalence, intensity and abundance of helminths in brown trout from fitted GLMMs.

Helminth	**Prevalence**		**Abundance**		**Intensity**	
	Significant factors	Direction	Significant factors	Direction	Significant factors	Direction
Pomphorhynchus tereticollis	Length** Year***	Decreases with increased host length Higher in 2015	Length* Year*** Region: Year**	Decreases with increased host length Higher in 2015 2015 higher in R. Barrow, Year ns in M. Blackwater	Region***	Higher in R. Barrow
Cucullanus truttae	Length*** Region* Year*	Increases with host length Higher in M. Blackwater Higher in 2017	Length*** Region** Year***	Increases with host length Higher in M. Blackwater Higher in 2017	Region** Year***	Higher in M. Blackwater Higher in 2017
Cystidicola farionis	ns	-	NA	-	NA	-
Rhabdochona sp.	ns	-	ns	-	Length*	Increases with length
Salmonema ephemeridarum	Region*** Year*** Region: Year***	Higher in M. Blackwater R Barrow: Higher in 2017 than 2015. Blackwater: Higher in 2015 than 2017	Length*** Region*** Year** Region: Year*	Increases with host length Higher in M. Blackwater R Barrow: Higher in 2017 than 2015. Blackwater: Higher in 2015 than 2017	Length*** Region*** Year*** Region: Year**	Increases with host length Higher in M. Blackwater R Barrow: Higher in 2017 than 2015. Blackwater: Higher in 2015 than 2017
Capillariidae gen. sp.	Year*** Sex*	Higher in 2015 Lower in hosts of unidentified sex	Year*** Sex*	Higher in 2015 Lower in hosts of unidentified sex	Region* Sex**	Higher in M. Blackwater Higher in male hosts
Unidentified digenean	Sex* Year*	Higher in male hosts Higher in 2015	NA	-	NA	-

Only helminths for which at least one model could be fitted are shown. Only significant factors and interactions are shown. *P < 0.05, **P < 0.01 and ***P < 0.001.

ns = non-significant. Cells are marked NA where models could not be satisfactorily fitted to the data

4.3.4 Similarity of brown trout communities

The helminth component communities of brown trout in the Munster Blackwater and the River Barrow were more similar to each other than to other brown trout populations previously surveyed for helminths in Ireland (Figure 4.3). Three of the four rivers included in the analysis (Munster Blackwater, R. Barrow, R. Shournagh) clustered together while the fourth river, Burishoole River (at the salmon leap downstream trap; Burishoole SLDT), clustered with Bunaveela Lough (2009 survey) and Lough Feeagh (2016 survey) which are in the same Burishoole catchment. The four loughs of the Rosses (Craghy, Waskel, Meela Owennamarve) in northwest Ireland were notably similar compared to other sites. The surveys of Bunaveela and Feeagh in the late 1980s and 1990s also cluster together but separate from the surveys of Bunaveela and Feeagh conducted in 2009 and 2016 (Figure 4.3). This suggests that helminth community composition within brown trout varies between locations and over time but that geographic distance and the type of system (lentic or lotic) effects the similarity of helminth community composition. The similarity of lentic systems to each other is likely driven by the frequent occurrence of common helminth species; the cestode, *Euthbothrium crassum* has been recorded at some point in brown trout in every lake investigated in Ireland and the digenean trematode, *Crepidostomum farionis* has been recorded in all but one (Table 4.7). Other species such as the cestodes, *Diphyllobothrium dendriticum* and *D. ditrenum,* and the monogenean, *Discocotyle sagittata* are also disproportionately represented in lentic helminth communities compared to lotic systems (Table 4.6). Conversely, the nematode *S. ephemeridarum* and the acanthocephalan *P. laevis s.l.* are more frequently reported in rivers compared to lakes (Table 4.6).

Table 4.6. Helminth species and taxa recorded in brown trout in the last 40 years in Irish lakes (n=9) and rivers (n=4). Data derived from 10 studies including the current study.

Helminth	Taxon	Intermediate host(s)	No. of records	% Lakes present	% Rivers present	% Lake & Rivers present
Acanthocephalus clavula	Acanthocephala	Isopod	8	55.56	25	50
Acanthocephalus lucii	Acanthocephala	Isopod	4	33.33	0	28.57
Pomphorhynchus laevis sensu lato	Acanthocephala	Amphipod	11	22.22	100	50
Diphyllobothrium dendriticum	Cestoda	Copepod	6	55.56	0	42.86
Diphyllobothrium ditrenum	Cestoda	Copepod	8	77.78	0	50
Diphyllobothrium sp.	Cestoda	Copepod	1	0	0	7.14
Eubothrium crassum	Cestoda	Copepod	15	100	25	78.57
Crepidostomum farionis	Trematode	Sphaeriid bivalve, Ephemoptera	11	88.89	25	71.43
Crepidostomum metoecus	Trematode	*Pisidium*/*Radix* sp., Ephemoptera/*Gammarus* sp.	8	66.67	25	57.14
Crepidostomum sp.	Trematode	Mollusc, aquatic arthropod	1	11.11	0	7.14
Diplostomum gasterostei	Trematode	Aquatic snail	2	22.22	0	14.29
Diplostomum spathaceum	Trematode	Lymnaeid snail	1	11.11	0	7.14
Diplostomum sp.	Trematode	Mollusc	2	0	25	14.29
Phyllodistomum conostomum (Phyllodistomum umblae)	Trematode	Sphaeriid bivalve	1	0	0	7.14
Anisakis sp.	Nematoda	Crustacea	3	33.33	0	21.43
Camallanus lacustris	Nematoda	Cyclopoid	1	0	0	7.14
Capillariidae gen. sp.	Nematoda	Aquatic invertebrate	2	0	50	14.29

Table 4.6 (contd.)

Helminth	Taxon	Intermediate host(s)	No. of records	% Lakes present	% Rivers present	% Lake & Rivers present
Cucullanus truttae	Nematoda	Lamprey	2	0	50	14.29
Cystidicola farionis	Nematoda	Gammarus	8	44.44	50	50
Hysterothylacium aduncum	Nematoda	Marine amphipod	1	11.11	0	7.14
Raphidascaris acus	Nematoda	Chironomid	5	44.44	25	35.71
Rhabdochona sp.	Nematoda	Ephemoptera (*Baetis rhodani*)	4	22.22	50	28.57
Salmonema ephemeridarum (Cystidicoloides tenuissima)	Nematoda	Ephemoptera	7	33.33	75	50.00
Discocotyle sagittata	Monogenea	-	12	88.89	0	64.29
Diplozooan paradoxum	Monogenea	-	1	0	25	7.14

Table 4.7. List of helminth species and taxa recorded in resident brown trout *Salmo trutta* in lentic (lake) and lotic (river) systems Ireland in the last 40 years. Conneely & McCarthy 1988 is not included due to inaccessibility of the original paper. Records from sea trout and migrating smolts and kelts are not included. *Pomphorhynchus laevis s.l.* (*sensu lato*) encompasses *P. laevis* and the more recently resurrected cryptic species, *P. tereticollis*. Species names in this table have been updated where applicable (i.e. *Cystidicoloides tenuissima* to *Salmonema ephemeridarum* and *Phyllodistomum umblae* to *Phyllodistomum conostomum*). Unidentified digenea recorded in the current study are not included. Due to the number of sites, Table 4.7 has been split vertically into two parts, (i) and (ii).

Table 4.7 (i)

System		**Lotic**				**Lotic & lentic**	**Lentic**		
Site		Shournagh	Burishoole SLDT	Blackwater	Barrow	Corrib	Bunaveela		
Author(s)		Fitzgerald & Mulcahy 1981	Maguire 2018	Tierney et al.	Tierney et al.	Conneely and McCarthy 1984	Molloy 1993	Molloy 1995	Maguire 2018
Year(s) of sampling		1980	2015	2015, 2017	2015, 2017	not stated	1989-1990	1991-1992	2009
n		242	15	137	112	26	36	285	36
Helminth	**Taxon**								
Acanthocephalus clavula	Acanthocephala		+			+			+
Acanthocephalus lucii	Acanthocephala					+	+		
Pomphorhynchus laevis s.l.	Acanthocephala	+	+	+	+	+	+	+	+
Diphyllobothrium dendriticum	Cestoda					+			
Diphyllobothrium ditrenum	Cestoda						+		
Diphyllobothrium sp.	Cestoda					+			
Eubothrium crassum	Cestoda		+			+	+	+	+
Crepidostomum farionis	Digenea	+				+		+	
Crepidostomum metoecus	Digenea	+				+			

Table 4.7 (i) (contd.)

System		**Lotic**				**Lotic & lentic**	**Lentic**		
Site		Shournagh	Burishoole SLDT	Blackwater	Barrow	Corrib	Bunaveela		
Helminth	**Taxon**								
Crepidostomum sp.	Digenea						+		
Diplostomum spathaceum	Digenea								
Diplostomum gasterostei	Digenea								
Diplostomum sp.	Digenea			+		+			
Phyllodistomum conostomum	Digenea					+			
Anisakis sp.	Nematoda								
Camallanus lacustris	Nematoda					+			
Capillariidae gen. sp.	Nematoda			+	+				
Cucullanus truttae	Nematoda			+	+				
Cystidicola farionis	Nematoda			+	+	+	+		
Hysterothylacium aduncum	Nematoda						+		
Raphidascaris acus	Nematoda				+				
Rhabdochona sp.	Nematoda	+		+					
Salmonema ephemeridarum	Nematoda		+	+	+	+			
Discocotyle sagittata	Monogenea					+	+	+	+
Diplozooan paradoxum	Monogenea			+					
No. of species		4	4	8	6	14	8	4	4

Table 4.7 (ii).

System		**Lentic**									
Site		Feeagh			Atorick	Craghy	Waskel	Meela	Owenn-amarve	Clogher	Dunlewy
Author(s)		Molloy 1995	Byrne 2002	Maguire 2018	Faherty & McCarthy 2006	Byrne 2000	Byrne 2000	Byrne 2000	Byrne 2000	Byrne 2004	McCarthy & McCarthy 2004
Year(s) of sampling		1991-1992	1997-1998	2016	1993	1997	1997	1997	1997	1997-1998	2001
n		264	217	7	40	40	32	45	23	161	64
Helminth	**Taxon**										
Acanthocephalus clavula	Acanthocephala		+	+	+					+	+
Acanthocephalus lucii	Acanthocephala				+						+
Pomphorhynchus laevis s.l.	Acanthocephala	+	+	+							
Diphyllobothrium dendriticum	Cestoda					+	+	+	+	+	
Diphyllobothrium ditrenum	Cestoda	+	+			+	+	+		+	+
Diphyllobothrium sp.	Cestoda										
Eubothrium crassum	Cestoda	+	+	+	+	+	+	+	+	+	+
Crepidostomum farionis	Digenea	+	+			+	+	+	+	+	+
Crepidostomum metoecus	Digenea				+	+	+	+	+		+
Crepidostomum sp.	Digenea										
Diplostomum spathaceum	Digenea				+						+
Diplostomum gasterostei	Digenea				+						

Table 4.7 (ii) (contd.)

System		**Lentic**									
Site		Feeagh			Atorick	Craghy	Waskel	Meela	Owenn-amarve	Clogher	Dunlewy
Diplostomum sp.	Digenea										
Phyllodistomum conostomum	Digenea										
Anisakis sp.	Nematoda					+			+	+	
Camallanus lacustris	Nematoda										
Capillariidae gen. sp.	Nematoda										
Cucullanus truttae	Nematoda										
Cystidicola farionis	Nematoda	+	+		+		+				
Hysterothylacium aduncum	Nematoda										
Raphidascaris acus	Nematoda					+	+	+	+		
Rhabdochona sp.	Nematoda		+							+	
Salmonema ephemeridarum	Nematoda		+						+		+
Discocotyle sagittata	Monogenea	+	+		+	+	+		+	+	+
Diplozooan paradoxum	Monogenea										
No. of species		6	9	3	8	8	8	6	8	8	9

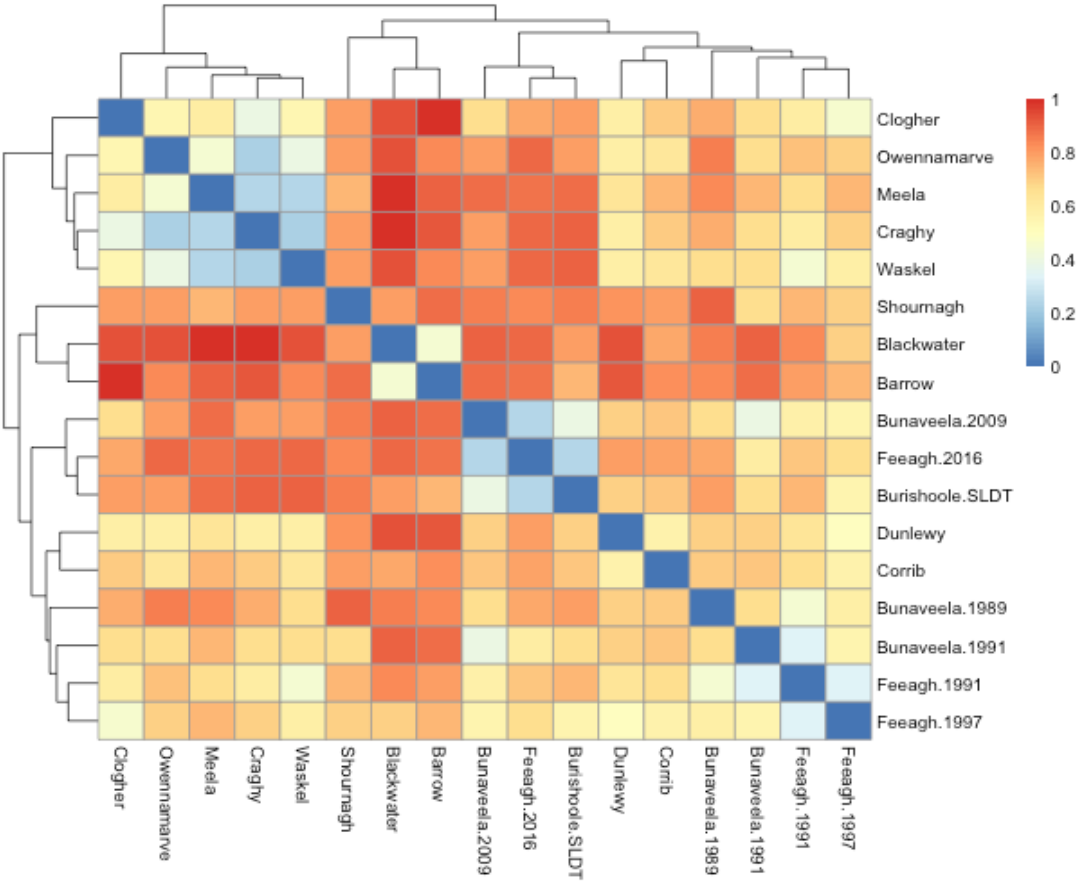

Figure 4.3. Heatmap of Jaccard dissimilarity of component communities of brown trout populations recorded in helminth surveys in Irish lakes and rivers in the last 40 years (1 indicates entirely dissimilar, 0 indicates entirely the same). See Table 4.7 for composition of the communities.

4.4 Discussion

A total of 31 helminth species have been previously reported in brown trout in Ireland (Holland & Kennedy 1997) although that figure varies somewhat depending on the inclusion or exclusion of helminth records from migrating kelts returning from the sea. For example, *Echinorhynchus truttae* was recorded in kelts by Molloy et al. (1993) which was not included in the current study. Six of those 31 species were recorded in brown trout in the Munster Blackwater (*S. ephemeridarum*, *P. tereticollis* (*P. laevis s.l.*), *C. truttae*, *Cystidicola farionis*, *Rhabdochona* sp. and *Diplostomum* sp.) along with two new helminth records for brown trout in Ireland, the monogean *Diplozooan paradoxum* and the Capillariid nematode species. In the River Barrow, five of the previously recorded 36 species were detected (*S. ephemeridarum*, *P. tereticollis* (*P. laevis s.l.*), *C. truttae*, *Cystidicola farionis*, *Raphidascaris acus*) as well as the new record for Capillariidae gen. sp.

D. paradoxum has previously been recorded in Ireland in gudgeon *Gobio gobio*, rudd *Scardinius erythrophthalmus* and roach *Rutilus rutilus* (Holland & Kennedy 1997). Gudgeon and roach are both non-native species which occur in the Munster Blackwater (Kelly et al. 2014). Within the Capillariidae, only *Capillaria* sp. has been previously reported in freshwater fish in Ireland, recorded in European eel *Anguilla anguilla*. Despite some evidence from morphological characters, I was cautious to identify the observed *Rhabdochona* sp. as *R. hellichi* without molecular data or scanning electron microscopy as the specific fish host of *R. hellichi*, barbel *Barbus barbus*, has never been recorded in Ireland. Without a satisfactory explanation for how a barbel specific helminth could have arrived in Ireland, to identify the *Rhabdochona* sp. as *R. hellichi* would imply both an unusual host switch and an entirely mysterious colonisation history. Fitzgerald & Mulcahy (1983) and Byrne et al. (2002), (2004) reported *Rhabdochona* sp. in brown trout but identified only to genus level.

Many of the helminth species recorded in brown trout in the Munster Blackwater and River Barrow were trout or salmonid specialists, namely the nematodes, *S. ephemeridarum*, *Cystidicola farionis* and *C. truttae*. Among the most abundant helminth species, *P. tereticollis* was the only species considered to be a generalist. Even so, given that *P. tereticollis* occurs as adults at such greater prevalence and in greater abundance in brown trout than in dace and fails to sexually mature in dace (Chapter 2), *P. tereticollis* is clearly not equally generalist across different species of definitive freshwater fish hosts. Given the broad palearctic distribution of *P. tereticollis,* it is possible that this parasite is generalist at a macro scale across its distribution range but displays some reginal specificity to local hosts at the local scale. The dominance of host-specific helminths in brown trout reflects previous studies in which trout specific parasites such as *S. ephemeridarum*, *Crepidostomum farionis, C. moteocus* and *Eubothrium crassum* were dominant helminths in brown trout helminth communities (Byrne et al. 2000; Kennedy & Hartvigsen 2000).

I observed strong differences in species richness and in the prevalence, mean abundance and mean intensity of several helminth species between the two study rivers in both dace and brown trout. For dace, this can be explained by time since colonisation and characteristics of invasion front populations (Tierney et al. 2020a). Host species tend to accumulate parasites over time such that longer established hosts have richer parasite communities and the establishment of parasites along an invasion corridor lags behind establishment of their hosts (Guégan & Kennedy 1993; Phillips et al. 2010). The Munster Blackwater has been invaded by dace for about a century longer than the upper River Barrow, so differences in the helminth community richness of dace between the two rivers are expected (Chapter 3; Tierney et al. 2020a). For brown trout, in the case of one of the species, *P. tereticollis,* Tierney et al. (2020b) (Chapter 2) found evidence that acquisition of the parasite by incompetent dace hosts dilutes infection in brown trout the Munster Blackwater where dace is long-established and *P. tereticollis* infection in dace is high. However, the differences between the Blackwater and Barrow I observed in the other helminth species (*S. ephemeridarum, C. truttae* and Capillariidae gen. sp.) are not readily explained by co-occupancy with invasive dace. These helminth are host-specific to salmonids (presumably, if the Capillariid is *P. salvelini*) and are not acquired by dace. Thus, the presence of a sympatric cyprinid is less likely to impact their populations in brown trout. The differences I observed between region in these species may be due to other stochastic effects or local processes such as the distribution of intermediate hosts, chance introductions or environmental variables.

Several helminth species were not stable over time, disappearing or appearing between the two sampling years of the study. I observed significant inter-year differences in *P. tereticollis*, *S. ephemeridarum*, *C. truttae, Rhabdochona* sp. and Capillariidae gen. sp. in brown trout. However, this compositional turnover may not be an unusual feature of brown trout communities. Considering Bunaveela Lough and Lough Feeagh, which have been studied three times within 25 years, only two species – *P. laevis* and *E. crassum* – have been present during all three brown trout helminth community studies. Byrne et al. (2002) studied the metazoan parasite community of wild brown trout in Lough Feeagh, conducting seven surveys between April 1997 and November 1998, and found the composition of the parasite community varied considerably between years with the number of parasite species ranging between five and nine.

Like Molloy et al. (1995), I found brown trout helminth communities were dominated by autogenic species in both the Munster Blackwater and River Barrow. Esch et al. (1988) defined autogenic species as parasites that utilise fish as definitive hosts and thus, are less readily able to disperse between unconnected catchments than allogenic species which utilise birds or mammals as definitive hosts. Of the helminth species identified in brown trout, all but *Diplostomum* sp. were autogenic and I observed that every brown trout population studied in the Blackwater and Barrow was strongly dominated by an autogenic species (either *S. ephemeridarum* or *P. tereticollis*). Esch et al. (1988) noted that brown trout helminth communities tend to be structurally similar, as they are characterised by the dominance of autogenic species, but that the richness of the community and identity of the dominant species varies between localities. As autogenic species have lower colonisation potential than allogenic species, the composition of mainly autogenic brown trout communities is much more likely to be shaped by chance introductions and stochastic processes than communities dominated by allogenic species (Esch et al. 1988). This may lead to considerable compositional variation in brown trout helminth communities between locations. Similar to Kennedy & Hartvigsen (2000), I found that helminth communities of brown trout in the Blackwater and Barrow were isolationist, exhibiting no pairwise associations between abundance of co-infecting helminth species. This indicated that interspecific effects among parasites are not important in structuring the helminth community of brown trout.

Brown trout had higher diversity of helminth parasites than invasive common dace at both the component and infracommunity levels in both the Munster Blackwater and River Barrow. Among freshwater fish families, salmonids, particularly resident salmonids like brown trout, tend to have the most diverse helminth communities due to their broad omnivorous diet (Kennedy et al. 1986). However, this alone does not account for the vast difference in helminth diversity observed in brown trout and dace, given that Kennedy et al. (1986) identified common dace as having the most diverse helminth communities within cyprinids and reported similar helminth community diversity between brown trout and dace in Great Britain. My findings of lower diversity in dace component and infracommunities support the hypothesis that dace in Ireland experience enemy release from helminth parasites as a result of invasion. Evidence for enemy release has previously been found when comparing helminth communities of invasive dace in Ireland to those of native populations of dace in Great Britain and continental Europe (Chapter 3; Tierney et al. 2020a) but this is the first evidence of reduced helminth diversity in invasive dace compared to a sympatric native species.

The helminth community of dace appears to follow a pattern of nestedness within the richer helminth community of brown trout (Poulin et al. 2001). The helminth community of dace in both regions was dominated by the only abundant generalist species infecting brown trout, *P. tereticollis*. Even if one assumes that dace and brown trout share food and habitat resources and are exposed to the same pool of parasites, it is unlikely that dace would acquire helminth species other than *P. tereticollis* from sympatric brown trout due to the salmonid specificity of most of the other helminths of brown trout and the rarity of non-specialists other than *P. tereticollis*. Faherty & Mc Carthy (2006) found a similarly nested pattern in the helminth communities of brown trout and non-native perch *Perca fluviatilis* in Lough Atorick where brown trout were infected with eight helminth species and perch were infected only with two acanthocephalan species which also occurred in brown trout.

The only other identified helminth species in dace, the monogenean *Discocotyle sagittata*, may also be an acquisition from the brown trout helminth community (albeit probably accidental). *D. sagittata* is typically parasitic on the gills of salmonids in the genera *Salmo* and *Oncorhynchus* (Yamaguti 1963). *D. sagittata* is a commonly recorded parasite of brown trout elsewhere in Ireland (Holland & Kennedy 1997; Table 4.7) but I did not detect it in brown trout in the present study. *D. sagittata* has not previously been recorded in species other than brown trout or Atlantic salmon *Salmo salar* in Ireland (Holland & Kennedy 1997) and outside of Ireland, there are few reliable records of *D. sagittata* infecting non-salmonids (Gibson et al. 2005). The appearance of *D. sagittata* in a cyprinid host is surprising and may reflect that it is present in *Salmo* populations in the Munster Blackwater at such a low prevalence as to go undetected in my survey of brown trout. Given the host-specificity of *D. sagittata*, it is dubious that its detection in dace represents true acquisition of native parasites by dace or host switching by *D. sagittata*, especially seeing as only a single individual in a single host was recorded.

The absence of cestodes was a surprising feature of the brown trout and dace helminth communities in my surveys given that cestodes are among the most commonly reported and dominant helminth species in previous brown trout helminth surveys in Ireland. Cestode parasites appeared to be disproportionately represented in lakes compared to rivers. This is probably a reflection of the distribution of their copepod intermediate hosts which prefer lentic systems (Fryer & Joyce 1981). There was a similarly conspicuous paucity of digeneans, particularly in brown trout in which the digenean *Crepidostomum farionis* is among the most frequently reported helminths in the Irish literature. Valtonen et al. (2001) attributed low numbers of digeneans in northern Bothnian Bay fish communities to a depauperate molluscan fauna which act as the first intermediate hosts. Sphaerid and *Pisidium* sp. bivalves, which are the reported first intermediate hosts of *Crepidostomum farionis* and *C. metoecus* are associated with slow flowing and standing water (Chadd 1999) which may limit the success of these helminth species in rivers. Helminth species which utilise only intermediate host species that tolerate fast flowing water (e.g. *S. emphemeridarum* which uses Ephemoptera and *P. tereticollis* which uses *Gammarus duebeni*) may have an advantage in lotic systems and are likely to be more common in rivers than lakes.

However, I must note the bias in the Irish helminth community literature towards lakes. Including the two rivers in the current study, helminth surveys of brown trout have been conducted in only four rivers in the last 40 years, one of which (Burishoole SLDT) had a low sample size. This raises the consideration that the previous literature may more likely be representative of western lakes than of Irish brown trout populations in general.

4.5 Conclusion

The helminth communities of brown trout in the two study rivers, the Munster Blackwater and the River Barrow, were variable over space and time and mainly dominated by host-specific and autogenic species. The helminth community of co-occurring invasive dace was species-poor, dominated by a single generalist species and represented a nested subset of the richer brown trout helminth community. While the brown trout helminth communities in my study population were similar in structure to the previous studies on brown trout helminth communities which were predominantly conducted in lakes, the composition of the helminth communities were considerably different. While some of these differences may be due to stochastic effects, it is likely that some aspects of helminth community composition in lentic and lotic systems are intrinsically different due to differing distributions of intermediate hosts.

5. Trophic niche overlap in invasive common dace and native brown trout

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Author contributions: PAT conducted host sampling in 2017, laboratory dissections and wrote the chapter. PAT conducted the analysis in consultation with BH. PAT, BH and CVH conceived the study design. JMC assisted in co-ordinated of host sampling. SMM conducted host sampling in 2015. BH and CVH commented on the chapter. CVH supervised the project.

Ethical statement: Electrofishing and euthanasia of fish was carried out by trained Inland Fisheries Ireland staff. This work was approved by the Trinity College Dublin School of Natural Science's Research Ethics Committee.

5.1 Introduction

Freshwaters are important and biodiverse ecosystems but are particularly vulnerable to the effects of introduced invasive species (Gozlan et al. 2010; Nunes et al. 2015). Invaders that succeed in integrating themselves into native ecosystems pose considerable ecological risk to native species. Many of the potential impacts of invasive species on native species such as trophic competition (Manchester & Bullock 2000) and direct mortality (Medina et al. 2011) are related to the trophic niche of the invader. The invader trophic niche may also be associated with more indirect impacts such as altered parasite transmission (Thieltges et al. 2009).

Parasite infection is variable within and among populations, with some hosts being infected with many species of parasites while others have few or no parasite infections (Kennedy 2009). The drivers behind these variations in parasite infections are still not fully understood (Bordes & Morand 2015), however the community of parasites infecting a host is generally acknowledged to be a product of exposure and host susceptibility (Wilson et al. 2002; Poulin 2013). Thus, the diet, or trophic niche, of a host is an important driver of infection patterns of trophically-transmitted parasites by exposing hosts to infected intermediate and paratenic host prey (Prati et al. 2020b). Many taxa of helminth parasites, namely the Acanthocephala, Cestoda, Nematoda and Digenea, are trophically transmitted (*i.e.* transmitted by ingestion of a previous, infected host) at some stage of the life cycle.

The trophic niche of a population describes the total extent of food sources exploited. Evaluating the trophic niche of a species can, however, be challenging. Traditional methods of gut contents analysis provide information on diet over only a short time scale and are weakened where there are high rates of empty stomachs and circadian and seasonal variability in diet composition (Nielsen et al. 2018; Nolan & Britton 2018). Similarly, while diet information is often used to understand the acquisition of trophically-transmitted parasites, helminths are accumulated and survive in their hosts over a much longer time frame (weeks to months: Kennedy 2006; Poulin & Lagrue 2015) than dietary items will persist in the alimentary tract of the host (6-48 hours: Nielsen et al. 2018). As stomach contents provide only a snapshot of diet, an intermediate invertebrate host could potentially form an important part of the trophic niche of a definitive host, as evidenced by high parasite burdens, but be missed from stomach analysis due to its temporal transience in the gastrointestinal tract. In contrast, stable isotope analysis (SIA) integrates trophic information of consumers over a much longer time frame, depending on the chosen study tissue (Nielsen et al. 2018). Isotopic niche is tightly correlated with trophic niche, making SIA a popular technique for evaluating the trophic niches of invasive and native species and assessing trophic interactions over a long time scale (Jackson et al. 2012; Tran et al. 2015; Britton et al. 2018). As trophically transmitted helminth parasites are accumulated over long periods of time and, for some helminths, initial infection events occur as seasonal peaks related to the availability of invertebrate intermediate hosts (Chubb 1979, 1982), SIA is also valuable for evaluating the role of trophic niche in the accumulation of helminth infections (Knudsen et al. 2014).

SIA can thus be leveraged as a powerful tool to assess both a) the potential for trophic niche overlap between native and invasive species and b) the relationship between trophic niche and trophically-transmitted helminth infection in native and invasive hosts. In this chapter, I use my established study system of invasive common dace *Leuciscus leuciscus* and native brown trout *Salmo trutta* to evaluate trophic niche overlap between the two species at the core (Munster Blackwater) and front (Upper River Barrow) of dace's invasive range in Ireland and to assess whether trophic niche is an important driver of infection patterns of trophically transmitted helminth parasites in these two species.

This chapter will address four main hypotheses:

1. Trophic niche overlap exists between native brown trout and invasive dace and is greater at the invasion front (River Barrow) than the invasion core (Munster Blackwater).
2. Trophically-transmitted parasites are associated with a wider host trophic niche.
3. Trophic niche specialisation exists between infected and uninfected cohorts of fish.
4. Infection with trophically-transmitted helminths is associated with higher trophic position.

5.1.1 Trophic niche overlap between invaders and natives

Although dace and brown trout are phylogenetically distinct, representing different fish families, there are similarities in habitat and diet that indicate the potential for trophic niche overlap in these species and that they may be exposed to a common community of local parasites. From a habitat perspective, Ireland has no native cyprinid species and widespread native salmonids occupy habitats such as lowland rivers (Delanty et al. 2017) that are more commonly associated with cyprinids elsewhere Europe (Kottelat & Freyhof 2007), so an invading cyprinid is likely to come into competition with native fish. Brown trout is a dominant species in every river system invaded by dace in Ireland (The Central and Regional Fisheries Board 2009;

Inland Fisheries Ireland 2010; Delanty et al. 2017) and both dace and brown trout are noted to prefer fast-flowing waters (Caffrey et al. 2007; Kottelat & Freyhof 2007). In the Munster Blackwater, co-occurring dace and brown trout show similar spatial ecology and patterns of habitat use (Barry et al. 2020) which could potentially signal similar dietary resource use. With regards to diet, aquatic macroinvertebrates and terrestrial insects are important in the diet of both species (Mann 1974; Sánchez-Hernández & Cobo 2012), although algae also forms a main component of the diet of dace (Helawell 1974), and brown trout shift towards more piscivorous behaviour with age (Jensen et al. 2012; Sanchez-Hernandez et al. 2012). The presence of the acanthocephalan parasite *Pomphorhynchus tereticollis* in both species (Chapter 2; Tierney et al. 2020b) indicates that they, at least, both feed on the amphipod intermediate host of the parasite, *Gammarus duebeni*.

While I hypothesise that the isotopic niches of dace and brown trout are likely to overlap due to the similarities in their habitat and dietary preferences, previous research on freshwater fish invaders by Tran et al. (2015) suggests that the isotopic niches of invasive and native fish may diverge over time in order to avoid trophic competition. Dace has been described as naturalised in the Munster Blackwater (Caffrey et al. 2007), having been present in the fish fauna there since 1889 (Went 1950), so adaptations to trophic competition between dace and brown trout may have developed in that region that are not yet developed at the invasion front in the River Barrow, where dace have been present for less than ten years.

5.1.2 Trophic niche width and helminth infection

The breadth of a host’s trophic niche has long been associated with structuring helminth infection, particularly in fish systems. Kennedy et al. (1986) reported that the broad omnivorous diets of resident brown trout, *Salmo trutta*, and Arctic charr, *Salvelinus alpinus,* was responsible for the high helminth species richness observed in the two species. Where dietary breadth of brown trout was narrow and fish fed intensively on a limited number of prey species, fish had low species richness, low diversity and high abundance of helminths (Kennedy & Burrough

1978). In a global analysis of macroparasite species richness in birds, Gutiérrez et al. (2019) found positive associations between trophic niche breadth and trophically transmitted helminths.

Previous research (Chapter 4) has found that the helminth communities of brown trout and dace in the River Barrow and Munster Blackwater are dominated by trophically transmitted helminths. While host competency (Chapter 2; Tierney et al. 2020b), and time since colonisation (Chapter 3; Tierney et al. 2020a), influence the differential patterns of helminth infection observed in dace and brown trout, and in the two study rivers, trophic niche is also likely to be a determining factor in trophically-transmitted helminth infection. I hypothesise that broader trophic niche will be associated with infected cohorts of dace and brown trout and that niche breadth will increase along with species richness of trophically transmitted helminths.

5.1.3 Trophic niche specialisation and helminth infection

In recent years, the population trophic niche is increasingly recognised as comprising smaller partitioned or specialised niches formed by subgroups of trophically specialised individuals (Bolnick et al. 2003; Quevedo et al. 2009). Cohorts within a population that utilise distinct food sources may experience different levels of exposure to trophically transmitted parasites resulting in intrapopulation variation in infection (Britton & Andreou 2016). Bertrand et al. (2008) found that brook charr, *Salvelinus fontinalis*, from the littoral zone of a lake consumed more benthic prey and were more infected with parasites that use littoral invertebrates as intermediate hosts than fish from the pelagic zone. If trophic niche specialisation similarly drives helminth infection in dace and brown trout then I expect divergence (i.e. specialisation) between the isotopic niches of infected and uninfected cohorts of each species.

5.1.4 Trophic position and helminth infection

Siwertsson et al. (2016) attributed differences in parasite species richness and abundance in *S. fontinalis* morphs to trophic specialisation where the piscivorous morph, feeding at a higher trophic position, accumulated more parasites than the benthivorous morph through consumption of parasitised fish prey. Pegg et al. (2015) also found an influence of trophic position on parasitism: European eels *Anguilla anguilla* with larger heads increased piscivory and were more likely to be infected with the nematode *Anguillicoloides crassus* due to increased exposure to paratenic fish hosts. As mentioned, dace and brown trout are both omnivorous with intrapopulation variation in diet associated with ontogenetic transitions to higher trophic positions (Helawell 1974; Jensen et al. 2012). Feeding at higher trophic positions is likely to increase exposure to helminth parasites such that, collectively, infected cohorts of dace and brown trout feed at a higher average trophic position than uninfected chohorts.

5.2 Methods

5.2.1 Field collection

Brown trout and common dace were collected from two river systems in Ireland, the Munster Blackwater (core of the dace invasive range) and the River Barrow (invasion front), in July and August, 2015 and 2017. Fish were collected by electrofishing at Fermoy, Glanworth and the River Funsion on the Munster Blackwater and at Monasterevin, Portarlington and the River Slate on the River Barrow (Appendix Table D1). In total, 112 brown trout and 73 common dace from the Munster Blackwater, and 95 brown trout and 144 common dace from the River Barrow were used in this study. Standard length (mm) and wet mass (g) were recorded for each fish. Scales were removed from between the dorsal fin and lateral line using a scalpel and a sample of muscle tissue for stable isotope analysis (SIA) was taken from each fish using a 8 mm biopsy punch.

Benthic macroinvertebrates were collected by kick-sampling using 1 mm mesh pond nets from wadeable sites surveyed in 2017 (Funsion, Monasterevin, Portarlington) and identified to order level for use as baseline isotope values. Macroinvertebrates were pooled by order and sampling region for SIA.

5.2.2 Stable isotope analysis

Muscle tissue was dried for 24 h at 60°C, ground to a powder and weighed into aluminium capsules. A mean weight (±SD) of 1.149 mg (± 0.161) of muscle tissue was analysed for each fish. The stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were analysed using a Costech 4010 elemental analyser (Costech, California, USA), coupled to a Thermo Finnigan Delta V Plus mass spectrometer (Thermo Finnegan Bremen, Germany). Fish $\delta^{13}C$ values were arithmetically lipid normalized to correct for variable lipid concentrations (Kiljunen et al. 2006). Samples of macroinvertebrate orders were also dried, ground and analysed for $\delta^{13}C$ and $\delta^{15}N$ values to provide an isotopic baseline for analysis of fish trophic position.

5.2.3 Parasitological examination

The swim bladder and gastrointestinal tract were removed from each fish, washed with 0.9% saline solution and examined for helminth parasites using a dissecting microscope. The body cavity was also washed with saline and examined. The number of trophically-transmitted helminth species (i.e. acanthocephalans, nematodes, cestodes and adult digenean trematodes) was recorded and the species were identified following methods in Chapter 4. Helminth individuals that could not be identified to species level were included in further analysis if they were identified as being a trophically-transmitted stage of that taxon. As this study is concerned with the effect of trophic niche on the acquisition of parasites, directly transmitted and non-trophically transmitted helminth parasites (*i.e.* monogeneans and larval digeneans) were not included.

5.2.4 Data analysis

5.2.4.1. Parasitological parameters

Percentage prevalence of infection, associated Pearson’s 95% exact confidence intervals, mean species richness (mean number of helminth species per host) and range of species richness of trophically transmitted helminths were calculated for brown trout and dace in both regions following Bush et al. (1997). Fish were categorised into infected and uninfected cohorts. There was only one infected dace in the River Barrow sample so infected River Barrow dace was excluded as a population from analyses of isotopic niche width and trophic position. Brown trout were further categorised into three species richness groups where species richness = 0, species richness = 1, and species richness > 1. Helminth species richness was selected as the measure of parasite infection as breadth of host diet has previously been associated with helminth richness (Gutiérrez et al. 2019). An abundance-based measure such as mean number of helminths per fish (mean parasite abundance; Bush et al. 1997) would be strongly influenced by intense infections of single helminth species and thus, could reflect either broad or narrow trophic niches. Dace were not categorised into species richness groups as the number of fish infected with more than one species was too small for statistical analysis.

5.2.4.2. Condition factors

Relative condition factor of each fish was calculated using $K_{rel}=W/(aL^b)$ (Froese 2006) where W is the body weight (g), L is the length (cm), a is the intercept of the regression and b is the slope. Parameters a and b were estimated by linear regression using the equation of the weight-to-length relationship, $W = aL^b$, transformed as $Log(W) = Log(a) + bLog(L)$ (Le Cren 1951). These parameters were calculated separately for each combination of fish species, region and sampling year. Generalised Linear Mixed Models (GLMMs) (Brooks et al. 2017) with site and year as random effects were used to assess the effect of infection and region on condition factor, length and weight in dace and brown trout. Models were validated by checking observed versus predicted residual deviance using the R package DHARMa (Hartig 2019).

5.2.4.3. Isotopic niche width

The R package SIBER (Jackson et al. 2011) was used to estimate the width of isotopic niches by calculating two metrics of standard ellipse area: SEA_c, sample size corrected Standard Ellipse Area, and SEA_b, Bayesian-estimated Standard Ellipse Area. A standard ellipse is to bivariate data as standard deviation is to univariate data; it encloses approximately 40% of the data and gives a bivariate measure of the distribution of individuals of a given group in isotopic space, thus indicating the core trophic niche of that group (Batschelet 1981; Ricklefs & Nealen 1998). Both metrics, SEA_c and SEA_b, are unbiased by sample size, when n is greater than 15 (Jackson et al. 2011; Syväranta et al. 2013). SEA_c gives a single estimate of ellipse area whereas SEA_b gives a distribution of estimates that reflects variability in the data and allows groups to be more robustly compared by calculating the probability associated with the difference in ellipses (Jackson et al. 2011). SEA_c and SEA_b were calculated for infected and uninfected brown trout and dace in each region, as well as for the overall populations of each species in each region. Isotopic niche widths of the three species richness categories of brown trout (species richness = 0, species richness = 1, and species richness > 1) were calculated as both SEA_c and SEA_b. Isotopic niche width was compared between groups by calculating the probability that the distributions of SEA_b differed from one another. All figures were produced using the SIBER package.

5.2.4.4. Isotopic niche specialisation

Isotopic niche specialisation was determined by assessing the overlap in the isotopic niches of the two populations or cohorts. Overlap in isotopic niches can be most easily assessed by calculating the area contained within the overlapping region of ellipses using SEA_c. However, this metric gives only a point estimate of overlap based on the maximum likelihood estimated SEA_c and neglects the variability around that estimate. A more robust method that incorporates variability is to use a Bayesian approach to create a distribution of multiple estimates drawn from the posterior distributions of the fitted ellipses (Jackson et al. 2011). The area of overlap between two ellipses, A and B, was represented as (i) the proportion of the total area of both ellipses (A + B – overlap) such that ellipses are entirely coincidental at 1 and entirely distinct at 0, (ii) the percentage of A that is overlapped by B and (iii) the percentage of B that is overlapped by A. The mode value and 95% credible intervals (95%CI) of the resulting distributions are reported for the overlap in brown trout and dace isotopic niches in each region and the overlap in infected and uninfected cohorts in each species and region.

5.2.4.5. Trophic position

The trophic positions of infected and uninfected dace and brown trout in the Munster Blackwater and River Barrow were calculated using the R package tRophic position (Quezada-Romegialli et al. 2018) (excluding infected River Barrow dace). Aquatic macroinvertebrates collected from the Munster Blackwater and River Barrow, comprising *Gammarus* sp., Trichoptera, Ephemoptera, Plecoptera, Odonata, Coleoptera (adult and larval), Chironomidae, and other Diptera (adult and larval), were pooled within their respective regions to form a separate isotopic baseline for each region. The isotopic baselines represent the primary consumers in the diet of secondary consumers, brown trout and dace. The trophic discrimination factors for fish muscle tissue used were mean 3.4 ± 0.98 standard deviation for $\delta^{15}N$ and mean 0.39 ± 1.3 standard deviation for $\delta^{13}C$ (Post 2002). Trophic discrimination factors represent the enrichment in carbon and nitrogen stable isotopes from one trophic level to another. Infected and uninfected fish were compared by calculating the probability that the estimated trophic position of infected fish was greater than that of uninfected fish.

5.3 Results

5.3.1 Trophically transmitted helminths

Brown trout were infected with between zero and five trophically transmitted helminths per fish in the Munster Blackwater and between zero and three trophically transmitted helminths per fish in the River Barrow (Table 5.1). The community of trophically transmitted helminths in the Munster Blackwater comprised seven species: the acanthocephalan *Pomphorhynchus tereticollis*, the nematodes *Cucullanus truttae, Cystidicola farionis, Rhabdochona* sp., *Salmonema ephemeridarum,* Capillariidae gen. sp. and Digenea gen. sp. The digeneans were included as trophically transmitted helminths despite being unidentifiable to species level because the presence of oral and ventral suckers and the location of infection within the gut lumen indicated that they were adult digeneans, not metacercariae, and thus likely trophically transmitted. In the River Barrow, the community of trophically transmitted helminths comprised six species: the acanthocephalan *Pomphorhynchus tereticollis*, the nematodes *Cucullanus truttae, Cystidicola farionis, Salmonema ephemeridarum, Raphidascaris acus,* Capillariidae gen. sp. and Digenea gen. sp. The nematode *Salmonema ephemeridarum* (transmitted by Ephemoptera) was the most dominant helminth in both regions. Both prevalence and mean species richness of trophically transmitted helminths were higher in brown trout in the Blackwater than in the Barrow (Table 5.1).

Dace were infected with between zero and two trophically transmitted helminth species in the Munster Blackwater and zero or one species in the River Barrow (Table 5.1). Both communities of trophically transmitted helminths were dominated by the acanthocephalan *P. tereticollis* which is transmitted by the amphipod *G. duebeni*, but the Munster Blackwater communities also contained a small number of nematode and adult digenean individuals which were unidentifiable to species level. Prevalence of trophically transmitted helminths in dace was 42% (31-53%) in the Munster Blackwater and under 1% in the River Barrow indicating that dace were more likely to be infected with trophically transmitted helminths at the core than the front of their range (Table 5.1).

Table 5.1. Overall infection parameters of trophically transmitted helminths in brown trout and common dace.

Host species	Region	n	n_{inf}	Prevalence (95%CI)	Mean species richness ± S.E	Species richness range	Dominant species
Brown trout	Munster Blackwater	122	116	95.10 (89.60-98.17)	1.93 ± 0.09	0-5	*Salmonema ephemeridarum*
	River Barrow	98	77	78.60 (69.13-86.22)	0.91 ± 0.06	0-3	*Salmonema ephemeridarum*
Dace	Munster Blackwater	86	36	41.90 (31.30-52.99)	0.43 ± 0.06	0-2	*Pomphorhynchus tereticollis*
	River Barrow	152	1	0.66 (0.02-3.61)	0.01 ± 0.01	0-1	*Pomphorhynchus tereticollis*

n = sample size, n_{inf} = number of infected fish, 95%CI = 95% Pearson's exact confidence interval, S.E = standard error.

Table 5.2. Isotopic niche width as Bayesian Standard Ellipse Area (SEA_b) modes with 95% credible intervals (95%CI) and sample size corrected Standard Ellipse Area (SEA_c).

		Brown trout			Dace		
		Uninfected	Infected	All	Uninfected	Infected	All
SEA_b (95%CI)	River Barrow	4.41 (2.93-7.13)	6.58 (5.21-8.14)	6.31 (5.12-7.67)	2.35 (2.00-2.78)	-	2.40 (2.06-2.81)
	Munster Blackwater	1.19 (0.46-3.16)	3.39 (2.82-4.07)	3.28 (2.75-3.92)	2.91(2.20-3.90)	2.75 (1.97-3.86)	2.96 (2.38-3.66)
SEA_c	River Barrow	4.89	6.67	6.40	2.39	-	2.43
	Munster Blackwater	1.55	3.44	3.33	3.02	2.89	3.01

5.3.2 Fish condition and size

I found no differences in relative condition factor between infected and uninfected fish in common dace or brown trout. Neither did relative condition factor of either species of fish differ between the Munster Blackwater (invasion core) and the River Barrow (invasion front).

Brown trout captured in the Munster Blackwater were overall significantly larger compared to the River Barrow (Length: Gaussian GLMM: df = 215, estimate ± standard error = 23.19 ± 5.59, Z = 4.15, P < 0.001; Weight: Gamma GLMM: df = 215, estimate ± standard error = 0.32 ± 0.15, Z = 2.18, P = 0.03) while, in dace, fish length or weight did not differ between the two study regions (Appendix Figure D1). Brown trout had overall greater length and weight than dace (Length: Gaussian GLMM: df = 452, estimate ± standard error = 13.15 ± 4.90, Z = 2.68, P = 0.007; Weight: Gamma GLMM: df = 451, estimate ± standard error = 0.37 ± 0.08, Z = 4.13, P < 0.001) but the interspecific difference in length and weight was significantly greater in the Munster Blackwater (Species:Region interaction; Length: Gaussian GLMM: df = 452, estimate ± standard error = 45.38 ± 7.17, Z = 6.33, P < 0.001; Weight: Gamma GLMM: df = 451, estimate ± standard error = 0.75 ± 0.14, Z = 5.51, P <0.001).

Infected dace had significantly greater standard length (Appendix Figure D1) and weight than uninfected dace (Length: Gaussian GLMM, df = 232, estimate ± standard error = -39.02 ± 4.98, Z = -7.84, P < 0.001; Weight: Gaussian GLMM, df = 232, estimate ± standard error = -40.96 ± 5.76, Z = -7.11 P < 0.001). In brown trout, there was no infection-associated difference in length (Gaussian GLMM: df = 215, estimate ± standard error = 5.24 ± 8.46, Z = 0.62, P = 0.54; Appendix Figure D1) or weight (Gamma GLMM: df = 215, estimate ± standard error = 0.14 ± 0.13, Z = 1.13, P = 0.26).

5.3.3 Isotopic niches of dace and brown trout

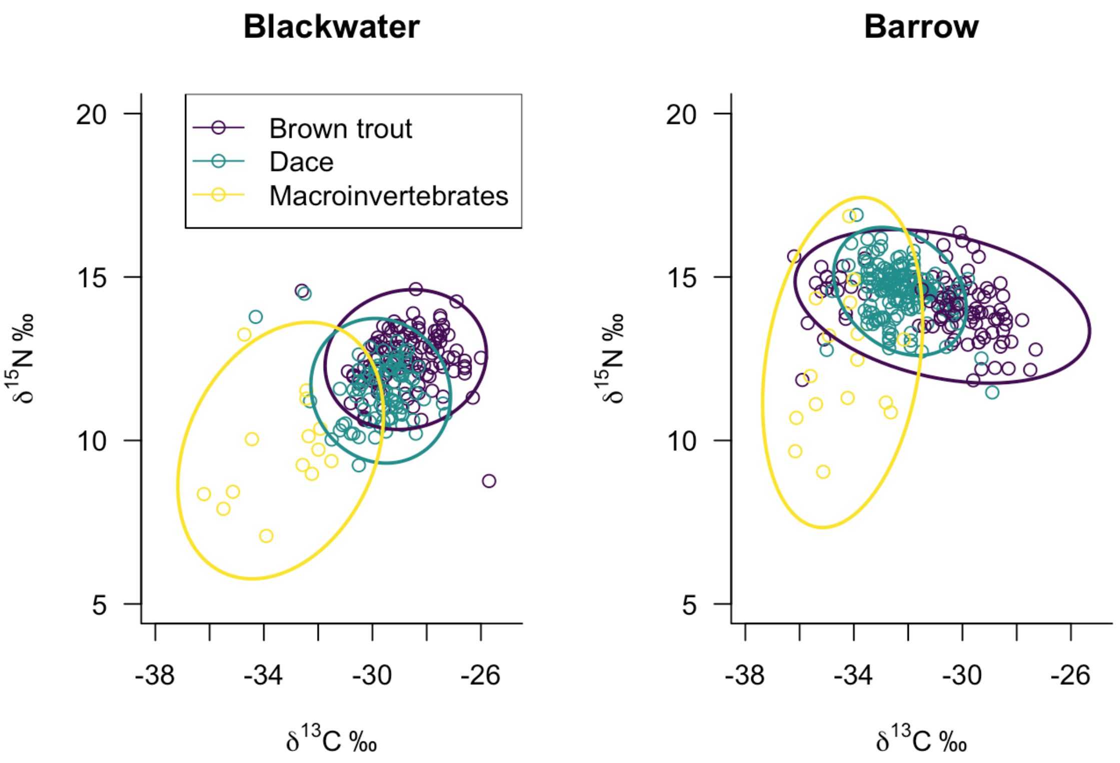

Figure 5.1. Isotope biplot depicting the raw isotopic data for brown trout *Salmo trutta*, dace *Leuciscus leuciscus*, and aquatic macroinvertebrates sampled from the Munster Blackwater (invasion core) and River Barrow (invasion front) along with their standard ellipses (SEAc for small sample size correction) based on maximum likelihood estimates.

Differences in niche width between dace and brown trout – as measured by sample size-corrected standard ellipse area (SEA$_c$; Figure 5.1) and Bayesian standard ellipse area (SEA$_b$; Figure 5.2) – varied between the two study regions (Table 5.2). In the Munster Blackwater (the core of the dace invasion range), the niche widths of dace and brown trout were not different (probability of brown trout standard ellipse area being greater than dace = 0.76). In this region, the proportion of overlap between brown trout and dace isotopic niches was 0.48 (95%CI = 0.40-0.57). 67% (95%CI = 56-81%) of the niche space of dace was overlapped by that of trout and 64% (51-73%) of the niche space of brown trout was overlapped by dace.

In the River Barrow, brown trout had a significantly larger isotopic niche than dace (probability = 1; Figure 5.2). The proportion of overlapping niche space between brown trout and dace in the River Barrow was 0.37 (0.29-0.46). The isotopic niche space of dace was nested within that of brown trout with 100% (95%CI = 93-100%) of the dace ellipse being overlapped by the trout ellipse. In contrast, 38% (95%CI = 29-47%) of the brown trout niche space was overlapped by dace.

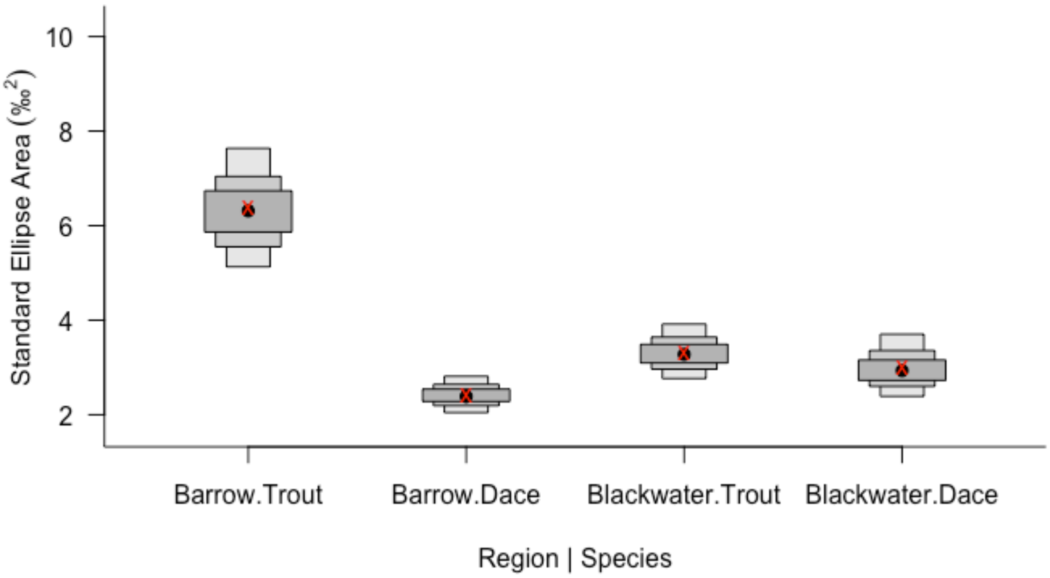

Figure 5.2. Isotopic niche width (estimated as Bayesian standard ellipse area, SEA$_b$) of brown trout and dace in the Munster Blackwater and River Barrow. The boxes represent the 95, 75 and 50% credible intervals in ascending order of size, with the mode indicated by the black circles. The sample size corrected standard ellipse area (SEA$_c$) calculated from the isotopic data is indicated by red crosses.

Within species, isotopic niche width varied by region (Figure 5.1, Figure 5.2). Brown trout in the Barrow had a larger niche than brown trout in the Blackwater (probability = 1) but dace had a smaller niche in the Barrow than in the Munster Blackwater (probability = 0.94). The niche size of the aquatic macroinvertebrate communities did not differ between the Munster Blackwater (SEA$_c$ = 8.12, SEA$_b$ mode = 7.17, 95%CI = 5.90-8.56) and the River Barrow (SEA$_c$ = 8.10, SEA$_b$ mode = 7.22, 95%CI = 6.15-8.70), with probability = 0.51 (Figure 5.1).

5.3.4 Isotopic niche and helminth infection

5.3.4.1. Niche width and specialisation in infected and uninfected cohorts

In brown trout, the infected cohort had a larger isotopic niche (SEA_b) than the uninfected cohort in the Munster Blackwater (probability = 0.96), but this was less pronounced in the River Barrow (probability = 0.89) (Figure 5.3). SEA_c of brown trout was larger for infected than uninfected cohorts in both regions (Table 5.3). In the Munster Blackwater, the proportion of overlap between infected and uninfected cohorts of brown trout was 0.28 (0.13-0.49). 29% (13-60%) of the infected ellipse was overlapped by the uninfected and 73% (44-100%) of the uninfected ellipse was overlapped by the infected. In the River Barrow, the proportion of overlap between infected and uninfected brown trout was 0.50 (0.39-0.62). 75% (58-91%) of the infected ellipse was overlapped by the uninfected ellipse and 60% (47-76%) of the uninfected ellipse was overlapped by that of the uninfected.

In contrast to brown trout, there was no difference in the size of the Bayesian ellipses between infected and uninfected cohorts of dace in the Munster Blackwater (probability = 0.42) (Table 5.2, Figure 5.3). SEA_c was higher for uninfected dace than infected dace in the Munster Blackwater (Table 5.2). The proportion of overlap in infected and uninfected ellipses was 0.31 (0.21-0.41). The overlap comprised 72% (48-99%) of the infected ellipse and 34% (25-47%) of the uninfected ellipse. Ellipses could not be calculated for infected dace in the River Barrow.

There was no interspecific difference in SEA_b between infected dace and infected brown trout in the Munster Blackwater (probability = 0.17) (Table 5.2, Figure 5.3). Interspecific differences in SEA_b between uninfected dace and trout differed by region (Figure 5.3). Uninfected dace had a smaller ellipse area than uninfected brown trout in the River Barrow (probability = 0.99) but did not differ from uninfected brown trout in the Munster Blackwater (probability = 0.07).

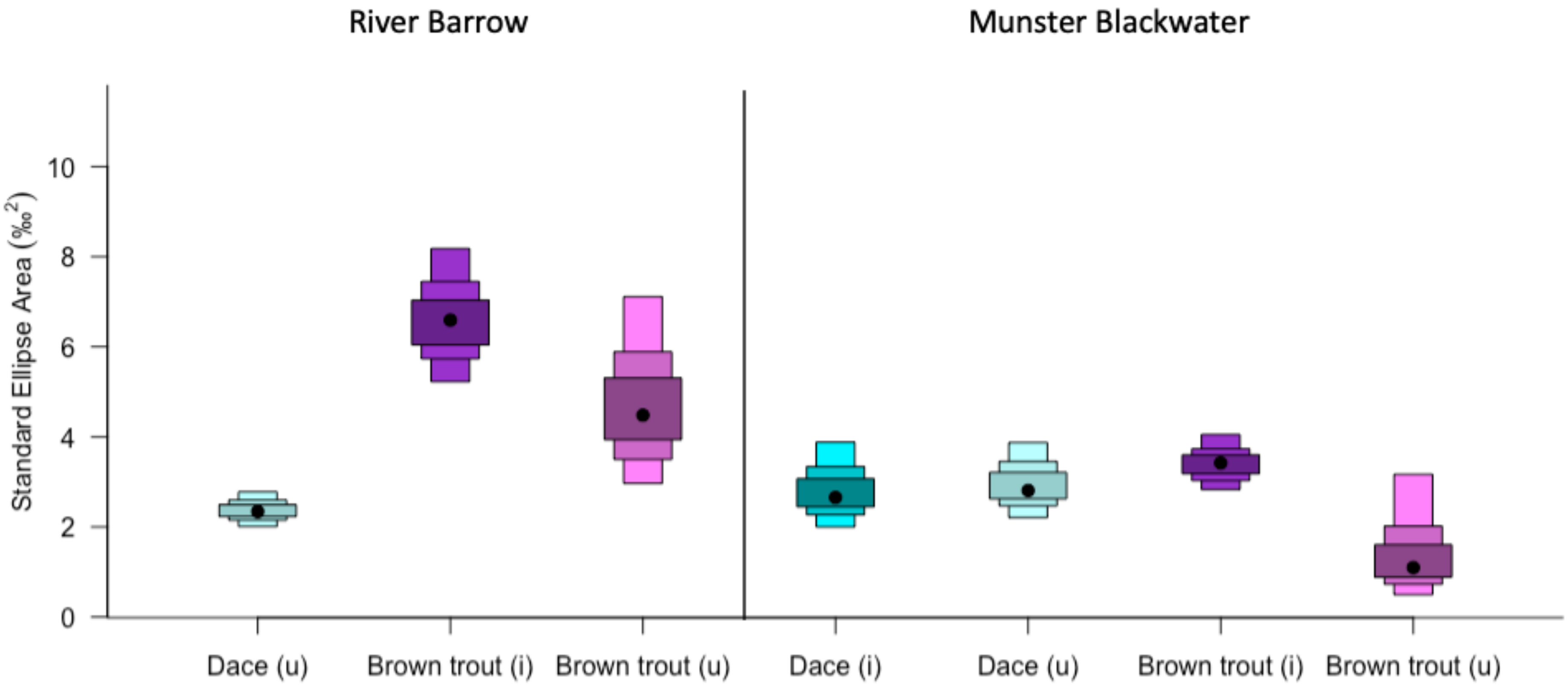

Figure 5.3. Isotopic niche width estimated by Bayesian standard ellipse area (SEA_b) of infected (i) and uninfected (u) brown trout and dace in the River Barrow and Munster Blackwater. The boxes represent the 95, 75 and 50% credible intervals in ascending order of size, with the mode indicated by the black circles. Dace populations are shown in turquoise and brown trout are shown in purple. Infected cohorts are indicated by the darker shades.

Table 5.3. Sample sizes, Bayesian Standard Ellipse Area (SEA_b) modes with 95% credible intervals (95%CI) and sample size corrected Standard Ellipse Area (SEA_c) of brown trout infection groups.

		Species richness = 0	Species richness = 1	Species richness > 1
n	River Barrow	21	66	11
	Munster Blackwater	6	39	77
SEA_b (95%CI)	River Barrow	4.50 (2.80-7.08)	5.29 (4.08-6.68)	7.45 (4.16-14.67)
	Munster Blackwater	1.24 (0.33-3.17)	3.11 (2.25-4.29)	3.32 (2.65-4.20)
SEA_c	River Barrow	4.89	5.36	8.69
	Munster Blackwater	1.55	3.27	3.41

5.3.4.2. Niche width and species richness in brown trout

The SEA_c values of brown trout in both study rivers were smallest when species richness = 0, intermediate when species richness = 1, and largest when species richness > 1 (Table 5.3). Comparisons using SEA_b (Table 5.3), also broadly supported the hypothesis that greater species richness was associated with larger isotopic niche. In the River Barrow, brown trout infected with zero or one species of trophically transmitted helminth had smaller isotopic niches than those infected with greater than one species (probability = 0.94 and 0.93, respectively, for zero and one species). In the Munster Blackwater, uninfected brown trout had smaller niches than brown trout infected with one or more species (probability = 0.94 and 0.96 respectively) (Table 5.3). However, no marked differences in isotopic niche width were detected between brown trout with zero and one in the River Barrow (probability = 0.70) or between brown trout with one species and greater than one species in the Munster Blackwater (probability = 0.61).

5.3.5 Trophic position

5.3.5.1. Dace and brown trout

In the Munster Blackwater, brown trout occupied a significantly higher trophic position than common dace (probability = 0.95) but in the River Barrow, I observed no interspecific difference in trophic position (probability of trout > dace = 0.25) (Figure 5.4). Brown trout had a mode estimated trophic position of 2.75 (95%CI = 2.52-2.98) in the Munster Blackwater and 2.53 in the River Barrow (95%CI = 2.22-2.83). Dace had a mode estimated trophic position of 2.47 in the Munster Blackwater (95%CI = 2.25-2.69) and 2.68 (95%CI = 2.36-2.99) in the River Barrow. Trophic position did not differ between the Munster Blackwater and the River Barrow for either fish species (probability of Barrow > Blackwater in dace = 0.83; in brown trout = 0.12).

5.3.5.2. Infected and uninfected cohorts

There were no differences in trophic position between infected and uninfected cohorts of either fish species, nor were there any regional differences in trophic position among infected or uninfected fish (Appendix Figure D2). In brown trout, the probability of the trophic position of the infected cohort being greater than uninfected was 0.58 in the Barrow and 0.47 in the Blackwater. In dace, the probability was 0.82 in the Munster Blackwater. Infected Barrow dace were not included in analysis but uninfected dace from the Barrow did not differ in trophic position from infected or uninfected Blackwater dace.

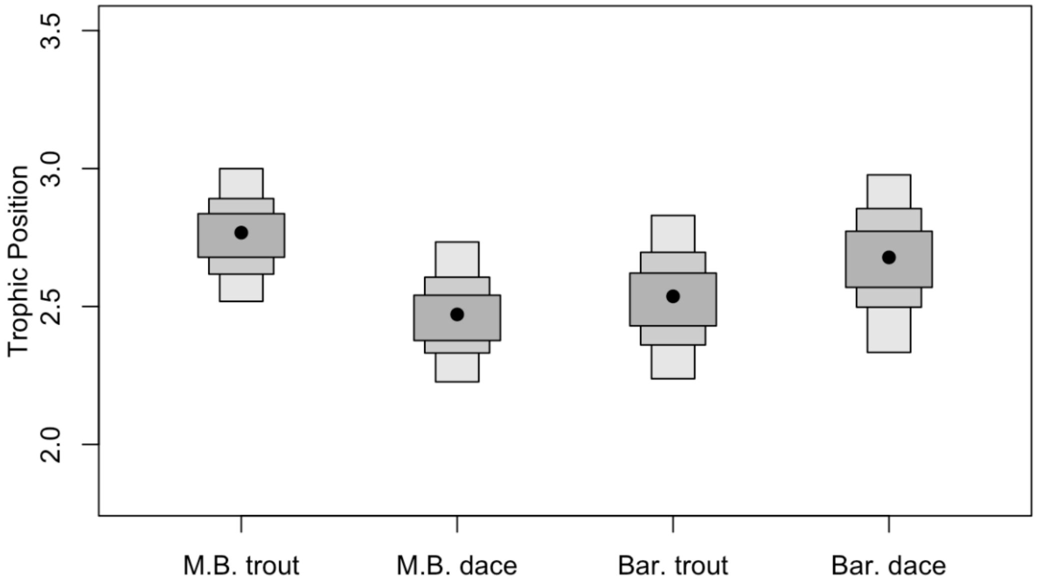

Figure 5.4. Estimated trophic positions of brown trout and dace collected from the Munster Blackwater (abbreviated M.B.) and River Barrow (abbreviated Bar.).

5.4 Discussion

Overlap in isotopic niches between dace and brown trout in both regions indicate that there is trophic overlap between the species, with greater niche overlap in the invasion core, the Munster Blackwater, where their isotopic niches overlapped by about half. With dace described as naturalised in the Munster Blackwater, having been present for over 100 years (Caffrey et al. 2007), compared to the recent invasion of the Upper Barrow, one might have expected greater trophic niche divergence between brown trout and dace in the Munster Blackwater, as observed between natives and invaders in other freshwater fish invasions (Tran et al. 2015). Tran et al. (2015) found that niche constriction in topmouth gudgeon, *Pseudorasbora parva,* led to a niche divergence from native fish which facilitated coexistence, although other studies have suggested that a species could also alleviate competition by diversifying its trophic niche (Svanbäck & Bolnick 2007). Native brown trout had a smaller niche in the invasion core relative to the front, which could reflect a competitive response to long-established dace invasion. However, if niche constriction in brown trout has occurred, the competitive benefit to brown trout is questionable, given that trout shared more of its niche with dace at the invasion core than at the front. Contrastingly, dace has a more diverged niche from brown trout at the core compared to the front, despite greater overall overlap, owing to brown trout having a smaller niche and dace having a larger niche. The isotopic niche width of a predator invariably reflects the isotopic niche width of their prey (Bearhop et al. 2004), such that narrower isotopic niche of prey populations is likely to result greater overlap in the niches of their consumers. The similar sizes of the aquatic macroinvertebrate niches in the Munster Blackwater and River Barrow therefore indicate that the differences in brown trout and dace niche width between the invasion front and core are unlikely to simply be due to differences in the isotopic niches of available prey.

Dace at the invasion front had a narrower niche compared to brown trout, and to conspecifics at the invasion core, and the dace niche at the invasion front was contained within a much larger brown trout niche. This indicates that at the front, brown trout exploits resources that dace do not and that dace feed on a limited subset of the resources utilised by trout. The size of the dace niche at the invasion front could suggest that invasive dace has diversified its niche at the core, while the niche of brown trout has contracted. However, if trophic competition between the two species differs between at the invasion front and core, this does not apparently impact the condition of the fish which did not differ between species or regions.

Brown trout fed at a similar trophic position to dace at the invasion front in the River Barrow but at a higher trophic position at the invasion core in Munster Blackwater. Brown trout is typically considered to have a higher trophic position than dace (Kottelat & Freyhof 2007) but this higher trophic position is driven by an ontogenetic niche shift to piscivory in brown trout

(Jensen et al. 2012; Sanchez-Hernandez et al. 2012). Given that, in the River Barrow, the collected brown trout used in this study were closer in size to dace than in the Blackwater, similar trophic positions in that region may be expected. The isotopic indication of an ontogenetic niche shift to piscivory in brown trout in the Munster Blackwater but not in the River Barrow is likely explained by the smaller size (and therefore younger age; Britton 2007) of brown trout collected from the River Barrow. Ontogenetic niche shifts to piscivory in brown trout have previously been shown to be associated with increased richness of trophically transmitted helminth species (Prati et al. 2020a). Evidence from my helminth community surveys of brown trout in Chapter 4 also support a shift to piscivory among Munster Blackwater individuals, with the nematode *Cucullanus truttae* – which is acquired through consumption of lamprey *Lampetra* sp. (Moravec 1979). – having significantly higher prevalence and abundance in the Munster Blackwater (Chapter 4, Table 4.5).

Results from brown trout generally supported the hypothesis that greater niche breadth is associated with parasite infection with the infected cohort having greater SEA_c and median SEA_b in both regions. There was also support for greater niche breadth driving parasite species richness, with niche width of brown trout generally increasing with species richness. Although the analyses in this chapter reveal only association and not causality, previous research (e.g. Kennedy & Burrough 1978, Gutiérrez et al. 2019) has strongly suggested that the trophic niche of hosts is a significant driver of trophically transmitted helminth diversity.

The narrow range of parasite species richness in Munster Blackwater dace (0-2) could explain why I did not observe a significant positive association between niche width and helminth infection status in this population. Previous research has demonstrated that feeding on a narrow range of resources can result in abundant infections of few helminth species (Kennedy & Burrough 1978). The helminth community survey in Chapter 3 demonstrated that the dace population in the Munster Blackwater is heavily dominated by a single helminth species, *Pomphorhynchus tereticollis*, which suggests intense feeding on gammarid intermediate hosts. Thus, it is plausible for infections in this populations to arise from a limited range of food resources such that a comparatively narrow niche is responsible for helminth infection. The inability to conduct analysis of helminth species richness and isotopic niche in dace (due to an insufficient range of species richness) highlights a methodological limitation that such analyses may not be possible or reliable in invasive populations with species-poor parasite communities.

I did not find compelling evidence that higher trophic position was associated with infection with trophically transmitted helminths; infected and uninfected cohorts in both species were found to be feeding at the same trophic position. Furthermore, brown trout and dace in the River Barrow had vastly different prevalence and species richness of helminths despite feeding at the same trophic position. That is not to say that trophic position does not influence the accumulation of parasites. For example, helminth species acquired through piscivory such as *Cucullanus truttae* are likely to be associated with higher trophic position, as suggested by the analyses in this chapter. Similarly, fish at lower trophic positions that incorporate more algal than invertebrate resources may escape infection with helminths transmitted by intermediate invertebrate hosts, as observed by Siwertsson et al. (2016). However, in the case of the brown trout and dace in this thesis, which had strongly overlapping isotopic niche but very different helminth communities, it appears that trophic position is relatively less important in structuring helminth parasite infection compared to other factors such as niche breadth and host competency (Chapter 2).

Although infected and uninfected cohorts occupied the same trophic positions, I found evidence for partial niche divergence between infected and uninfected niches. This can also be described as niche specialisation in infected and uninfected individuals. Particularly in the Munster Blackwater, the invasion core, there was greater niche divergence between infected and uninfected cohort of fish than between brown trout and dace. In all the paired comparisons of infected and uninfected cohorts – in Blackwater trout, Barrow trout and Blackwater dace populations – there was some degree of divergence between the niches of infected and uninfected fish. This illustrates that there were some resources utilised by one cohort which were not present in the diet of the other and indicates that trophic niche ha a role in intrapopulation variation in helminth infection.

The extent of divergence between infected and uninfected niches differed between the three populations with the most divergence occurring in trout at the invasion core (0.28 proportion overlap) and the least divergence occurring in trout at the invasion front (0.5 proportion overlap). The greater divergence in the core population of brown trout may be associated with the helminth community of that population being more species rich, however, in this system, it is not possible ascertain causation between niche specialisation and helminth infection. Differences in infection status in a population can arise from differential exposure to intermediate hosts caused by niche specialisation (e.g. Bertrand et al. 2008; Pegg et al. 2015) but observed differences in dietary niche can also result from helminth infection causing dietary shifts in their hosts (Britton & Andreou 2016). For example, Hutchings et al. (1998) found sheep infected with gastro-intestinal nematodes avoided feeding on contaminated swarths, Médoc et al. (2011) determined that infection with the acanthocephalan *Polymorphus minutus* caused reduced feeding on live isopod prey in the amphipod *Gammarus roeseli,* and Britton et al. (2011) found wild common carp *Cyprinus carpio* infected with the cestode *Bothriocephalus acheilognathi* shifted to lower trophic status prey items as a result of pathological damage to the gastrointestinal tract. A shift in host diet as a result of infection is usually associated with some parasite-induced cost to the host which, in previous studies, has detectibly manifested as reductions in host growth and condition (Britton et al. 2011; Pegg et al. 2015b). The absence of any difference in body condition between infected and uninfected fish in my study may therefore cautiously suggest that helminth infection in this system is a result of dietary preferences rather than a cause of them.

Nonetheless, the mechanistic role of trophic niche in helminth infection is difficult to assess with surveys of wild hosts alone. Hypothetically, one could determine the importance of exposure as opposed to susceptibility in a case where an infected niche is entirely coincident with the uninfected niche. In this case, one could be confident that the intermediate host prey causing infection is present within the trophic niche of both infected and uninfected cohorts, meaning that despite exploiting the same resources, intrinsic factors must be at play in some hosts that allow them to escape infection. In my study, despite about three quarters of the infected niche being overlapped by the uninfected niche in Barrow brown trout and Blackwater dace populations, it remained a possibility that infection in those populations resulted from the one quarter of trophic niche space that is exploited only by infected individuals. Furthermore, the role of diet in host infection is multifaceted and may be influenced or superseded by other, more important factors. While exposure is certainly necessary for infection with a parasite, intrinsic host factors such as host immunity and competency determine whether an infection is established. Helminths sometimes show different rates of establishment in different species of host, even if hosts are fed on the same diet and experimentally infected with identical doses of helminth infective stages (Taraschewski 1989; Paterson et al. 2013b). Therefore, the mechanistic influence of trophic niche on helminth infection (or vice versa) is best inferred by combining field data with experimental research (e.g. Britton et al. 2011; Médoc et al. 2011).

In conclusion, this study demonstrated considerable isotopic niche overlap between invasive common dace and native brown trout although the extent of that overlap differed between the core and front of the invasive range. Infection with trophically transmitted helminths was associated with partial niche specialisation in both species. In fact, at the core, there was greater niche divergence evident between infected and uninfected conspecifics than between dace and brown trout. My results generally support that greater niche breadth is associated with helminth infection status and helminth richness. However, I did not find compelling evidence that co-occurrence of invasive and native species leads, in time, to niche constriction by the invader or to overall niche divergence. While the design of my study did not allow me to causatively attribute patterns of helminth infection to trophic niche, the similar condition of infected and uninfected individuals may suggest that niche specialisation influences helminth infection as opposed to established helminths inducing trophic shifts. This study of trophic niche in an invasion context offers insight both into trophic competition between sympatric invasive and native species and drivers of parasite infection in different hosts. However, I acknowledge that the interactions between invasion, trophic niche and parasitism are complex and acknowledge the limitations of inferring patterns of helminth infection from trophic niche when other factors such as host competency are also likely to be important.

6. Discussion

With the incidence of species invasions set to increase in the coming decades (Seebens et al. 2020), it is increasingly important that research on invasive species impacts captures the complexity of natural ecosystems (Mack et al. 2000; Galiana et al. 2014; Gallardo et al. 2016; Walsh et al. 2016), including the influence of parasites in invasions (Poulin 2017). Many hypotheses in invasion biology have mixed or no robust empirical support, indicating that these hypotheses may not satisfactorily capture the complex nature of species invasions (Jeschke et al. 2012; Moles et al. 2012; Heger & Jeschke 2014). The outcomes of invasions are frequently context-dependent, varying with the traits of the invaders (Brandner et al. 2013; Grimm et al. 2020), the recipient community (Busst & Britton 2017; Britton et al. 2018; Crane et al. 2020), dispersal pathways (Wilson et al. 2009), and environmental context (Boets et al. 2019; Gozlan et al. 2020), among other variables. The influence of parasites in invasions is equally likely to be context-dependent, modified by factors such as invasive and native host traits (Dallas et al. 2019; Llopis-Belenguer et al. 2020) and environmental variables (Loxton et al. 2017; Wells & Clark 2019; Stuart et al. 2020). Thus, while theoretical and conceptual work are necessary for advancing understanding of parasites in invasions, it is critical that conceptual frameworks are challenged with empirical data to facilitate a truly general and mechanistic understanding of invasion impacts across contexts.

The aim of this thesis was to leverage the 100-year range expansion of an invasive freshwater fish as a natural experiment to uncover empirical patterns in helminth parasite communities of invasive and native sympatric hosts, and investigate parasite-mediated impacts of long-established and recently-established invasive host populations. I used a model system of invasive common dace (*Leuciscus leuciscus*) and native brown trout (*Salmo trutta*) to explore how helminth parasite communities in invasive and native hosts are influenced by host competence (Tierney et al. 2020b; Chapter 2), host colonisation history (Tierney et al. 2020a; Chapter 3), habitat type and the parasitology of co-occurring hosts (Chapter 4), and host trophic niche (Chapter 5). I also synthesise existing records, and update these with new records of helminth parasites infecting brown trout in Ireland and common dace across its invasive and native ranges.

6.1 Enemy release

The loss of parasites from invasive species during biological invasions is well-documented in the invasive species literature (e.g. Mitchell & Power 2003; Torchin et al. 2003; Dunn et al. 2012; Gendron et al. 2012; Goedknegt et al. 2016; Loxton et al. 2016; Wilson et al. 2019). Most research on enemy release compares the parasites of invaders to native sympatric species (a community approach e.g. Roznik et al. 2020; Stuart et al. 2020) or, less frequently, to native conspecifics (a biogeographic approach e.g. Torchin et al. 2001; Costa et al. 2018). A minority of research combines community and biogeographic comparisons in the assessment of enemy release (Torchin et al. 2003; Sarabeev et al. 2017; Gozzi et al. 2020). The combination of community and biogeographical approaches in this thesis allowed me to provide insight into reduced parasitism in invaders from both biogeographic and community points of view. My systematic review (Chapter 3) demonstrated that dace are infected with fewer species of helminth parasite in their invasive range in Ireland than conspecifics in the native range in continental Europe and Great Britain. My helminth community survey (Chapter 4) showed that invasive dace have lower helminth community diversity than sympatric native brown trout.

Further, this thesis provides a nuanced insight into aspects of enemy release commonly neglected in the literature. My finding of a lower overall prevalence of *Pomphorhynchus tereticollis* in dace than brown trout (Chapter 2) supports the prediction that parasite prevalence is lower in invasive than native populations (Torchin et al. 2003), which is a less frequently investigated aspect of enemy release than parasite species richness or diversity. Moreover, the helminth community survey (Chapter 3) evidenced variation in enemy release within an invasive range and with time since invasion, demonstrating that the parasite diversity of the invasive species is lower at the invasion front than at the invasion core. Greater enemy release at invasion fronts has long been predicted (Phillips et al. 2010; White & Perkins 2012) but empirical studies rarely compare front and core populations (Barnett et al. 2018; Stuart et al. 2020). Recognising that invasive species' parasite dynamics and their consequences for native species are likely to differ along invasion corridors and change over time is important to invasive species management, as initial impact assessments may not accurately capture their longer-term impacts.

Identifying the precise mechanisms driving reduced parasitism in invaders is difficult and sometimes impossible by means of parasite community surveys alone (Blackburn & Ewen 2017). My work does, however, provide important indicators of why helminth parasitism in invasive dace populations in Ireland may have been initially very low and remained so over time. I found no convincing evidence for the co-introduction of helminth parasites, generalist or cyprinid-specific, with dace (Chapter 3), indicating that the helminth community of invasive dace is likely composed of acquired native species. The low diversity and low prevalence of native helminth parasites in dace compared to native hosts (Chapter 4) is probably more likely due to host competence (Chapter 2), few generalist helminths (Chapter 4), and a lack of cyprinid-specific parasites and sympatric cyprinid hosts (Chapter 3) in the invasive range, rather than to dace being less exposed to helminth infective stages than brown trout (Chapter 5).

If dace are predominantly uninfected due to low competency for native parasites, then parasite adaptations to dace hosts may emerge with longer time since introduction, as with *P. tereticollis* in Ireland adapting to utilise brown trout as its main host (O'Mahony et al. 2004a, b). It is possible that the higher *P. tereticollis* abundance in dace at the invasion core relative to dace at the invasion front already indicates some degree of adaptation by the parasite. Additionally, given that dace also seem to escape infection due to the dearth of co-occurring cyprinid hosts and cyprinid specialist parasites, then future introductions of non-native freshwater fish to Ireland and range expansion of existing non-native species may facilitate increased helminth infection in dace. This would potentially represent an invasional meltdown scenario (Simberloff

2006) for co-introduced parasites, where the existing presence of an invasive host species could facilitate the establishment of non-native parasites.

Although reduced parasitism in invaders is predicted to release invaders from deleterious effects of parasites and thus, confer an advantage over natives (Torchin & Mitchell 2004; Dunn et al. 2012), I did not find that helminth infection negatively impacted host condition in either native brown trout or invasive dace (Chapter 5). It is, however, possible that helminth infection has deleterious impacts on other elements of host physiology and functioning not measured here (see Future perspectives) (Plaistow et al. 2001; Wood et al. 2007; Timi & Poulin 2020).

6.2 Native parasite dynamics

The impact of free-living invasive species on the parasite dynamics of native parasites is often neglected in the study of parasites in invaded systems (Kelly et al. 2009; Poulin 2017). Given that I found little evidence for the co-introduction (Chapter 3) or co-invasion (Chapter 4) of helminth parasites with dace, I had the opportunity to focus on the potential for dace invasion to alter native parasite dynamics. I found that the potential for invasion-altered native parasite dynamics varied among helminth species, contingent on the specificity of the parasite.

The generalist acanthocephalan *P. tereticollis*, which is adapted to brown trout in Ireland, had the capacity to infect common dace, especially dace in the invasion core (Chapter 2). This sharing of *P. tereticollis* between invasive dace and native brown trout populations in the invasion core, coupled with dace's incompetency as a definitive host, likely leads to reduced overall transmission and diluted infection in brown trout (indicated by lower parasite abundance in trout in the invasion core versus the front). As discussed in Chapter 2, different feeding preferences by dace and trout for the amphipod intermediate host, *Gammarus duebeni*, in the River Barrow and Munster Blackwater may play a role in the different patterns in *P. tereticollis* infection observed for the two species. For example, Paterson et al. (2011) found that low competence of invasive brown trout hosts for a native acanthocephalan (*Acanthocephalus galaxii*) had little effect on infection in native galaxias (*Galaxias anomalus*), and that lower parasite burdens in sympatric galaxias were likely caused by reduced galaxias density or reduced exposure due to displacement to poorer quality habitats, rather than by dilution. However, my findings on the isotopic niche of dace and brown trout indicate considerable similarity in their trophic niches (Chapter 5). While not necessarily conclusive, the overlap observed in the isotopic niches of dace and brown trout suggests that, in this system, a combination of host competency and dilution effects is a more convincing explanation for differing prevalence of *P. tereticollis* in the two fish species than is trophic niche separation.

For the other helminth species recorded in the community surveys (Chapter 4), I concluded that altered native parasite dynamics as a result of dace invasion was unlikely. The high host specificity of most of the recorded helminth species to brown trout or Salmonidae (Chapter 4) suggests that the presence of invasive dace hosts in the ecosystem is unlikely to perturb the parasite dynamics of specialist helminth species. The fish fauna of Ireland is characterised by anadromous euryhaline species—salmonids, eels etc. (Fitzsimons & Igoe 2004)—the helminth communities of which tend to be variable and heavily composed of autogenic species (species that use fish as definitive hosts; Esch et al. 1988). The helminth communities of cyprinids, on the other hand, tend to be dominated by a few widespread allogenic species (species that use birds or mammals as definitive hosts; Esch et al. 1988). If Ireland therefore has lower overall prevalence of generalist and allogenic helminth species, there would be little potential for native helminths to infect dace (Chapter 3) and low risk to native parasite dynamics through parasite acquisition. However, while this was not apparent in my study system, there remains the potential for an invader to disrupt native parasites dynamics by reducing native host density or altering native host distributions, even if invasive species do not acquire native parasites (Paterson et al. 2011). In a fish stock assessment of the River Barrow in 2015, Delanty et al. (2017) speculated that brown trout distribution in the river may be impacted by dace invasion, indicating that the disruption of native parasite dynamics through invader-induced changes to host density or distribution may become important in future as dace invasion progresses.

6.3 Implications for invasive species management

6.3.1 Baseline knowledge

Many authors, academic and governmental, have emphasised the importance of establishing baselines of various aspects of species invasions to the management of current and future invasive species (Gardner et al. 2016; Tsiamis et al. 2017; Pagad et al. 2018). This need for baseline knowledge also applies to parasite interactions in biological invasions. Introductions of non-native freshwater fish are predicted to rise into the future (Gozlan et al. 2010; Seebens et al. 2017). Despite strong advances in biosecurity in recent years (Sutcliffe et al. 2018; Coughlan et al. 2019), control measures remain at risk of being outpaced by the intensification of global trade which often provides introduction opportunities and vectors (Levine & D'antonio 2003; Nunes et al. 2015; Essl et al. 2020).

My work provides important empirical baseline data on the helminth communities of invasive dace populations and native trout populations in two catchments that were previously largely unstudied. These data are likely to prove valuable in the face of future freshwater fish invasions and are already useful to the assessment of potential parasite-mediated impacts of recent fish invasions in Ireland. In 2017, the unprecedented widespread occurrence of non-native Pacific pink salmon, *Oncorhynchus gorbuscha*, in rivers along the west of Ireland raised concerns of impacts on native salmonids (Millane et al. 2019). Research from other European regions where non-native pink salmon occurs indicated the strong potential for this species to share helminth parasites with native Atlantic salmon, *Salmo salar*, and sea trout and brown trout, *S. trutta* (Ieshko et al. 2016, 2018; Sandlund et al. 2019). My research identified that the salmonid swim bladder nematode, *Cystidicola farionis*, occurred in both native riverine brown trout populations and non-native pink salmon captured in Irish rivers (Millane et al. 2019), as well as demonstrating the potential for other shared helminths such as *Salmonema ephemeridarum, Eubothrium crassum* and *Crepidostomum farionis* between native *Salmo* species and non-native pink salmon (Appendix Table E1). Fortunately, the 2017 invasion of pink salmon seems to have been an isolated occurrence, with very few pink salmon detected in Ireland in subsequent years (M. Millane, pers. comm.).

The non-native cyprinid, chub *Leuciscus cephalus*, was previously introduced and eradicated from Ireland (Caffrey et al. 2008, 2018), but was, once again, detected in Ireland in August 2020 (O'Neill 2020). In its native range in Great Britain and continental Europe, chub acts as one of the preferred definitive hosts of *P. laevis s.l.* (the other preferred definitive host being barbel, *Barbus barbus*; Bates & Kennedy 1991; Perrot-Minnot et al. 2019, 2020). *P. laevis s.l.* in Great Britain and continental Europe occurs in chub in particularly high abundance (Hine & Kennedy 1974a; Perrot-Minnot et al. 2020). This raises the obvious concern that establishment of chub and subsequent acquisition of *P. tereticollis* in Ireland could result in amplification of *P. tereticollis* transmission and spillback into native freshwater fish hosts. In that respect, the limited success of *P. tereticollis* in infecting invasive dace may be reassuring, as it suggests that in adapting to brown trout (O'Mahony et al. 2004a), the Irish strain of *P. tereticollis* may have reduced capacity to infect other hosts.

As case studies, the pink salmon invasion event and the introduction of chub demonstrate that in light of the likely future non-native species introductions, information on the composition of native parasite communities is vital for predicting the potential for parasite-sharing between invaders and natives. Such information allows researchers and stakeholders to assess the potential for invasion impacts on native fauna such as spillback and dilution when planning actions to manage and mitigate invasion impacts. Moreover, in the event of successful invasions, baseline knowledge on natural spatial and temporal variation in the structure and composition of native host-parasite systems will be essential for evaluating disruption of native parasite dynamics caused by an invader.

6.3.2 Ecosystem effects of altered parasite dynamics

From the point of view of most fisheries managers, the introduction of invasive parasites and spillback to native fish would likely be the most concerning parasite-associated impact of invasion; for example the introduction of the salmon gill fluke (monogenean), *Gyrodactylus salaris* (Forseth et al. 2017; Lucy et al. 2020)*,* or the eel swim bladder nematode, *Anguillicola crassus* (Weclawski et al. 2014). I demonstrate that, even without co-introducing parasites, invasive species have the capacity to disrupt native parasite dynamics and affect native species (Chapter 2). My findings demonstrating that the presence of invasive dace over time reduces infection in native hosts (Chapter 2), could potentially be considered a rare, positive effect of invasion. However, given the complexities of host-parasite interactions and the prominent roles that parasites play in ecosystems, I must note that the reduced *P. tereticollis* infection observed in brown trout does not necessarily mean that the presence of invasive dace is beneficial to native fish. My results do not indicate that helminth infection reduces the body condition of fish hosts (Chapter 5). Moreover, the highly connected nature of parasites in food webs (Lafferty et al. 2006; Dallas et al. 2019) means that perturbations to one stage of the parasite life cycle may have ecosystem-wide consequences (Mehlhorn et al. 2015). For example, acanthocephalans such as *P. tereticollis* can act as ecosystem engineers by mediating the behaviour of their amphipod intermediate hosts and therefore have important functions in ecosystems in their own right (Sures et al. 2017; Giari et al. 2020). Acanthocephalans are known to bio-accumulate heavy metals (Sures & Siddall 1999; Sures et al. 2003; Erasmus et al. 2020), mediate trophic interactions (Fielding et al. 2003; Macneil et al. 2003), and can mediate host impacts on habitat structure (Williams et al. 2019). Indeed, *P. tereticollis* modifies the behaviour of its amphipod intermediate hosts to make them easier prey for fish hosts (Fayard et al. 2019). Accordingly, it is difficult to weigh the possible benefit to native fish of diluted parasitism as a result of invasive species against potential reductions to important parasite-mediated ecosystem functions, not to mention against other effects of invasive species such as competition and habitat loss.

6.4 Future prospects

6.4.1 Mechanistic approaches to enemy release and parasite-mediated invasion impacts

Enemy release is increasingly viewed as a collection of sub-hypotheses (the hierarchy of hypotheses approach) in which reduced infection with parasites is one part, which may or may not lead to benefits to the invader in the form of reduced damage or increased performance (Heger & Jeschke 2014, 2018). Some authors (e.g. Blakeslee et al. 2013; Prior et al. 2015) make the specific distinction between "parasite loss" as the loss of parasites from invasive populations and "enemy release" as the benefit gained by the invader from reduced parasitism. While this thesis provides ample evidence for the reduction of helminth parasitism in invasive compared to native populations, the data I collected do not allow me to investigate whether loss of parasites benefits invasive dace or contributes to invasion success. This is not uncommon in invasion biology studies; the hypothesis that loss of parasites during invasion gives invasive species a competitive advantage over native species is one of the most referenced concepts in parasite ecology in the invasion context, but remains rarely tested. Studies that do test whether enemy release determines invasion success overwhelmingly focus on terrestrial plants (Mitchell & Power 2003; Prior et al. 2015; Blackburn & Ewen 2017). The challenge lies in empirically demonstrating that native populations are controlled by parasites whereas invasive populations are not, and that this escape from parasite control significantly contributes to their invasion success as opposed to other factors (Prior et al. 2015). One could test the enemy release hypothesis by comparing whether successfully established invasive populations experienced greater parasite loss than unsuccessful invaders, but data on the parasites of introduced species that fail to establish has simply never been recorded (Van Kleunen et al. 2010; Blackburn & Ewen 2017).

A recent study by Gozzi et al. (2020) circumvented these issues by comparing parasite richness between different introduced populations of Pallas' squirrel, *Callosciurus erythraeus*, with varying invasion success (measured by invader density and spread). Although parasite richness of *C. erythraeus* was lower in introduced populations than native conspecific populations and other native sympatric mammals, parasite richness in introduced ranges was not associated with invasion success (Gozzi et al. 2020). Another recent study on an animal invasion managed to mechanistically test the effect of parasite loss on invasion success through perturbation experiments in invaded systems. In this experiment, Roznik et al. (2020) treated native and invasive species of treefrogs with an anthelminthic drug, hypothesising that if the enemy release hypothesis is supported, native hosts would benefit more from parasite removal due to native hosts' greater investment in anti-parasite defences. While the treatment reduced helminth burdens in both species, invaders and native species responded similarly to the treatment and frogs treated with the anthelminthic had similar growth and survivorship to untreated frogs, indicating that parasitism was not an important driver of invasion success (Roznik et al. 2020).

Neither of these studies provided convincing evidence that parasite loss drives invasion success, but they demonstrate the application and value of mechanistic approaches and perturbation experiments for evaluating the processes that drive invasions and parasite interactions. Mechanistic approaches to invasion-modified native parasite dynamics (e.g. dilution, spillback) using experimental infections and population modelling have already been valuable in disentangling the roles of native and invasive hosts in parasite dynamics (Paterson et al. 2013b). However, many parasite taxa lack basic descriptions of their life histories (Blasco-Costa & Poulin 2017), hindering the development of protocols for experimental infections and the parameterisation of infection models. Poulin & Maure (2015) noted that most experimental work on host manipulation by parasites is restricted to a few model host-parasite systems—a trend that also seems true for empirical invasion research. Lack of reliable experimental infection protocols for the Irish strain of *P. tereticollis* prevented me from experimentally verifying the differences in host competency of brown trout and dace. Perturbation experiments on invaded communities such as that of Roznik et al. (2020) are particularly promising for the field of parasite ecology in invasions as they allow for mechanistic investigations of parasite and invader effects in less well-studied host-parasite systems. Future work should use experimental manipulations of invasion systems to mechanistically test long-standing hypotheses such as enemy release, and newer hypotheses such as parasite-mediation of propagule pressure (for a given propagule pressure, invasion success is greater for species that are more impacted by parasites in their native range; Prior et al. 2015) and suppressive spillover (deleterious effects of acquired native parasitic infection limit invasion success; Chalkowski et al. 2018).

6.4.2 Host immunology and genetics in parasite ecology of invasions

Unaddressed thus far in this thesis and generally neglected in the invasion literature, is the effect of host factors such as genetic diversity and immunology on the helminth communities of native and invasive populations. The genetics of invaders is often discussed in the context of invasions, as a potential mediator of invasion success (Allendorf & Lundquist 2003). Genetic diversity in invasive populations is predicted to be low as a result of bottlenecks from small founding populations of invaders, potentially limiting the capacity of invaders to survive and adapt to novel environments (Allendorf & Lundquist 2003). This has since been challenged as Roman & Darling (2007) found that high propagule pressure in aquatic invasions resulted in 63% of aquatic invasive populations having similar genetic diversity to native populations, and Gozlan et al. (2020) found that genetic diversity did not affect fitness-related trait shifts in the invasive freshwater fish, topmouth gudgeon *Pseudorasbora parva*.

Genetics also play a role in the susceptibility of wild hosts to metazoan parasites, influencing the immune function of their hosts (Blanchet et al. 2010). In cases of vertebrate invasions where genetic diversity is reduced in invasive populations, loss of diversity at the major histocompatibility complex (MHC) loci can result in a reduced capacity for rapid adaptive immunity (Lee & Klasing 2004; White & Perkins 2012). For example, Biedrzycka et al. (2020) found that random genetic drift in invasive raccoon, *Procyon lotor*, populations led to two distinct genetic clusters with different MHC-parasite associations, which in turn influenced the infection status of populations. Invasive cane toads, *Rhinella marina*, rapidly evolve shifts in immune functions in response to dispersal, particularly at the invasion front where parasite are likely to be less prevalent (Phillips et al. 2010; Brown & Shine 2014; Brown et al. 2015).

The ecoimmunology of invasive species is a relatively new but growing sub-field of biology with clear applications to understanding the structure and composition of helminth communities in native and invasive species, and parasite-mediated invasion impacts in invasions (Lee & Klasing 2004; White & Perkins 2012; Cornet et al. 2016; Becker et al. 2020). In my study system, ecoimmunological investigations would benefit the understanding of parasite dynamics in dace and brown trout, particularly the evolution of host competency and the infection dynamics of helminths in front and core populations of invasive dace. The newly-coined field of macroimmunology—the study of variation in host immunology across spatial scales—establishes the interactions of immunology with range expansions and biological invasions as a priority for future research (Becker et al. 2020). This new field of study is likely to bring together evidence in different host taxa for immune variation across an invasive range and further illuminate the role of parasitism in invasion front and core populations (Becker et al. 2020). For example, Diagne et al. (2017) reported increased inflammatory responses in front populations of invasive black rats, *Rattus rattus*, suggesting higher parasitism at the front, contrary to expectations of the evolution of increased competitive ability (EICA) refined hypothesis (White & Perkins 2012). Given that helminth parasite prevalence in dace is greater at the core of than the front, I would expect immunological adaptations to native parasites to evolve in dace over time since invasion. On the other hand, the low diversity of parasites in dace at the front of the invasive range could facilitate a shift away from energetically costly immune functions in favour of investing in growth, reproduction, or other adaptations that enhance dispersal (Evolution of increased competitive ability refined hypothesis; White & Perkins 2012). Similar to the enemy release hypothesis, ecoimmunological hypotheses relating to invasive species such as EICA suffer from a lack of supporting empirical and mechanistic evidence (Cornet et al. 2016) so already well-studied host-parasite systems such as those of brown trout and dace in Ireland would be a valuable framework on which to build further evidence of immunological determinants of parasite patterns in invasions.

6.4.3 Stable isotope approaches to parasite-mediated invasion interactions

Much of the work to date on stable isotope analysis (SIA) in parasite ecology is concerned with the role of parasites in food webs and focusses on the trophic position of parasites relative to their host (e.g. Nachev et al. 2017). Stable isotope analysis is also used alongside parasitological information to infer dietary shifts and the trophic ecology of individual species (Sinisalo et al. 2006; Bertrand et al. 2008; Lynggaard et al. 2018; Gilbert et al. 2020). Despite the large body of work using SIA as a tool in invasion biology to evaluate trophic interactions between native and invasive species (e.g. Olsson et al. 2009; Hayden et al. 2013; Tran et al. 2015; Britton et al. 2018, 2019; Gutmann Roberts & Britton 2018; Nolan & Britton 2018), very few studies utilise SIA to explore the role of parasites in those interactions. Research that does incorporate stable isotope approaches and parasite ecology in the study of invasive species is limited to a narrow taxonomic range and is often purely conceptual (Britton et al. 2011; Britton & Andreou 2016). Given the advantage of SIA over traditional methods of trophic analysis when integrating dietary data over long time scales (Nielsen et al. 2018), the lack of research utilising SIA in the investigation of parasite acquisition by invasive species through time is a missed opportunity. Chapter 5 demonstrated the clear potential for stable isotope approaches to help disentangle the drivers of helminth infection in native and invasive species, shedding new light on variation in helminth communities across invaded ranges. Future work should leverage SIA approaches to provide empirical data on the dietary drivers of parasite infection and the effect of invasion-induced niche diversification or niche specialism on the parasite communities of invasive and native populations.

6.5 Concluding remarks

This thesis builds upon existing theoretical frameworks of the role of parasites in biological invasions to provide empirical evidence of important parasite interactions in invaded ecosystems. This research fills knowledge gaps in the helminth community of two important freshwater fish in Ireland—the dominant native fish, brown trout (*S. trutta*), and the rapidly spreading invasive fish, common dace (*L. leuciscus*)—providing helminth community data from two previously unstudied river systems. With the incidence of freshwater fish introductions only set to rise in future (Seebens et al. 2020), these data advance our understanding of the helminth parasites of freshwater fish in Ireland, and provide invaluable baseline information for the assessment of parasite-mediated impacts of future freshwater fish invasions. By combining parasitological surveys of fish at the host community level, a systematic literature review at the biogeographical level, and stable isotope analysis of trophic ecology, I was able to illuminate the processes that shape helminth communities of dace and brown trout in Ireland, including enemy release in dace and parasite dilution in brown trout. However, robust, mechanistic investigations of parasite dynamics in invasions are still needed across taxa and ecological contexts. Future research should seek to exploit the potential of perturbation experiments and the growing field of ecoimmunology to gain a deeper understanding of the mechanisms that shape helminth parasite communities in biological invasions.

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8. Appendix A – Supplementary information to Chapter 2

Table A1. Structure and output of the generalised linear mixed model (binomial family) for prevalence of adult *P. tereticollis*. Significance codes as follows: 0 '***', 0.001 '**', 0.01 '*' 0.05 '.'.

Model: Adult.Prevalence ~ species*region + species*length + (1|year) + (1|region:site)					
Random effects					
	Variance	Std.Dev.			
Year	0.98	0.99			
Region:Site	2.78	1.67			
Fixed effects					
	Estimate	Std. Error	z value	P value	
Species	-13.42	2.58	-5.20	1.99e-07	***
Region	4.21	1.85	2.27	0.0232	*
Length	0.05	0.01	4.83	1.37e-06	***
Species:Region	-2.50	1.25	-1.99	0.0462	*
Species:Length	-0.06	0.01	-5.35	8.72e-08	***

Table A2. Structure and output of the refined generalised linear mixed model (negative binomial family) for intensity of adult *P. tereticollis*. Significance codes as follows: 0 '***', 0.001 '**', 0.01 '*' 0.05 '.'.

Model: Adult.Intensity ~ species*region + species*length + (1|region:site), ziformula=~0					
Random effects					
	Variance	Std.Dev.			
Region:Site	6.813e-10	2.61e-05			
Fixed effects					
	Estimate	Std. Error	z value	P value	
Species	-0.02	0.38	-0.05	0.9629	
Region	-1.00	0.46	-2.19	0.0286	*
Length (scaled)	0.37	0.28	1.31	0.1916	
Species:Region	-0.10	0.48	-0.20	0.8396	
Species:Length	-0.31	0.29	-1.07	0.2840	

Table A3. Structure and output of the generalised linear mixed model (binomial family) for prevalence of subadult *P. tereticollis*. Significance codes as follows: 0 '***', 0.001 '**', 0.01 '*' 0.05 '.'.

Model: Subadult.Prevalence ~ species*region + species*length + (1|year) + (1|region:site)					
Random effects					
	Variance	Std.Dev.			
Year	5.05e-09	7.10e-05			
Region:Site	1.63	1.28			
Fixed effects					
	Estimate	Std. Error	z value	P value	
Species	9.56	2.52	3.80	0.0001	***
Region	3.93	1.46	2.70	0.0070	**
Length	0.06	0.01	5.87	4.25e-09	***
Species:Region	-3.82	1.37	-2.80	0.0050	**
Species:Length	-0.06	0.01	-4.76	1.98e-06	***

Table A4. Structure and output of the refined generalised linear mixed model (negative binomial family) for intensity of subadult *P. tereticollis*. Significance codes as follows: 0 '***', 0.001 '**', 0.01 '*' 0.05 '.'.

Model: Subadult.Intensity ~ species*region + species*length + (1|region:site), ziformula=~0					
Random effects					
	Variance	Std.Dev.			
Region:Site	1.39	1.18			
Fixed effects					
	Estimate	Std. Error	z value	P value	
Species	-1.10	0.57	-1.92	0.0546	.
Region	1.97	1.24	1.59	0.1113	
Length (scaled)	1.62	0.24	6.66	2.71e-11	***
Species:Region	-1.05	0.81	-1.29	0.1957	
Species:Length	-1.65	0.36	-4.60	4.16e-06	***

Table A5. Structure and output of the generalised linear mixed model (binomial family) for proportion *P. tereticollis* relative to total *P. tereticollis*. Significance codes as follows: 0 '***', 0.001 '**', 0.01 '*' 0.05 '.'.

Model: Proportion.Adults ~ species*region + species*length + (1|year) + (1|region:site)					
Random effects					
	Variance	Std.Dev.			
Year	1.22	1.11			
Region:Site	1.18e-09	3.43e-05			
Fixed effects					
	Estimate	Std. Error	z value	P value	
Species	4.36	0.47	9.28	< 2e-16	***
Region	0.25	0.40	0.63	0.52984	
Length (scaled)	-0.94	0.29	-3.28	0.00104	**
Species:Region	0.87	0.67	1.31	0.19086	
Species:Length	0.71	0.38	1.87	0.06186	.

Table A6. Structure and output of the final (simplified) generalised linear mixed model (gamma family) for weight of adult *P. tereticollis*. Significance codes as follows: 0 '***', 0.001 '**', 0.01 '*' 0.05 '.'.

Model: Weight ~ sex + host.length * fish.species + (1|observer) + (1|region:site) + (1|host.id)					
Random effects					
	Variance	Std.Dev.			
Observer	0.04	0.20			
Region:Site	0.04	0.19			
Host ID	0.21	0.46			
Fixed effects					
	Estimate	Std. Error	z value	P value	
Sex	-0.37	0.07	-5.21	1.87e-07	***
Host length	0.01	0.01	1.40	0.16	
Host species	2.48	1.00	2.49	0.01	*
Host species:Host length	-0.01	0.01	-1.76	0.08	.

9. Appendix B – Supplementary information to Chapter 3

Table B1. Number of helminth species recorded in common dace (*Leuciscus leuciscus*) per site for a subset of records in the systematic review where site data were obtained (full dataset available at https://doi.org/10.1017/S0022149X20000759). Abbreviations for the ranges as follows: I = Ireland, GB = Great Britain, N = Nordics, CE = Continental Europe (excluding Nordics).

Site	Country/ region	Range	Helminth species	No. of papers	Source(s)
Kanturk, Co. Cork	Ireland	I	1	1	(Kane 1966)
Anglian and Thames regions	England	GB	1	1	(Kirk & Lewis 1994)
Boston, Lincolnshire	England	GB	3	1	Kennedy 1974
Cambridgeshire	England	GB	2	1	(Baylis 1939)
Ravensthorpe Reservoir, Northampton	England	GB	1	1	(Orr 1968)
River Avon, Hampshire	England	GB	10	12	(Stranack 1966; Kennedy 1968, 1969, 1972, 1974; Chappell & Owen 1969; Kennedy & Hine 1969; Hine 1970; Hine & Kennedy 1974; Kennedy & Rumpus 1977; Chubb 1979; Esch et al. 1988)
River Bain, Lincolnshire	England	GB	4	3	(Chappell & Owen 1969; Kennedy 1974; Kirk & Lewis 1994)
River Blackwater, Essex	England	GB	2	1	Kennedy 1974
River Colne, Essex	England	GB	1	1	Kennedy 1974
River Lugg, Herefordshire	England	GB	10	1	(Davies 1967)
River Roding, Essex	England	GB	7	2	(Shillcock 1972; Kennedy 1974)
River Stour, Hampshire	England	GB	2	2	(Stranack 1966; Esch et al. 1988)
River Thames	England	GB	1	2	(Crowden 1976; Crowden & Broom 1980)
River Tone, Somerset	England	GB	1	1	Kennedy 1974
River Severn	England/ Wales	GB	1	1	(Esch et al. 1988)
Bothnian Bay	Finland	N	1	1	(Valtonen & Crompton 1990)
River Kivijoki system	Finland	N	14	1	(Ieshko et al. 1997)
Lake Øyeren	Norway	N	1	1	(Hansen & Brabrand 1979)
Rivers Leira, Nitelva and Glomma	Norway	N	13	1	(Sterud & Appleby 1997)
Albertcanal, close to Genk	Belgium	CE	1	1	(Thomas & Ollevier 1992)
Ilova River	Croatia	CE	1	1	(Zrnčić et al. 2009)

River Bystricka near Hradec Králové	Czech Republic	CE	1	1	(Scholz 1989)
River Morava	Czech Republic	CE	9	2	(Gelnar et al. 1994; Matejusová et al. 2002)
Rokytná River, Danube Basin	Czech Republic	CE	5	4	(Moravec 1989, 1992; Scholz 1989; Moravec & Scholz 1991)
Vltava River	Czech Republic	CE	4	1	(Moravec et al. 1997)
River Vingeanne	France	CE	1	1	(Perrot-Minnot et al. 2019)
Lake Constance at Langenargen	Germany	CE	2	1	(Balling & Pfeiffer 1997)
River Lippe near Hamm	Germany	CE	1	1	(Taraschewski 1988)
Kiviniemi, Bothnian Bay	Gulf of Bothnia	CE	1	1	(Karvonen & Seppälä 2008)
North east Baltic Sea	Gulf of Bothnia	CE	6	2	(Valtonen et al. 2001; Seppälä et al. 2011)
Danube	Hungary	CE	3	1	(Molnar 1970)
Ipoly	Hungary	CE	2	1	(Molnar 1970)
Tisza , Lake Balaton	Hungary	CE	2	1	(Molnar 1970)
Velence, Bäche, Zagyva	Hungary	CE	1	1	(Molnar 1970)
Magyarkút and Kemence Brooks	Hungary	CE	2	1	(Molnar 1970)
Lake Onega	Karelia	CE	19	1	(Rumyantsev et al. 1984)
Lakes Kimas, Luv and Vendurskom	Karelia	CE	1	1	(Ieshko et al. 1976)
Vistula Lagoon	Poland	CE	2	2	(Rolbiecki et al. 1999; Rolbiecki 2004)
Rozhjan and Vychegda Rivers	Russia	CE	1	1	(Dorovskich 1991)
Volga Basin	Russia	CE	1	1	(Tyutin et al. 2013)
Tamis River region, Danube, Sjenicko-Pesterska Plateau	Serbia	CE	10	2	(Kiskaroly & Tafro 1988; Cakic 1992)

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10. Appendix C – Supplementary material for Chapter 4

Table C1. GLMM output for significant models of identified helminths in brown trout *Salmo trutta*.

Salmonema ephemeridarum								
Infection parameter (response variable)	Model	df	Explanatory variable	Coefficient	Standard error	Z	P	
Prevalence	Binomial GLMM	244	Region	4.0218	0.6294	6.39	1.66E-10	***
			Year	2.2113	0.4546	4.864	0.00000115	***
			Region:Year	-3.6948	0.7578	-4.875	0.00000109	***
Abundance	Negative binomial GLMM	239	Host length (scaled)	0.5346	0.1174	4.555	0.00000523	***
			Region	3.7585	0.7652	4.912	9.03E-07	***
			Year	1.5276	0.5185	2.946	0.00322	**
			Sex (male)	0.364	0.196	1.857	0.06334	.
			Sex (unidentifed)	0.1179	0.2123	0.555	0.57867	
			Region:Year	-1.9197	0.9091	-2.112	0.03472	*
Intensity	Negative binomial GLMM	175	Host length (scaled)	0.5874	0.0954	6.157	7.42E-10	***
			Region	2.601	0.5365	4.848	0.00000125	***
			Year	1.4883	0.4305	3.457	0.000546	***
			Sex (male)	0.2176	0.1708	1.274	0.202677	
			Sex (unidentifed)	0.1076	0.1935	0.556	0.578224	
			Region:Year	-1.7363	0.6167	-2.816	0.004868	**

Table C1. (contd.)

Pomphorhynchus tereticollis

Infection parameter (response variable)	Model	df	Explanatory variable	Coefficient	Standard error	Z	P	
Prevalence	Binomial GLMM	241	Region	0.367976	1.121287	0.328	0.74278	
			Year	-4.682755	1.407852	-3.326	0.00088	***
			Host length	-0.013017	0.004679	-2.782	0.0054	**
			Sex (male)	-0.14369	0.415912	-0.345	0.72973	
			Sex (unidentifed)	-0.449188	0.434197	-1.035	0.30089	
			Region:Year	3.279717	1.719078	1.908	0.05641	.
Abundance	Negative binomial GLMM	239	Host length (scaled)	-0.28304	0.12615	-2.244	0.024856	*
			Region	-0.26836	0.91502	-0.293	0.769308	
			Year	-4.71978	1.26848	-3.721	0.000199	***
			Sex (male)	0.06222	0.23717	0.262	0.793062	
			Sex (unidentifed)	-0.24627	0.26324	-0.936	0.349501	
			Region:Year	4.04878	1.50084	2.698	0.006982	**
Intensity	Negative binomial GLMM	97	Host length (scaled)	-0.02961	0.08817	-0.336	0.737	
			Region	-1.27433	0.174	-7.324	2.41E-13	***
			Year	-2.20246	1.16127	-1.897	0.0579	.
			Sex (male)	0.21043	0.18174	1.158	0.2469	
			Sex (unidentifed)	-0.17893	0.20773	-0.861	0.389	
			Region:Year	2.19819	1.17508	1.871	0.0614	.

Table C1. (contd.)

Cucullanus truttae

Infection parameter (response variable)	Model	df	Explanatory variable	Coefficient	Standard error	Z	P	
Prevalence	Binomial GLMM	244	Region	2.898411	1.152339	2.515	0.011895	*
			Year	1.950643	0.902746	2.161	0.030712	*
			Host length	0.029536	0.008725	3.385	0.000712	***
Abundance	Negative binomial GLMM	243	Host length (scaled)	2.0931	0.6262	3.342	0.00083	***
			Region	3.0999	0.9797	3.164	1.56E-03	**
			Year	3.7564	0.9678	3.881	0.000104	***
Intensity	Negative binomial GLMM	13	Region	2.7683	1.0195	2.715	0.00662	**
			Year	1.5156	0.3535	4.287	1.81E-05	***
			Sex (male)	-0.3871	0.3569	-1.085	0.27808	
			Sex (unidentifed)	0.9804	0.5162	1.899	0.05754	.

Rhabdochona sp

Infection parameter (response variable)	Model	df	Explanatory variable	Coefficient	Standard error	Z	P	
Intensity	Negative binomial GLMM	27	Host length (scaled)	0.78358	0.33789	2.319	0.0204	*
			Sex (male)	-0.09684	0.56066	-0.173	0.8629	
			Sex (unidentifed)	0.29762	0.55714	0.534	0.5932	

11. Appendix D – Supplementary material for Chapter 5

Table D1. Sample sizes (n) of brown trout *Salmo trutta* and common dace *Leuciscus leuciscus* used in this study by sampling site and year. "-" indicates that the site was not sampled that year

River system	Site	Co-ordinates		Brown trout (n)			Common dace (n)		
		Latitude	Longitude	2015	2017	Total	2015	2017	Total
Munster Blackwater	Fermoy	52.140556	-8.268333	21	35	56	13	43	56
	Funsion	52.157385	-8.242280	-	26	26	-	0	0
	Glanworth	52.188056	-8.353333	29	-	29	17	-	17
				50	61	111	30	43	73
River Barrow	Monasterevin	53.145833	-7.070556	1	1	2	36	5	41
	Portarlington	53.162222	-7.192222	2	70	72	16	81	97
	Slate	53.219722	-6.995833	21	-	21	6	-	6
				24	71	95	58	86	144

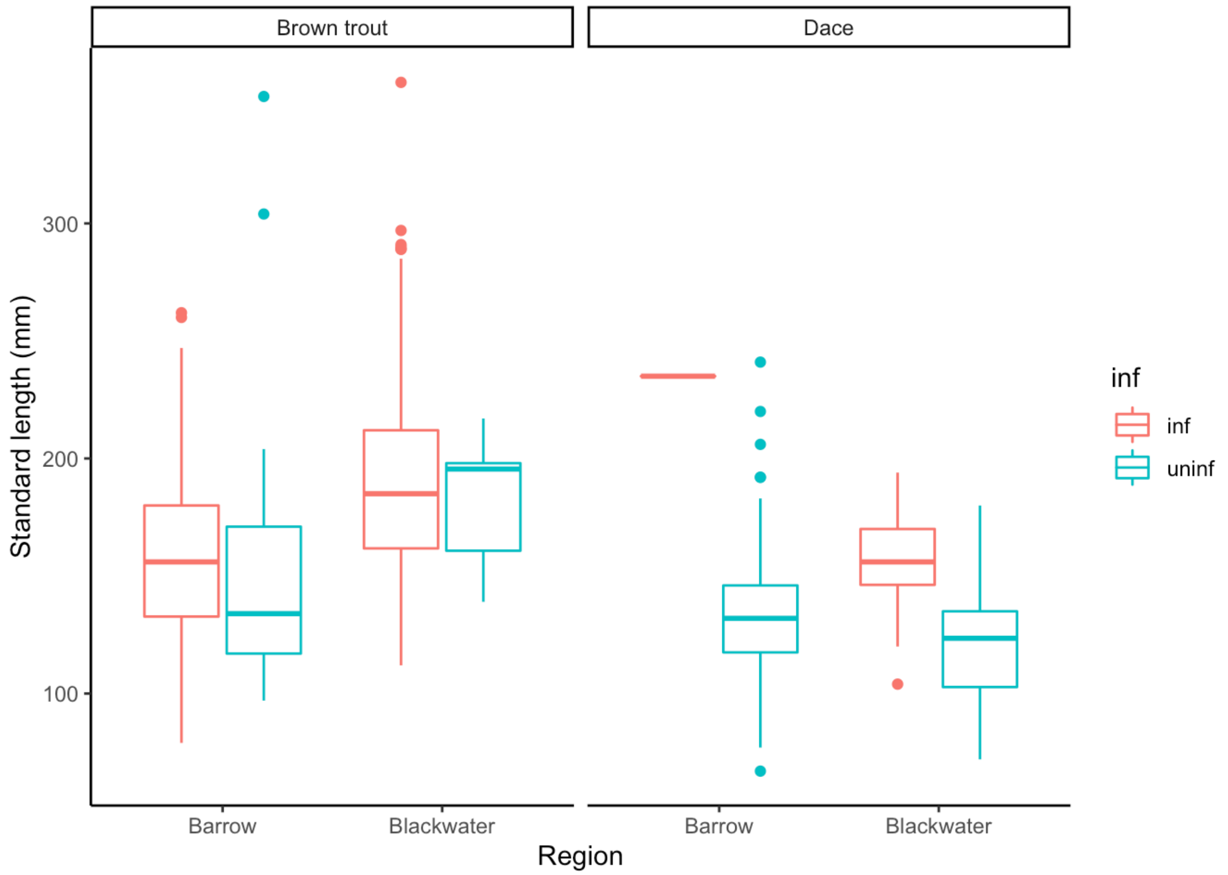

Figure D1. Boxplot of standard length (mm) invasive common dace *Leuciscus leuciscus* and native brown trout *Salmo trutta* from the Munster Blackwater (invasion core) and River Barrow (invasion front), grouped by status of infection with trophically transmitted helminths. Box represents the interquartile range and the horizonal line represents the median value. Abbreviations: inf = infected, uninf = uninfected.

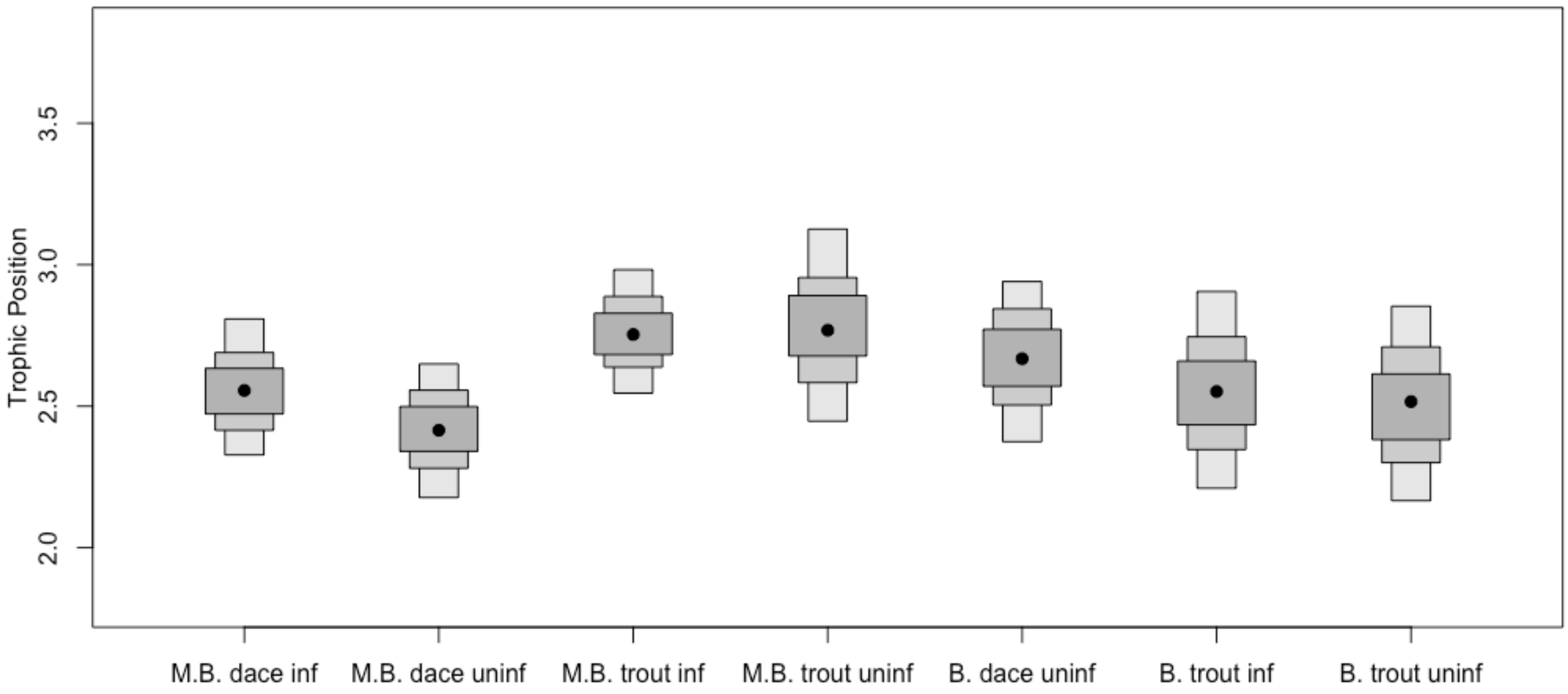

Figure D2. Density plot of the estimated trophic position of dace and brown trout from the Munster Blackwater (M.B.) and River Barrow (B.) that were infected (inf) and uninfected (uninf) with trophically-transmitted helminths.

12. Appendix E – Supplementary information to Chapter 6

Table E1. Checklist of metazoan parasite species recorded in pink salmon *Oncorhynchus gorbuscha* in its native Pacific range (Alaska, British Columbia, Japan) and non-native Atlantic range (Great Lakes, White Sea, Barents Sea). Helminth species that also occur in the helminth communities of native brown trout *Salmo trutta* and Atlantic salmon *Salmo salar* in Ireland are highlighted in bold.

Taxa	Location	Host tissue	Reference
CESTODA			
Diphyllobothrium latum	N. America	body cavity/viscera	(Chai et al. 2005; Scholz & Kuchta 2016)
Diphyllobothrium nihonkaiense	Japan	body cavity/viscera	(Yamane et al. 1986)
Diphyllobothrium klebanovskii	Russia	body cavity/viscera	(Muratov & Posokhov 1988)
***Diphyllobothrium* spp**	Great Lakes, Alaska	body cavity/viscera	(Muzzall & Peebles 1986; Muzzall 1995; Moles 2007)
Proteocephalus parallacticus	Great Lakes	GI tract	(Muzzall & Peebles 1986; Muzzall 1995; Moles 2007)
Cyathocephalus truncatus	Great Lakes	GI tract	(Muzzall & Peebles 1986; Muzzall 1995; Moles 2007)
Eubothrium salvelini	Great Lakes	GI tract	(Muzzall & Peebles 1986; Muzzall 1995; Moles 2007)
Eubothrium crassum	White & Barents Sea basins	GI tract	(Ieshko et al. 2016, 2018)
Eubothrium sp.	Alaska	Body cavity, mesentery, muscle	(Moles 2007)
Triaenophorus crassus	Great Lakes	muscle	(Muzzall 1995)
Triaenophorus nodulosus	Great Lakes	body cavity/viscera	(Muzzall 1995)
Scolex pleuronectis	White & Barents Sea basins		(Ieshko et al. 2018)
Bothrimonus sturionis	Alaska	intestine, pyloric cecae	(Moles 2007)

Phyllobothrium caudatum	Alaska	intestine, pyloric cecae	(Moles 2007)
Phyllobothrium sp.	Alaska; British Columbia	intestine, pyloric cecae, gall bladder	(Boyce 1969; Moles 2007)
NEMATODA			
Anisakidae sp.	NW Pacific, Alaska	body cavity, muscle, mesentery, intestinal wall, stomach wall	(Bilska-Zajac et al. 2016)
Anisakis simplex l.	Keret River, White Sea		(Ieshko et al. 2016)
Ascarophis sp.	Alaska	intestine, stomach	(Moles 2007)
Capillaria salvelini	Great Lakes	GI tract	(Muzzall & Peebles 1986; Muzzall 1995)
Cystidicola farionis	Great Lakes	swim bladder	Muzzall & Peebles 1986; Muzzall 1995)
Salmonema ephemeridarum	Great Lakes	stomach	Muzzall & Peebles 1986; Muzzall 1995)
Philonema oncorhynchi	Great Lakes	body cavity/viscera	(Muzzall 1995)
Rhabdochona canadensis	Great Lakes	GI tract	(Muzzall & Peebles 1986; Muzzall 1995)
Spinitectus gracilis	Great Lakes	GI tract	(Muzzall & Peebles 1986; Muzzall 1995)
Haplonema sp.	Great Lakes	GI tract	(Muzzall & Peebles 1986)
Hysterothylacium gadi aduncum	White & Barents Sea basins		(Ieshko et al. 2018)
Hysterothylacium aduncum	Keret River, White Sea		(Ieshko et al. 2016)
Pseudoterranova decipiens	Keret River, White Sea; Alaska	mesentery, muscle	(Moles 2007; Ieshko et al. 2016)
Contracaecum sp.	Alaska; British Columbia	intestinal wall, mesentery, stomach wall	(Boyce 1969; Moles 2007)
Philonema oncorhynchi	Alaska	body cavity, swim bladder	(Moles 2007)

TREMATODA			
Crepidostomum farionis	Great Lakes	GI tract	(Muzzall & Peebles 1986; Muzzall 1995)
Derogenes varicus	White & Barents Sea basins		(Ieshko et al. 2016, 2018)
Brachyphallus crenatus	White & Barents Sea basins		(Ieshko et al. 2016, 2018)
Lecithaster gibbosus	White & Barents Sea basins, Alaska; British Columbia	intestine, pyloric cecae, stomach	(Boyce 1969; Moles 2007; Ieshko et al. 2016, 2018)
Lecithophyllum botryophorum	Alaska	intestine, stomach	(Moles 2007)
Podocotyle atomon	White & Barents Sea basins		(Ieshko et al. 2018)
Podocotyle reflexa	Keret River, White Sea		(Ieshko et al. 2016)
Diplostomum volvens	Keret River, White Sea	eye	(Ieshko et al. 2016)
Ichthyocotylurus erraticus	Keret River, White Sea		(Ieshko et al. 2016)
Hemiurus levenseni	Keret River, White Sea		(Ieshko et al. 2016)
Parahemiurus merus	Alaska; British Columbia	intestine, pyloric cecae, stomach	(Boyce 1969; Moles 2007)
Pentagramma petrowi	British Columbia	intestine	(Boyce 1969)
Pronoprymna petrowi	Alaska	intestine, pyloric cecae	(Moles 2007)
Prosorhynchoides basargini	Alaska	intestine, pyloric cecae, stomach	(Moles 2007)
Tetracotyle sp.	Alaska	heart, mesentery, muscle, kidney tubules	(Moles 2007)
Tubulovesicula lindbergi	Alaska	intestine, stomach	(Moles 2007)
ACANTHOCEPHALA			
Echinorhynchus salmonis	Great Lakes	GI tract	(Muzzall & Peebles 1986; Muzzall 1995)

Echinorhynchus sp.	Great Lakes	GI tract	(Muzzall & Peebles 1986; Muzzall 1995)
Neoechinorhynchus tumidus	Great Lakes	GI tract	Muzzall & Peebles 1986; Muzzall 1995)
Neoechinorhynchus sp.	Alaska	intestine	(Muzzall 1995)
Echinorhynchus gadi	Keret River, White & Barents Sea basins, Alaska	intestine, mesentery	(Moles 2007; Ieshko et al. 2016, 2018)
Bolbosoma sp.	Alaska	intestine	(Moles 2007)
Corynosoma strumosum	Alaska	body cavity, intestinal wall, liver, mesentery	(Moles 2007)
Corynosoma villosum	Alaska	body cavity, intestinal wall, mesentery	(Moles 2007)
Corynosoma sp.	Alaska	body cavity, intestine, mesentery	(Moles 2007)
Rhadinorhynchus trachuri	Alaska	intestine	(Moles 2007)
ARTHROPODA			
Lepeophtheirus salmonis	British Columbia, White & Barents Sea basins, Alaska	skin, fins, gills	(Beamish et al. 2005; Saksida et al. 2012; Ieshko et al. 2016, 2018)
Caligus clemensi	British Columbia	skin	(Beamish et al. 2005)
Salmincola salmoneus	White & Barents Sea basins	gills	(Ieshko et al. 2018)
Ergasilus sieboldi	Keret River, White Sea		(Ieshko et al. 2016)
Bomolochus cuneatus	Alaska	gill cavity	(Moles 2007)
Rocinela belliceps	Alaska	skin	(Moles 2007)

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